

# ORALS

## Oral session: Diagnosis and risk factors

### Subgingival – *in vivo* – calculus detection using 655 nm diode laser irradiation.

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**Background:** Clinical subgingival calculus detection has a poor test sensitivity. The aim of this *in vivo* study was to compare a diagnostic laser with an explorer in their ability of subgingival calculus detection.

**Methods:** Fifteen teeth with untreatable periodontitis were analysed directly before their extraction. At the mesial and distal surface of each tooth calculus was diagnosed either by a thin explorer (EXD 11/12) or by a diagnostic InGaAsP diode laser (KaVo KEY 3 laser) with a headpiece for the irradiation (655 nm wavelength) of the root surface. The intensity of the resulting fluorescence was analysed and a peak value  $> 30$  was counted as presence of calculus. The clinical detection was performed before or after laser detection. On each surface five areas were assessed separately. After extraction, digital images were taken and the presence of calculus was determined.

**Results:** The result shows that 64 out of 150 surfaces were covered with calculus. The explorer method discovered 24 of the 64 surfaces (sensitivity 37.5%) and the laser method was positive on 52 of the 64 areas with calculus (sensitivity 81.3%). Areas free of calculus were detected on 64 out of 86 (explorer: specificity 74.4%) vs. 69 out of 86 (laser: specificity 80.2%) surfaces.

**Conclusion:** Within the limits of this study it can be concluded, that the sensitivity of subgingival calculus detection by laser induced fluorescence (655 nm excitation wavelength) is significantly higher than the detection sensitivity of an explorer.

### Quantification of the periodontal attachment area in fully dentate humans by computed tomography

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Current literature does not provide data of the total periodontal attachment area measured in single individuals. This presentation focuses on a study using Computed tomography (CT)-data of fully dentate, periodontal healthy subjects to determine the total root surface area of humans. CT-data of 57 patients, who underwent CT-scanning for other than periodontal indications, were analysed. After image processing the root surface of every single tooth was calculated. In the next step every root area was vertically divided in thirds (coronal, middle, apical) and the surface area of each third calculated. Statistic processing of the data was performed to determine mean values and standard deviations for the root surface area of single teeth, the root thirds, the upper and lower jaw separately and the total of all 28 teeth. The results for the total periodontal attachment area show a mean value of  $75.4 \text{ cm}^2$  (SD

$18.3 \text{ cm}^2$ ).  $39.3 \text{ cm}^2$  and  $36.9 \text{ cm}^2$  are the mean values for the upper and lower jaw respectively (SD  $9.3 \text{ cm}^2$  and  $8.7 \text{ cm}^2$ ). The mean value for the coronal root thirds is  $33.3 \text{ cm}^2$  (SD  $7.8 \text{ cm}^2$ ), for the middle thirds  $28.3 \text{ cm}^2$  (SD  $6.3 \text{ cm}^2$ ) and for the apical thirds  $14.3 \text{ cm}^2$  (SD  $3.9 \text{ cm}^2$ ). On the basis of the single teeth-data it seems to be possible to get a better estimation of the area of attachment loss in patients with periodontal disease. It needs to be shown whether the extent of inflamed periodontal area plays a role for the severity of systemic response.

### Periodontal disease in 3-generation Brazilian families – genetic power estimation

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Linkage analysis is a powerful method to detect predisposing gene(s), providing there is sufficient pedigree information. The aim was to estimate the power of 3-generation families to detect linkage for future detection of genetic effects in periodontal disease (PD). Three Brazilian families were selected after confirmed diagnoses of generalized aggressive periodontitis in the probands. A 6 site/tooth full-mouth probing was performed by a calibrated examiner in 58 family members (all non-smokers). The phenotype was dichotomized based on questionnaire (edentulous cases) and clinical attachment loss of 4 mm due to pocketing in at least 4 sites of different teeth. A dominant mode of inheritance (autosomal) and recombination fraction  $\Theta = 0$  were adopted in the model. Penetrance values (F) of 0.98, 0.75, 0.5 and phenocopy rates (P) 0 and 0.02 were tested using the SLINK simulation method in 100 replicas. A lod score of 3 is considered significant for genetic linkage analysis. The highest values were found in  $F = 0.98$  and  $P = 0$ , followed by  $F = 0.98$  and  $P = 0.02$ . The average/maximum expected lod scores were 5.90/9.67 and 4.80/8.83 respectively. Bearing in mind the heterogeneity of periodontal disease (genetic and/or environmental contributors), the authors conclude that the analysed pedigrees provide sufficient power for future genotyping.

### Genetic influences on neutrophil function as risk factors for aggressive periodontitis

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**Background:** Neutrophils (PMN) in Aggressive Periodontitis (AgP) patients are hyper active especially with regards to superoxide production. Polymorphisms in genes influencing PMN function have been proposed as candidate risk factors for AgP. The aim of this study was to test the association of specific gene polymorphisms affecting PMN functions with AgP.

**Methods:** Two hundred and twenty four patients with confirmed diagnosis of AgP and 231 subjects with healthy periodontium took part in the study. A blood sample was collected from subjects and genotypes for C242T p22<sup>phox</sup> NADPH oxidase, FP, Fc $\alpha$  and Fc $\gamma$  receptors were analysed in a blind fashion.

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## Oral session: Diagnosis and risk factors

**Results:** The C242T p22<sup>phox</sup> NADPH oxidase T allele was significantly associated with AgP in a multiple logistic regression model adjusting for confounders, both in Caucasian ( $P = 0.009$ , OR = 2.07, 95% CI = 1.20–3.59) and Black subgroups ( $P = 0.032$ , OR = 3.16, 95% CI = 1.28–7.76). The same genotype and FcγR IIIb polymorphism were associated with the Generalised AgP phenotype both in all subjects and Caucasians. FcγR haplotypes were associated with AgP in Blacks ( $P = 0.034$ ).

**Discussion:** The C242T p22<sup>phox</sup> NADPH oxidase and FcγR polymorphisms may predispose to Aggressive Periodontitis, probably through a modulation of neutrophil superoxide production.

### VDR, osteoprotegerin and IL-6 gene polymorphisms in Turkish population with aggressive and chronic periodontitis

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Epidemiologic studies suggest that there is a genetic component of susceptibility to periodontitis. In this study, the VDR, osteoprotegerin (OPG) and IL-6 gene polymorphisms, which are thought to play roles in bone metabolism, were evaluated in Turkish population with aggressive periodontitis (AP) patients, chronic periodontitis (CP) patients and periodontally healthy controls (HC). Sixty-two AP patients, 63 CP patients and 64 HC were enrolled in the study. A full-mouth periodontal examination including probing depth (PD), clinical attachment level (CAL), plaque index (PI) and bleeding on probing (BOP) were performed. DNA's isolated from peripheral blood were analysed for the polymorphisms in VDR, OPG and IL-6 genes by PCR followed by restriction enzyme digestion and gel electrophoresis. There were no significant differences between AP, CP and HC groups for VDR, OPG and IL-6 genotype analysis or allele frequencies investigated. When the patient groups (AP and CP) were classified as mild-moderate (CAL < 4 mm) and severe (CAL > 4 mm) according to CAL, genotype 1/1 and allele 1 were found significantly higher in CP patients severe group in OPG gene polymorphism and although it was not significant allele 1 was found to be higher in CP severe group in IL-6 gene polymorphism. The investigated polymorphisms may not be the markers to designate susceptibility to periodontitis but when the disease is present OPG gene polymorphism might be related with the severity of attachment loss in CP.

### RANKL and OPG mRNA expression in chronic and aggressive periodontitis

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**Aim:** The receptor activator of NF-kappa B ligand (RANKL) and its decoy receptor osteoprotegerin (OPG) are the main regulators of osteoclast formation. Their relative expression ratio is a key factor for the occurrence of bone destruction in periodontitis. The aim of this study was to investigate RANKL and OPG mRNA expression in gingival tissues of patients with different periodontal diseases.

**Methods:** Gingival biopsies were obtained from five patients with generalized aggressive periodontitis (G-AgP), five with chronic periodontitis (CP), five with gingivitis and five periodontally healthy subjects. RANKL and OPG mRNA levels were quantified by real time quantitative RT-PCR.

**Results:** Osteoprotegerin expression levels were significantly lower in Gingivitis and CP patients, compared to healthy subjects (13% and 1%, respectively). In contrast, the G-AgP group showed less

marked reduction in OPG expression (66% of healthy subject levels). However, the G-AgP group showed strong induction in RANKL expression, whereas in CP patients there was no increased RANKL expression. When RANKL/OPG ratios were calculated, this was markedly increased in both AgP and CP compared with controls, there was no significant difference between CP and G-AgP in RANKL/OPG ratios.

**Conclusion:** These preliminary results suggest that the increased RANKL/OPG ratio in CP patients may account for a down-regulation of OPG expression, whereas in G-AgP patients this may be due to up-regulation of RANKL expression.

### Salivary pro-inflammatory cytokine arrays for diagnosis of periodontitis

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**Aims:** Increased levels of pro-inflammatory cytokines may be found in oral fluids of patients afflicted with chronic periodontitis. The identification of multiple analytes from the same biological sample remains a challenge in the evaluation of periodontal disease biomarkers. The aim of the present cross-sectional study was to measure salivary-derived pro-inflammatory cytokines in periodontally healthy and diseased subjects.

**Materials and methods:** Clinical attachment levels (CAL), probing depths (PPD), bleeding on probing (BOP) were assessed from a total of 20 patients with periodontal health or periodontitis. Whole saliva (WS) was collected and analysed using protein microarray techniques for IL-6, IL-1β, IL-2, IL-13, IL-4, IFN-γ, IL-5 and TNF-α. These assays are based on a miniaturized immunoassay using microarrays of biotinylated monoclonal antibodies and streptavidin-conjugated fluorophores.

**Results:** In WS, a > 20 fold increase of IL-1β was found in subjects with periodontal disease compared to healthy individuals ( $P < 0.05$ ), while > 2 fold increases in IL-4, IL-6 and IL-10 were found in disease vs. health. A statistical significant correlation between PPD and CAL, was found in WS with multiple cytokines evaluated (Spearman's rank correlation rho: 0.57–0.72).

**Conclusion:** The evaluation of WS via multiplexed cytokine arrays may give valuable information about the pathophysiological processes in periodontitis and provide a more robust assessment of periodontal disease risk.

### Gingival squamous cell carcinoma (GSCC): diagnostic delay or rapid invasion?

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**Background:** The similarity between GSCC and common periodontal lesions may cause delay in diagnosis or misdiagnosis. These cancers are frequently diagnosed at an advanced stage.

**Methods:** To assess the relative time from when the patient first became aware of the problem to histopathological diagnosis (total diagnostic time), 59 consecutive oral cancer cases were examined. Variables studied: age, gender, smoking habits, tumour stage at diagnosis and total diagnostic time. The median of the patients' total diagnostic time (1.5 months) was used as a cut-off point to distinguish delayed from non-delayed cases. Statistical analysis was undertaken using the Chi square and Student *t*-tests, with a confidence interval of 95%.

**Results:** The total diagnostic time was less than 1.5 months for 75% of GSCC, 50% of tongue cancers and 78% of floor of the

mouth carcinomas. It was more than 1.5 months for 25% of GSCC, 50% of tongue carcinomas and 21% of floor of the mouth cancers. No significant differences in time before diagnosis were found when GSCC were compared to other oral tumours ( $\chi^2 = 0.21$ ; 95% CI = -0.40–0.26). However, by the time of diagnosis, GSCCs had invaded adjacent structures more

frequently than other oral cancers ( $\chi^2 = 13.51$ ; 95% CI = 0.18–0.85).

**Conclusions:** Gingival squamous cell carcinomas were associated with advanced stages at diagnosis, due to early invasion of contiguous bone tissue (T4-primary tumour). This would indicate that even earlier referral and diagnosis was necessary.

## Oral session: Antimicrobial therapy

### The effects of a 0.1% chlorhexidine mouthwash on the subgingival biofilm in patients with gingivitis: a 3-month study

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**Aim:** To compare the microbiological efficacy and safety of a 0.1% chlorhexidine (CHX) mouth rinse during a 3-month trial using a placebo and a 0.2% CHX solutions as controls.

**Materials and methods:** A prospective, parallel, randomised clinical trial was designed to evaluate the microbiological effects of the test mouth rinse. 37 patients were assessed at baseline, and days 7 and 84. Pooled microbiological samples were taken from the mesial sites of the upper first molars, from the lingual side for culturing and from the buccal side for DNA probes analysis. DNA probes identified *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythensis* and *T. denticola*. Statistical comparisons were performed by ANOVA and Dunnett's test.

**Results:** The total bacterial counts (expressed in log) were reduced in the three groups after 84 days, and the 0.1% CHX achieved the highest level of reduction (0.19) vs. the placebo (0.13). With culture, a reduction in the percentage of flora for *F. nucleatum*, *P. intermedia* and *M. micros* was observed for the CHX groups. No statistically significant differences were observed when comparing the results obtained with DNA probes. No overgrowth of opportunistic species was detected by culture.

**Conclusion:** A limited microbiological effect of the test mouth rinse on the subgingival flora was observed, and no microbiological adverse effects were detected.

Supported by Pierre-Fabre.

### Antimicrobiological photodynamic therapy apt for treatment of chronic periodontitis

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The effective reduction of colonisation of periodontal pockets is the success parameter for chronic periodontitis. A clinical study with 62 patients and a minimum recall for 12 month was evaluated. The intra-oral disinfection was performed by photodynamic therapy and for 12 patients with additional administration of antibiotics. A thiazin based photosensitizer is applied to the infected area for 1 min. After the coloration of the bacteria the activation of the photosensitizer is performed with non-thermal laser light of 660 nm for 1 min for each area. The photodynamic reaction leads to a singlet oxidation at the membrane of the bacteria and performs a selective cell death. The amount of AA; TG; TI and PG was determined prior, 1 week, 6 and 12 months after therapy. An average of 23.2 teeth were treated for each patient. A significant reduction of the bacterial load occurred in all tested sides for the

1 year control. All patients showed immediately a reduction of bacterial load for all marker specimens. The combination therapy of APT and antibiotics showed no improvement in comparison to APT alone. The reduction of clinical pathological signs could be reached with an average reduction of SBI by 0.84. Attachment level gain was 0.9 mm in average. The APT is an efficient method for bacterial load reduction in periodontal therapy. The treatment is non invasive and can be repeated without the risk of allergies and resistance in comparison to antibiotic therapy.

### Adjunctive amoxicillin and metronidazole in the treatment of generalized aggressive periodontitis: response in molars and anteriors.

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**Aim:** To assess the adjunctive benefit of amoxicillin and metronidazole in generalized aggressive periodontitis (GAP) in terms of reduction in the need for corrective periodontal therapy in molar and anteriors 6 months post treatment.

**Methods:** Forty one GAP patients were randomly assigned to either test (TG) or placebo group (PG). 20 subjects (TG) received full-mouth root instrumentation (FRI) with systemic amoxicillin (500 mg/TID/7 days) and metronidazole (500 mg/TID/7 days). 21 subjects (PG) received FRI with placebo medication. Clinical parameters were evaluated at baseline and 2–6 months.

**Results:** Median percentage of pockets converting from  $\geq 5$  mm to  $\leq 4$  mm at 6 months was, in anteriors, 89.4% and 69.2% in the TG and PG, respectively ( $P = 0.002$ , Mann–Whitney). In molars, the figures were 56.6% and 34.6% in TG and PG, respectively ( $P = 0.011$ ). In anteriors, median percentage of pockets converting from  $\geq 4$  mm at baseline to  $\leq 3$  mm at 6 months was 75.6% and 50% for TG and PG, respectively ( $P = 0.007$ ). The same figure in molars was 24.1% and 20.1% in TG and PG, respectively ( $P = 0.220$ ). The additional benefit of the antibiotic treatment was larger in anteriors (25.6%) as compared to molars (4.1%).

**Conclusions:** The adjunctive use of amoxicillin and metronidazole resulted in higher additional reductions in the need for corrective periodontal therapy in anteriors as compared to molars when using 4 mm as the discriminant value.

### Adjunctive systemic antibiotics in patients with aggressive and severe chronic periodontitis

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**Aims:** The goal of this study was to find out if the treatment results in patients with aggressive (AgP) and severe chronic periodontitis (sCP) can be improved with adjunctive systemic antibiotics if

## Oral session: Periodontal molecular mechanisms of tissue damage

patients have been tested positive for *Actinobacillus actinomycetemcomitans* (A.a.).

**Methods:** A total of 39 patients with either AgP ( $n = 10$ ) or sCP ( $n = 29$ ) participated in the study. Subgingival samples of 22 patients were positive for A.a. at the beginning of the study.

**Results:** The patient groups with and without A.a. did not differ significantly at the beginning of the study, with regard to their hygiene (PCR) and inflammation parameters (GBI, BOP). Mean pocket probing depths (PD) and clinical attachment levels (AL) were comparable in both groups. During periodontal therapy, a significant overall reduction of GBI (from 16% to 3%), PCR (from 44% to 21%) and BOP (from 34% to 14%) could be observed. Mean increase of AL was 0.96 mm in patients with A.a. and 0.50 mm in patients without A.a. The mean reduction of pocket probing depths was significantly higher in patients with A.a. (1.65 mm) compared to patients without A.a. (0.72 mm).

**Conclusion:** In patients with A.a., adjunctive systemic antibiosis can help to establish a better clinical outcome compared to patients without A.a. that receive no adjunctive antibiosis.

### Reinfections with Aa and Pg after adjunctive antibiotic therapy in aggressive and chronic periodontitis

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Out of the large variety of subgingival bacterial species only a few have biochemical abilities, which are of importance for the pathogenesis of Periodontitis. All molecular biological mechanisms, host responsibility and non-acquired risk-factors are genetically determined. Microbiological testing is important for the determination of adjunctive antibacterial treatment. During periodontal non surgical treatment 230 patients with aggressive ( $n = 82$ ) or generalised progressive chronic periodontitis ( $n = 148$ ) got adjunctive systemic antibiotic regime and were followed by periodontal maintenance for 2–10 years. In 10 cases (20.4%) of an *actinobacillus actinomycetemcomitans* (Aa)-associated aggressive periodontitis and 15 cases (22.7%) of an Aa-associated chronic periodontitis an Aa-reinfection and additional loss of attachment 2–5 years postoperatively made periodontal retreatment necessary. In three cases (9.1%) of a porphyromonas *gingivalis* (Pg)-associated aggressive periodontitis and 23 cases (28.0%) of a chronic Pg-associated periodontitis with later

Aa-infection 3.5–10 years postoperatively caused also immediate retreatment. Reinfections with Aa were diagnosed in 25 patients (21.7%) with former Aa-associated periodontitis. In 98 patients (85.2%) with a former Pg-associated periodontitis Pg-reinfections were found. Microbiological testing in the daily periodontal practise is only sufficient, if therapeutical consequences follow and a positive cost-effect relation is realized.

### Effect of adjunctive low-dose doxycycline therapy on clinical parameters and gingival crevicular fluid t-PA levels in chronic periodontitis

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**Objectives:** The present study examined effectiveness of low-dose doxycycline (LDD) in combination with mechanical therapy on gingival crevicular fluid (GCF) tissue plasminogen activator (t-PA) levels and clinical parameters in chronic periodontitis over 12-month period.

**Methods:** Gingival crevicular fluid samples were collected, probing depth (PD), clinical attachment level (CAL), gingival index (GI) and plaque index were recorded at baseline, 3, 6, 9 and 12 months. 65 chronic periodontitis patients were randomized either to LDD or to placebo groups. LDD group received LDD (20 mg) b.i.d for 3 months plus scaling and root planing (SRP), while placebo group was given placebo capsules b.i.d for 3 months plus SRP. GCF t-PA levels were determined by ELISA. Friedman and Wilcoxon test was used to test significance of changes over time. Differences between LDD and placebo groups were analysed by Mann–Whitney test.

**Results:** Significant improvement was observed in all clinical parameters in both groups over 12-month period ( $P < 0.01$ ). LDD group had lower PD, CAL and GI scores compared to placebo group at 6, 9 and 12 months ( $P < 0.05$ ). GCF t-PA levels was significantly reduced in both groups over 12-month period ( $P < 0.01$ ). However, GCF t-PA levels in LDD group was significantly lower than that of placebo group at 6 and 9 months ( $P < 0.05$ ).

**Conclusion:** These results provide additional information about the usefulness of LDD therapy as an adjunct to mechanical therapy in the long-term management of periodontal disease.

## Oral session: Periodontal molecular mechanisms of tissue damage

### Hydrocortisone affects the mRNA expression for matrix metalloproteinases and tissue inhibitors of metalloproteinases in gingival fibroblast cells

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**Background:** Disruption of the balance between matrix metalloproteinases (MMPs) and endogenous tissue inhibitors of metalloproteinases (TIMPs) production results in periodontal breakdown. The aim of this study was to evaluate the effect of the hydrocortisone, which is important in the hypothalamic-pituitary-adrenal axis regulation of the periodontal inflammation,

in the mRNA expression for MMPs and TIMPs by human gingival fibroblasts.

**Materials and methods:** Human gingival fibroblast cells were stimulated with com hydrocortisone at  $10^{-7}$  M,  $10^{-9}$  M,  $10^{-12}$  M for 24, 48 and 72 h in DMEM without fetal calf serum. Untreated cells in DMEM without fetal calf serum served as controls. The cells were lysed and the RNA was extracted. Alterations in expression of MMP-1, MMP-2, MMP-3, MMP-7, MMP-11, TIMP-1 and TIMP-2 mRNA were determined using real-time RT-PCR. b-actin was used as a housekeeping gene to normalize the results.

**Results:** It was observed an increased MMP-1, MMP-2, MMP-3 and MMP-7 expressions independently of the concentration or

time of exposition. MMP-11, TIMP-1 and TIMP-2 expressions were dose- and time-dependents, in general, MMP-11 and TIMP-2 expressions were up-regulated at longer exposition to the drug, and TIMP-1 was down-regulated at lower hydrocortisone concentrations.

**Conclusion/Discussion:** Hydrocortisone can affect the balance of the mRNA expression for MMPs and TIMPs, and is likely involved in the periodontal breakdown.

### Stromelysin-1 gene expression is up-regulated with the progression of periodontal disease

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**Background and Aim:** It was previously described that collagenases (MMP-1, MMP-8, MMP-13) play an important role in periodontal destruction. In the present study we have investigated the regulation of the coordinated expression of other metalloproteinases (MMPs), gelatinases and stromelysin, and tissue inhibitor of MMPs (TIMP-1) gene expression in gingival tissues with increased severity of periodontal damage.

**Materials and methods:** Normal gingival tissues (control) and diseased tissues (from patients with periodontal disease) were obtained during periodontal resective procedures. Diseased tissues were clinically classified according to disease severity. Tissues were immediately frozen in liquid nitrogen and stored at -80 °C. Tissues were processed to obtain m-RNA and gene expression of gelatinases (MMP-2 and MMP-9), stromelysin-1 (MMP-3) and TIMP-1 analysed by real time PCR microfluidic cards (ABI-PRISM 7000).

**Results:** Expression of gelatinases was not significantly modified but expression of stromelysin-1 was positively correlated with disease severity ( $P < 0.05$ ). TIMP-1 expression was significantly reduced in inflamed tissues ( $P < 0.05$ ).

**Conclusion:** In addition to collagenases, tissue destruction in periodontal disease is also associated to up-regulation of stromelysin and down-regulation of TIMP-1 gene expression, hence favouring un-inhibited tissue degradation. Future studies will have to address the study of MMP-3-protein expression levels and their functional implications.

### Elastolysis triggers collagenolysis in gingiva equivalent

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Elastin derived peptides (EDPs) were shown to enhance the expression of MMP-3 in human gingival fibroblasts (HGF) cultures while having no influence on levels of uPA, MMP-2, MMP-13, MMP-14, TIMP-1 and TIMP-2. EDPs-inducing effect on MMP-3 production was S-Gal mediated since it was significantly inhibited by lactose and reproduced by peptides containing the VGVAPG sequence recognized by this plasma membrane receptor. To assess the contribution of EDPs in collagenolysis, collagen lattices were used; in contracted lattices, EDPs were found to synergize the influence of Plasminogen (Plgn) on collagen degradation and MMPs-activation. Those effects could be accounted for by EDPs-mediated up-regulation of MMP-3/MMP-1 cascade associated with significant down-regulation of TIMP-1 and TIMP-2 production. EDPs or Plgn alone did not significantly induced collagenolysis in attached collagen lattices; however, combination of both agents led to collagenolysis that could be attributed to MMP-3 over expression, MMP-1 activation and TIMP-2 down-regulation.

### The mitogenic effect of TGF $\beta$ 1 and 17- $\beta$ estradiol on human periodontal ligament cells. An *in vitro* study

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Growth factors and hormones are known to play a major role in the periodontal healing procedures. TGF- $\beta$ 1 is a polypeptide growth factor considered to affect mitogenesis of periodontal ligament (PDL) cells in a dose- and time-dependent manner. This factor is regulated by hormones as estrogens, while these hormones seem to act directly on the PDL cell proliferation. The aim of this *in vitro* study was to assess the effect of TGF- $\beta$ 1 and 17- $\beta$  estradiol on the proliferation of human PDL cells when these factors are applied in various dosages and time periods. Cell cultures were developed from the periodontal ligament of three human premolars extracted for orthodontic reasons. TGF- $\beta$ 1 in concentrations of 1 ng/mL  $\kappa$  25 ng/mL and 17- $\beta$  Estradiol (E2) in concentration of 1 nM  $\kappa$  100 nM were applied on PDL cells. The mitogenic effect of these factors on PDL cells was tested at 48 and 72 h. The Trypan Blue exclusion assay was used for determining absolute cell numbers. An increase in the cell number was obtained at 48 h and 72 h. Compared to control, this was statistically significant ( $P < 0.05$ ), for TGF- $\beta$ 1 1 ng/mL and E2 100 nM at 48 h and for E2 100 nM at 72 h. Concluding, TGF- $\beta$ 1 and E2 affect PDL cell proliferation in a dose and time-dependent manner. Further investigation of the exact mechanism of action of these factors on human PDL cells may give useful information about the healing of the periodontal tissues *in vivo*.

### Functional significance of female sex hormone receptors in the periodontium.

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**Aim:** Several studies have addressed the association between changes in estrogen and progesterone levels and changes in parameters of periodontitis. The purpose of this project is to investigate the mechanisms by which estrogen influence structure and function of the periodontal ligament by affecting the properties of periodontal ligament cells (PDL cells).

**Materials and methods:** Periodontal ligament cells were obtained from teeth extracted for orthodontic reasons. The cells were cultured from periodontal tissue explants and used in passages 3–5. Estrogen (ER) and progesterone receptor (PR) expression was investigated by immunocytochemistry. Subcellular distribution of ER $\beta$  was determined using immunogold labeling and electronmicroscopy. DNA and collagen synthesis was measured using incorporation of [<sup>3</sup>H]-thymidine and L-[<sup>3</sup>H]-proline, respectively.

**Results:** ER $\beta$  but not ER $\alpha$  or PR immunoreactivity was observed in the PDL cells. Preliminary results show that ER $\beta$  is distributed not only in the nucleus but also in the mitochondria and cytosol. Estrogen increased DNA synthesis in human breast cancer cells but had no effect on PDL cell DNA or collagen synthesis.

**Conclusions:** Human PDL cells express ER $\beta$  suggesting that estrogen affects PDL cellular function via this ER subtype. Estrogen has no effect on DNA and collagen synthesis, showing that estrogen has no beneficial effect on the periodontium via this mechanism. Instead estrogen via ER $\beta$  acts on the periodontium via another still unknown mechanism.

### The effect of age on prostaglandin-synthesising enzymes in the development of gingivitis

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The aim of the study was to evaluate the difference in the expression of Cox-1, Cox-2, Cox-3 and mPGES-1 in experimental gingivitis of periodontally healthy young and aging test persons. The test persons were divided into two groups aged from 18 to 30 years ( $n = 7$ ) and from 46 to 77 ( $n = 7$ ). After establishing a high level of oral hygiene, clinical data were recorded and a tissue biopsy was taken. After establishing an experimental gingivitis the clinical examination was repeated and a new tissue biopsy was taken. Cox-1, Cox-2 and mPGES-1 were analysed in the connective tissue and epithelium. In both periodontally healthy age groups, Cox-1 and mPGES-1 was demonstrated in epithelium cells, endothelium and fibroblast-like connecting tissue cells. Additionally Cox-1 was found in Langerhans cells. Cox-2 was discovered in cells exhibiting the morphology of epithelial mitosis cells. In the older test persons the amount was statistically significantly lower ( $P = 0.014$ ). During the experimental gingivitis there were no significant differences in Cox-1 and m-PGES-1. In contrast, the Cox-2 expression increased statistically ( $P < 0.05$ ). Cox-3 was not found in either age group. In conclusion, there were no significant differences in Cox-1 and m-PGES-1 to be seen in either of the age groups. Cox-1 was demonstrated in Langerhans cells. In both the older and the younger age groups, the experimental gingivitis was accompanied by a significant increase Cox-2 expression.

### The effect of AGE on MIF and PGE2 in the development of gingivitis

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The aim of the study was to evaluate the difference in the expression of MIF and PGE2 in experimental gingivitis of periodontally healthy young and aging individuals. These persons were divided into two groups aged from 18 to 30 years ( $n = 11$ ) and from 46 to 77 ( $n = 10$ ). After establishing a high level of oral hygiene, clinical data were recorded and sulcus fluid samples were taken. The samples were analysed using ELISA with regard to PGE2 and MIF. After establishing an experimental gingivitis immunological examinations were repeated on days 7 and 14. In both age groups there was a increase in PGE2 (statistically not

significant) during the course of the study (group 1: from  $12.74 \pm 12.62$  ng/mL to  $17.87 \pm 10.61$ ; group 2: from  $18.26 \pm 12.62$  ng/mL to  $23.17 \pm 35.88$ ). At baseline, high concentrations of MIF were detected in both groups ( $1542.48 \pm 792.96$  ng/mL and  $1503.81 \pm 804.27$  respectively). During the course of the study, MIF increased in the younger group ( $+652.39 \pm 1315.58$  ng/mL), while in the older persons a decrease was observed ( $-46.66 \pm 795.49$  ng/mL). In conclusion, the study showed for the first time that high concentrations of MIF are present in the sulcus fluid of periodontally healthy persons. During the course of an experimental gingivitis, MIF increased in the younger age group while it decreased in the older group. PGE2 did not significantly increase in either group. It was not possible to prove a correlation between MIF and PGE2.

### Osteoprotegerin (OPG) levels in gingival crevicular fluid (GCF) during orthodontic tooth movement in human

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Osteoprotegerin is an inhibitor of osteoclast differentiation. Tooth movement is mediated by coupling of bone resorption on the compressed side of PDL and bone formation on the stretched side of PDL. Aim was to determine OPG levels in GCF during tooth movement. Six patients requiring extraction of the 1 pm participated. At time points, PI, GI, PD, BOP were recorded. A canine of each patient undergoing distal movement served as test whereas the contralateral, free from any orthodontic force, were used as control. GCF was collected from mesial and distal sides before activation, at six time points after activation. OPG was measured by ELISA. At baseline while concentration median (CM) was 73.0 (13–205) at test mesial, 56.3 (10–180) at distal ( $P > 0.05$ ), it was 38.2 (3–156) at control mesial, 32.1 (2.6–212) at distal ( $P > 0.05$ ). CM decreased to 18.7 at 24 h and to 10.3 at 3 month, at test mesial sides and to 11.6 and 10.3 respectively at control. There was a time-dependent decrease in GCF OPG levels in groups and difference was significant compared to baseline at all time points ( $P < 0.001$ ). No significant difference was observed between groups and sides at time points. Multiple analyses demonstrated that while time is an independent factor, side or orthodontic force are not risk factors. During the treatment, GI and PI were increased by time in groups ( $P < 0.001$ ). These findings suggest that during the tooth movement, OPG may not be an indicator or a specific marker for evaluating alveolar bone remodelling.

## Oral session: Clinical tips and cases – implants

### A CT scan-derived customized surgical template and fixed prosthesis for flapless surgery and immediate loading utilizing the electroforming technique

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Numerous techniques on immediate occlusal loading of the edentulous patient have been presented. However, very few preconize the placement of the final fixed restoration on the day of fixture installation. The first oral implant protocol with which it was possible to provide patients with a successful on-the-day permanent treatment result, was the Branemark Novum Concept, (1999). Recently, the Nobel Guide concept has been described.

Based on three-dimensional implant planning software for computed tomographic (CT) scan data, customized surgical templates and final dental prostheses can be designed to ensure high precision transfer of the implant treatment planning to the operative field and an immediate rigid splinting of the installed implants, respectively. Here a novel type of rehabilitation is presented, in which utilizing three-dimensional implant planning software for CT scan data, and a flapless implant placement approach, the implants are placed, and custom made screw-retained zirconia abutments are connected in order to support a final fixed bridge utilizing the electroforming technique. An ideal intraoral passive fit and an extremely rigid fixation of the implant abutments to the prosthesis can be achieved. Once the osseointegration process has

taken place, the patient is instructed to retrieve the removable-fixed bridge regularly in order to maintain an excellent hygiene, and soft tissue health, better than any other type of fixed implant-supported restoration.

### A protocol for immediate implant placement in the multirooted molar teeth sites

J. Y. Kim\*

*UCLA School of Dentistry*

A Protocol for Predictable Immediate Implant Placement in Multi-Rooted Molars Despite numerous clinical advantages of immediate implant placement following extraction, there are many limitations to this approach, often due to anatomical limitations. In teeth with divergent roots, inter-radicular osseous septum can be utilized to achieve primary anchor for the implant. However technical challenges are often met due to the narrow and sometimes angled shape of the septum or septa. The molar tooth is decoronated by sectioning with high speed rotary instrument, at the level of bone crest. Sequence of osteotomy is initiated at the ideal anticipated implant position. Osteotomy is stopped at drill diameter that is approximately 0.7–1.0 mm less than the implant diameter, as to preserve as much inter-radicular bone as possible. At this point the remaining roots are sectioned using high-speed rotary instrument. The separated roots are atraumatically luxated out by using periostomes and root forceps. Tapering osteotome or bone dilator may also be utilized. Tapping may be necessary in denser bone. Implant is placed and remaining socket space grafted if necessary. Initiating osteotomy steps whilst roots of the molars are intact allows predictable drilling, in bi-rooted and tri-rooted teeth. This technique is contraindicated in teeth with fused roots. Successful clinical outcomes have been observed in excess of 43 cases, including maxillary and mandibular molars over the past 3 years.

### Flapless implant placement in the esthetic zone: technique evaluation and long term clinical results

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**Purpose:** In implant dentistry, care should be taken in the esthetic zones before planning treatment sequences. Potential changes, following implant placement, such as bone resorption and/or soft tissue retraction can jeopardize the esthetic outcome of the future prosthesis. In this presentation we will discuss implant placement immediately after tooth extraction without incision or flap elevation and to correlate it with tissue preservation.

**Materials and methods:** Thirty four patients aged 18 to 62 years old received 52 implants placed immediately into extraction socket with a flapless technique. Temporization is performed immediately after surgery and final restoration is delivered 3 months after implant placement. Clinical and radiographic evaluation is done in order to monitor hard and soft tissue outcome and establish implant follow-up for a period of 6 to 54 months.

**Results:** All implants healed uneventfully but one leading to a 98.2% success rate. All restorations were satisfying regarding implant osseointegration, tissue preservation and patient satisfaction. Minor complications were noted.

**Conclusion:** This clinical study suggests the predictability of the flapless technique. Advantages of this procedure are mainly esthetic but it also gives the patient minimal post-operative morbidity. Nevertheless care is to be taken in order to either prevent or manage potential complications encountered in our cases.

### Clinical and Microbiological determinants of failing dental implants.

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This cross-sectional split-mouth study aimed to identify clinical radiographic, and bacterial characteristics of peri-implant disease. In 15 patients with bilateral implants, sites with peri-implantitis (radiographic bone loss beyond the third implant thread) and peri-implant healthy tissues (radiographic bone level above the first implant thread) were identified in periapical radiographs using a long-cone paralleling projection technique. Brånemark and 3i implants were studied. Microbiological identification was carried out using established anaerobic culture techniques. Logistic regression analysis determined significant predictors of disease. Peri-implant pocket depth was positively associated with bleeding on probing and the diameter of the implant ( $P < 0.03$ ), and was negatively associated with the presence of radiographic crestal lamina dura ( $P = 0.02$ ) and the length of the implant ( $P = 0.006$ ). Significant predictors of peri-implant bone loss were the absence of radiographic crestal lamina dura ( $P = 0.000$ ), peri-implant pocket depth ( $P = 0.001$ ) pain ( $P = 0.004$ ), and the submucosal presence of the periodontopathogens *Tamarella forsythia* ( $P = 0.008$ ), *Campylobacter* species ( $P < 0.05$ ), and *Peptostreptococcus micros* ( $P = 0.014$ ). Pain was associated with *P. micros* ( $P = 0.04$ ), *Fusobacterium* species ( $P < 0.02$ ), *Eubacterium* species ( $P < 0.05$ ). The absence of radiographic crestal lamina dura and the presence of suspected periodontal pathogens seem to be important predictors of peri-implantitis.

### Functional and aesthetic considerations for anterior tooth replacement with immediate or delayed implants placement.

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**Aim:** To report the clinical success of 189 immediate implants in the anterior region of maxilla.

**Methods:** Between January 1994 and November 2002, 101 immediate implants and 88 delayed implants were placed in 104 patients. 26 Brånemark, 67 Straumann and 96 Ankylos Implants. Bone augmentation procedures were combined with implant placement. After a conventional healing period of 6 months, all implants were osseointegrated; subsequently, the abutments were connected and fixed restorations were inserted. In different time intervals mPII and mSBI, standardized peri-apical radiographs, technical complications and patients' satisfaction, have been registered.

**Results:** After a total observation period of 2–10 years six implants were lost and the cumulative success rate was 96.8%. The majority part of implants presented healthy peri-implant soft tissue conditions, showing low values of clinical parameters.

**Conclusion:** Immediate implants show a high survival rate which is similar to those associated with conventional implant placement. Technical or prosthetic complications and compromised soft tissue outcome can have a negative influence on the patient's comfort. These complications seem to be related to different designs of the investigated implant-abutment connections. The internal-tapered implant-abutment connection have been reported to provide more mechanical stability, moreover this connection can have a positive influence on the healing and long term stability of peri-implant tissues.

### Evaluation of CRESCO-TI system in the reduction of the gap between implant and abutment

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**Introduction:** The aim of this poster is to explain the CRESCO-TI system, as a tool to reduce or eliminate the gap between the implant, the abutment and the crown. The exposure of the implant threads can be produced by the microfiltration of the gap between the abutment and the implant, so the reduction of the gap and the improvement in the marginal fit can reduce this microfiltration which can lead to the failure of the implant. We

present the CRESCO-TI SYSTEM as a system to reduce the microgap.

**Materials and methods:** Five cases from the Department of Prosthesis of the Faculty of Dentistry of Madrid are presented. By means of Laser weldings and cold-worked soldered joints we optimize the reduction of the gap between the implant and the abutment. The space is analysed by visual adaptation on the model, patient, and by photographic magnification.

**Results:** The CRESCO-TI SYSTEM provides a clinical and photographic reduction of the gap between the implant and the abutment.

## Oral session: Therapy and healing

### Influence of different treatment approaches on nonsubmerged and submerged healing of ligature induced peri-implantitis lesions in dogs

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The aim of the present study was to evaluate wound healing following nonsubmerged (ns) and submerged (s) healing of ligature induced peri-implantitis in beagle dogs. Peri-implantitis was induced by ligature placement and plaque accumulation in five beagle dogs for 3 months following implant installation ( $n = 30$  implants). The defects were randomly treated either (a) nonsurgically + ns healing or (b) surgically + s healing ( $n = 15$  implants each) using an Er: YAG laser (ERL), an ultrasonic device (VUS), or plastic curets and local application of metronidazole gel (PCM). The animals were sacrificed after 3 months of healing. Clinical, radiological and histological (e.g. new bone-to-implant contact – BIC) parameters were assessed. After 3 months of both ns- and s- healing, all treatment procedures resulted in statistically significant improvements of all clinical parameters. Histomorphometrical analysis showed that ns-implants of ERL, VUS and PCM groups generally exhibited comparable low amounts of new BIC, ranging from 1.3% (ERL, PCM) to 1.6% (VUS). In contrast, mean BIC values were statistically significant higher in the respective ERL-s (49.7%), VUS-s (11.4%) and PCM-s groups (22.4%). Within the limits of the present study, it might be concluded that (i) ERL seemed to be more suitable to promote re-osseointegration of contaminated implant surfaces than VUS and PCM, and (ii) s- healing seemed to improve the outcome of treatment in comparison to ns- healing.

### The effect of periodontal treatment on IL-6 production of peripheral blood monocytes among aggressive periodontitis and chronic periodontitis patients

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**Background:** The aim of present study was to evaluate the effect of periodontal treatment on IL-6 production of peripheral blood monocytes (PBM) among aggressive periodontitis patients (AG) and chronic periodontitis patients (CP) before and after stimulation by *E. coli* LPS.

**Materials and methods:** Fifteen AP patients, 15 CP patients and 15 periodontal healthy (PH) took part in the study. PBM IL-6 production was measured before and after stimulation of cultured PBM cells by 0.1 µg/mL LPS of *E. coli* using ELISA. Following

periodontal surgical full-mouth treatment of AP and CP groups, the same measurements were repeated for these two groups.

**Results:** The LPS-stimulated IL-6 production was significantly greater than non-stimulated IL-6 for all three groups. Before periodontal treatment, LPS-stimulated IL-6 production of AP group was significantly greater than the other two groups. Periodontal treatment did not result in significant decrease in LPS-stimulated IL-6 production or unstimulated IL-6 production of PBM cells in AP and CP groups. No correlation existed between IL-6 levels and changes in clinical parameters or baseline clinical parameters.

**Discussion and Conclusion:** Peripheral blood monocytes cells in AP and CP patients are hyper-responsive in terms of IL-6 production. This hyper-responsiveness does not return to normal even after a successful periodontal treatment.

### Evaluation through cultural analysis of the bactericidal effect of the radiation of two different Nd:YAG laser systems ( $\lambda = 1064$ nm) on a dental biofilm

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**Background and Aim:** The laser radiation can constitute a valuable aid on the removal of the dental plaque. The aim of the present work is to establish a natural biofilm model which allows the formation of dental plaque and to compare the bacterial reduction on the accumulated dental biofilm after laser irradiation with two different Nd:YAG laser.

**Materials and methods:** An acetate splint was designed to maintain four sterile disks of hydroxyapatite in contact with the teeth of the maxillary arch of a patient. The disks were used to accumulate dental plaque during 12 h. Immediately after the acetate splint was removed, the disks were irradiated with two different Nd:YAG laser systems [Q-switched Spectron SL282G and Diodium (Shütz Weil-Dental)] operating at 1064 nm. Next, the microbiological analysis of the disks and the bacterial count by means of CFU/mL were carried out by culture. A double blind study was carried out with 34 cases and 26 controls.

**Results:** The design employed allowed the accumulation of plaque without any type of manipulation and its immediate examination. The laser irradiation of the biofilm induced considerable bacterial elimination. The statistical analysis of the results obtained by ANOVA, KRUSKAL-WALLIS and DUNCAN, confirmed a significant reduction of aerobic and anaerobic microorganisms with the different laser systems employed ( $P < 0.05$ ).

**Conclusions:** The Nd:YAG Diodium clinical laser which delivers radiation by optical fibre is showed to be more efficient.



### Impairment of wound healing and cell migration by targeted water sensor AQP3 gene disruption

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Membrane water channel protein aquaporin3 (AQP3) is implicated in water absorption in epithelial cells. The aim of this study is to explore the role of AQP3 in re-organization of keratinocyte cytoskeleton and its related-downstream mechanism in wound healing process.

**Materials and methods:** Here, we show *in vitro* and *in vivo* model of wound healing, with particular attention to cytoskeleton structures in activated keratinocytes by using small interfering RNA (siRNA) duplexes targeted against *aqp3* gene.

**Results:** The TNF- $\alpha$  promote AQP3 expression as well as changes in microtubule structure and induce increase in keratinocyte motility seen typically at 24 h. Transfection of cells with siRNA selectively 'knocked-down' the expression of AQP3 as demonstrated by FACs analysis. AQP3-siRNA not only inhibits BrdU labelling indices but also changes in cell shape and enhances assemble of microtubule arrangement. The RNA interference level-induced change in the arrangement of cytoskeleton and preceded significant impairment of wound healing in AQP3 knockdown mice. The molecular dialogue between TNF- $\alpha$  and keratinocytes involves modulation of the activity of MAPK family proteins along with up-regulation of AQP3 expression.

**Conclusion:** These observations support a direct association between *aqp3* gene in cytoskeleton change and distribution though MAPK-mediated signal transduction pathway. This study provides novel therapies for speedy gingival wound healing in Diabetes and aging patients.

### Evaluation of MMP-1 levels in gingival fibroblasts of CSA-treated patients with gingival overgrowth and healthy periodontium

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Gingival overgrowth is a common side effect following the administration of CsA and the pathogenesis of this condition is not fully understood. The aim of this study was to compare the matrix metalloproteinase-1 (MMP-1) levels in gingival fibroblast cultures derived from two groups of renal transplant patients receiving CsA who exhibit gingival overgrowth and who have healthy periodontium. Gingival fibroblasts obtained from four

patients with CsA-induced gingival overgrowth (CsA GO) and four with healthy periodontium were incubated with increasing concentrations of CsA (200, 400 and 800 ng/mL) and cultured for 72 h. The expression of MMP-1 levels of all the groups were measured for four repeated times on 0, 24, 48 and 72 h by Rapid Collagenase Assay Kit. There was no statistically significant difference between the MMP-1 levels of two patient groups at the baseline. As the CsA concentration and the duration in the cell media increases, the CsA GO derived fibroblasts presented significantly suppressed MMP-1 levels with respect to the baseline where fibroblasts from CsA using patients with healthy periodontium exhibited the same result at the highest CsA concentration. Since the overall pathogenesis of drug-induced gingival hyperplasia has been accepted as multifactorial, it is thought that the down regulation of MMP-1 expression has a role in the play.

### Effect of shockwaves on fibroblasts of periodontal ligaments *in vitro*

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Charles University, Czech Republic

The aim of the present study was to explore the influence of energy density and the number of applied shockwaves on the viability and transformation of fibroblasts of periodontal ligaments (PDL). PDL fibroblasts were cultivated in a humidified incubator and 5% CO<sub>2</sub>. The cell suspension samples were subjected to SW energy densities of 0.15 mJ/mm<sup>2</sup> and the samples were treated with 100, 250, 500 and 1000 shockwaves. After the SW treatment the cells were incubated at 37 °C and after 1, 24 and 48 h the viability of cells was assessed by spectrophotometrical analysis. We investigated the effect of various SW energy flux densities on osteogenic transformation of PDL fibroblasts with and without SW treatment (0.15 mJ/mm<sup>2</sup>; 200, 500 impulses). Cells were cultivated in osteogenic medium for 21 days and bone alkaline phosphatase activities and bone nodule formations were measured. Seeding of viable cells after the SW application showed that the decrease in the growth potential was statistically dependent on the number of applied SW only. We further demonstrated promotion of osteogenic differentiation of PDL fibroblasts by SW treatment. Increased bone alkaline phosphatase activity coincided with elevated osteogenic transformation. Enhancement of bone mineralized matrix by SW treatment (0.15 mJ/mm<sup>2</sup> flux density, 200 impulses) was visibly demonstrated by an increase in bone nodule formations after culture for 21 days.

## Oral session: Perio-systemic interactions

### A prospective study to investigate the relationship between periodontal disease and preterm and low birth weight infants

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School of Dentistry, University of Barcelona, Spain

**Objectives:** To determine the influence of mother's periodontal status on low birth weight and preterm births.

**Materials and methods:** One thousand three hundred pregnant women were enrolled between the 20th and the 24th week of gestation. Demographic data, state of health, pregnancy outcome variables and information on others factors which may influence

adverse pregnancy outcomes were collected. Dental plaque, probing depth, bleeding on probing and clinical attachment level were recorded in all teeth, except third molars. Pregnancy outcome data was collected retrospectively

**Results:** The incidence of preterm birth and LBW infants was 6.8 and 6.3 respectively. Preterm birth was related to mother's age, systemic diseases, complications of pregnancy and body mass index (BMI) Low birth weight was related to mother's smoking habits, race, BMI, systemic diseases and complications of pregnancy. No relation was observed between mother's periodontal condition and LBW. In the group of women without periodontal disease, the infant mean weight (MW) was 3278 g, whereas in women with periodontitis MW was 3175 g ( $P = 0.001$ ) This difference

## Oral session: Perio-systemic interactions

remained significant after adjusting for age, systemic diseases, complications of pregnancy, race, smoking and BMI.

**Conclusions:** No relationship was observed between mother's periodontal condition and preterm birth or LBW. However, the average birth weight was significantly lower in infants born to mothers with periodontal disease after adjusting for confounding factors.

### Periodontal pathogens in human placentas of women with severe preeclampsia

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**Background:** Preeclampsia (PET) is a pregnancy-specific syndrome being one of the leading causes of maternal and fetal morbidity and mortality. Lately, we and others reported a clinical and immunological association between periodontal disease and PET. Recent evidence also showed the presence of several periodontal bacteria in atherosclerotic plaques. Since the pathogenesis of PET remains elusive, and since the placental histopathologic lesions of acute atherosclerosis, seen in PET, bear resemblance to those seen in atherosclerosis, we decided to examine the possibility that periodontal bacteria might contaminate the placentas of women with PET, and thus play a role in the pathogenesis of the syndrome. **Materials and methods:** Sixteen placentas obtained from women suffering from PET were dissected and transferred into transport medium. Microbiological analysis was performed by real-time PCR technique for the detection and quantification of *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum* sp., *Porphyromonas gingivalis* (Pg), *Prevotella intermedia*, *Tannerella forsythensis* (Tf) and *Treponema denticola* (Td).

**Results:** A total of 50% of the placentas were positive to one periodontal pathogen or more. All the examined periodontal bacteria were detected in one or more of the placentas. The most prevalent bacteria was Td (43% of samples), followed by both Pg and Tf (31%).

**Conclusion:** Periodontal pathogens are present in placentas of women suffering from PET and thus might play a role in the pathogenesis of this disorder.

### Advanced glycosylation end products (AGES) induce modulation of local monocytic cytokine release in diabetes type II and periodontitis patients

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**Aims:** Diabetes is an established risk factor for periodontitis. Still cellular and molecular association is unclear. Therefore we investigated AGES distribution in human gingival tissues and monocyte cytokine expression in diabetic patients with chronic periodontitis (DII/cP).

**Materials and methods:** After informed consent a periodontal exam comprising missing teeth (mt), bleeding on probing (BoP/PBI), clinical and radiographic attachment level (cAL/rAL) was carried out on 12 subjects. Within indicated surgical procedures gingival biopsies were taken from 10 DII/cP and two healthy controls. Tissue samples were prepared for immunohistochemistry using a monoclonal antibody specific for AGES (6D12, TransGenic Inc.) and IMAGES J was used for quantification. GCF PGE<sub>2</sub> and IL-1 $\beta$  levels were determined by ELISA. For statistical analysis the 2-tailed student's *t* test was used.

**Results:** Mean clinical parameters in DII/cP presented typical for severe chronic periodontitis (mt: 10.5; BoP: 84%/PBI: 2.9; cAL: 5.5 mm; rAL: 6.9 mm). Enhanced accumulation of AGES (17%) compared to controls (13%) was found. PGE<sub>2</sub> and IL-1 $\beta$  levels in

the GCF were  $292.2 \pm 0.7$  and  $658.9 \pm 9.5$  ng/mL in DII/cP and  $25.5 \pm 7.6$  and  $18.5 \pm 9.3$  ng/mL in controls.

**Conclusion:** This shows that AGES interaction with monocytes may lead to an increased cytokine production providing a molecular mechanism for mutual diabetes periodontitis interaction suggesting periodontitis to be a risk factor in regard to pre-diabetes becoming diabetes type II.

### Sub-clinical atherosclerosis in young patients affected by severe periodontitis

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**Background:** A significant association between the extent and severity of periodontal disease and cardiovascular diseases has been reported in aged populations. The aim of this controlled study was to evaluate the possible relationship between severe periodontitis and sub-clinical atherosclerosis in young patients ( $\leq 40$  years) with no systemic signs of atherosclerosis.

**Method:** Carotid artery intima-media wall thickness was assessed by means of carotid ultrasonography in patients affected by severe periodontal disease (test group) and sex and age-matched, healthy periodontal patients with a similar risk profile for cardiovascular disease (control group).

**Results:** Twenty patients (mean age 35.5 years) were enrolled in the test group and 20 patients (mean age 33.3 years) in the control group. Carotid intima-media thickness was higher in the test group ( $0.88 \text{ mm} \pm 0.14$ ) than in the control group ( $0.73 \text{ mm} \pm 0.07$ ). This difference (0.15 mm) is statistically significant (CI 95% 0.08; 0.22), *P*-value 0.0004 (*t*-test for matched cases).

**Conclusions:** This result suggests that severe periodontal disease in young patients may be associated to sub-clinical atherosclerosis. This association should be confirmed in a larger sample and may have clinical relevance in preventing cardiovascular risks in young periodontal patients.

### Eradication of periodontitis leads to reduced plasma interleukin-6: correlation with cardiovascular risk markers

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**Background:** Periodontal disease has been associated with increased risk of cardiovascular disease (CVD), but the causal relationship is uncertain. We previously reported that full mouth extraction (FME) leads to reduced C-reactive protein and fibrinogen, inflammatory CVD risk markers.

**Aim:** To further study the link between periodontitis (PD) and systemic inflammation, by evaluating the effect of FME on interleukin-6 (IL-6), a cytokine that promotes inflammatory marker production.

**Methods:** We studied 67 adults (age  $57 \pm 11$  years, 66% male) with advanced PD requiring FME. Blood samples were collected at three time points: T1, at initial presentation, before treatment of presenting symptoms; T2, 1–2 weeks later, before FME; T3, 12 weeks after FME. Levels of IL-6 and CRP (high sensitivity) were measured using commercial ELISA. Fibrinogen was assayed by the Clauss method. IL-6 levels were compared before and after FME using the Wilcoxon Signed Rank test. Associations between IL-6, CRP and fibrinogen levels were sought using the Spearman test.

**Results:** Interleukin-6 levels fell from T2 to T3 (from  $3.46(2.49–4.41)$  to  $2.93(2.23–3.92)$  ng/L, *P* = 0.006). Levels of IL-6 were also highly correlated with CRP (*r* = 0.59, *P* < 0.001) and fibrinogen (*r* = 0.39, *P* < 0.001).

**Conclusion:** Periodontitis has systemic inflammatory and prothrombotic consequences. Its treatment leads to reduced inflammatory markers of CVD risk, mediated in part through reduced IL-6 production.

### Local periodontal therapy with subgingival controlled release of chlorhexidine – systemic influences

L. Harnack, J. Gonzales and J. Meyle\* et al.

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Studies on inflammatory mediators during active periodontal treatment are scarce. The aim of this randomized, prospective, double-blinded, placebo-controlled study was to evaluate systemic effects of the local periodontal therapy with the adjunctive use of subgingival release of CHX (PerioChip®) before, during and after active treatment. 24 adults with severe chronic periodontitis ( $\geq 12$  teeth with PPD  $\geq 5$  mm) were randomized in two groups ( $n = 12$ ). After a prophylaxis phase, in the test group (PC) PerioChips® and in the control group (PL) placebo chips were inserted in pockets with PPD  $\geq 5$  mm. After 10 days, SRP was done and new chips were placed. Venous blood was taken at baseline (T0), at the beginning (T1a), 1 h after (T1m), at treatment's end (T1e), and after 6 months (T4). The serum levels of high sensitive C-reactive (hs-CRP) and Lipopolysaccharide-binding (LBP) proteins were measured. At T0,  $>25\%$  of patients showed hs-CRP levels  $> 3$  mg/L (means: 2.67 mg/L in PC and 2.40 mg/L in PL). At T4 mean hs-CRP was reduced to 1.33 mg/L in the PL and to 1.34 mg/L in the PC groups. Elevated levels of LBP were found at T0 (means: 11.80  $\mu\text{g/mL}$  in PC and 10.95  $\mu\text{g/mL}$  in PL). After 6 months, a significant reduction of LBP was determined in both groups (means: 6.45  $\mu\text{g/mL}$  in PC and 7.07  $\mu\text{g/mL}$  in PL,  $P < 0.05$ ,  $U$ -test,  $\alpha$  level:  $P < 0.05$ ). These results clearly demonstrate that local periodontal therapy has an influence on systemic inflammatory factors. This study was supported by DEX-CEL® PHARMA, Germany.

### Obesity and periodontal disease progression: cohort study

N. Brodala\*, J. Beck and D. Paquette et al.

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Current population statistics indicate that 30% of US adults are obese and at increased risk for chronic, inflammatory conditions including cardiovascular disease, diabetes and periodontal disease. **Methods:** Sample included 452 dentate community-dwelling subjects, 65 years or older, participating the Piedmont 65 + Study of the Elderly. At baseline, demographics medical information and body mass index (BMI in  $\text{kg/m}^2$ ) were evaluated. Periodontal exams, which included pocket depth (PD), clinical

attachment level (CAL) and number of teeth, were performed at baseline and repeated at 36 months. Periodontitis progression was assessed as mean changes in PD, CAL and number of teeth over the 36 months. Data were analysed using frequency and regression procedures.

**Results:** Overall, 50.7% were classified as obese (BMI  $\geq 30$ ). Although no significant differences in PD or CAL changes were observed overall for obese vs. nonobese subjects, subgroup analyses showed significantly greater PD and CAL worsening for obese black subjects vs. nonobese black subjects ( $P < 0.05$ ). In contrast, the incidence of tooth loss over the 36 months was significantly greater among obese subjects overall (49.3%) vs. nonobese subjects (33.2%,  $P < 0.001$ ).

**Conclusions:** These cohort data further implicate an association between obesity and inflammatory periodontal disease that extends beyond the 7th decade of life.

### Obesity and inflammatory periodontal disease: cross-sectional human study

D. Paquette\*, S. Offenbacher and J. Beck et al.

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Although obesity is commonly associated with insulin resistance, new evidence indicates that obesity extenuates chronic inflammation.

**Objectives:** To compare levels of inflammatory periodontal disease in obese (O) and non-obese (N) subjects.

**Methods:** Population included 6,731 adults (Dental Atherosclerosis Risk in Communities Study). Demographics, medical status and body mass index ( $\text{kg/m}^2$ ) measures were collected. Periodontal outcomes included plaque (PI) and gingival (GI) indices, bleeding on probing (BOP), pocket depth (PD) and clinical attachment level (CAL). Data were analysed using linear and logistic regression procedures.

**Results:** Obese subjects exhibited significantly greater periodontal disease ( $P < 0.01$ ). The mean extent (%) of sites with PI  $\geq 1$ , GI  $\geq 1$ , BOP, PD  $\geq 4$  mm and CAL  $\geq 3$  mm was 51.6, 43.4, 27.0, 8.3 and 24.9% respectively for O vs. 37.1, 29.1, 23.4, 6.9 and 22.5% for N subjects. No significant differences in periodontal parameters were detected for diabetic O vs. N subjects. For nondiabetics, O subjects exhibited significantly greater PI, GI, BOP, PD extent scores and fewer teeth ( $P < 0.001$ ) as compared to N controls. The odds ratios for obesity and moderate ( $\geq 2$  sites with CAL  $\geq 4$  mm) or severe periodontal disease ( $\geq 2$  sites with CAL  $\geq 6$  mm and PD  $\geq 5$  mm) among nondiabetics were 1.23 (95% CI 1.03–1.48) and 1.31 (95% CI, 1.04–1.66) respectively.

**Conclusions:** These data indicate a modest but significant association between obesity and periodontal disease among nondiabetics.

## Oral session: Prognostic factors

### Tobacco use and rate of tooth loss among U.S. dentists and other health professionals

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The purpose of this study was to estimate the effect of tobacco use on risk of tooth loss. We used data on 43 518 participants of the Health Professionals Follow-Up Study, an ongoing longitudinal cohort study begun in 1986, who completed mailed questionnaires every

2 years. We evaluated the association between tobacco use and time until first incident tooth loss using Cox proportional hazards models, adjusting for age, race, diabetes, BMI, caloric intake, alcohol consumption, use of multivitamins, use of vitamin C supplements, profession (dentist vs. non-dentist), and routine health examination during the previous year. We found a strong, dose-dependent, positive association between current cigarette smoking and risk of tooth loss (45 + cigarettes/day, HR 3.14, 95% CI 2.45–4.01). Compared to never smokers, former smokers had increased risk for

## Oral session: Prognostic factors

tooth loss, dependent on time since cessation (< 10 years: HR 1.66, 95% CI 1.57–1.76; 10 + years: HR 1.20, 95% CI 1.16–1.25). Ever use of pipes or cigars (HR: 1.20, 95% CI 1.11–1.30) and smokeless tobacco (HR: 1.13, 95% CI 1.04–1.23) also increased the risk for tooth loss. Results were very similar in an analysis restricted to dentists. In conclusion, tobacco use (including pipe, cigar and smokeless tobacco) increases the risk for tooth loss. For cigarette smokers, risk of tooth loss is a function of dose and time since cessation, and remains elevated > 10 years after cessation.

Supported by NIH R03DE016357, K24DE00419.

### Effects of smoking on clinical and microbiological outcomes of adjunctive antibiotic periodontal therapy – a 24-months prospective study

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The effect of smoking on the prevalence of periodontal pathogens after periodontal therapy is still unclear. Aim of this study was to evaluate the influence of smoking on clinical and microbiological outcomes of an adjunctive antibiotic periodontal therapy in smokers with aggressive periodontitis.

**Methods:** The study included 40 patients, 20 smokers and 20 non-smokers with the clinical symptoms of aggressive periodontitis. Clinical and microbiological data were obtained before, 6 months and 24 months after treatment. Subgingival plaque samples were collected from five randomly selected sites  $\geq 4$  mm in each subject. PCR was used to detect the presence of *A.a.*, *P.g.*, *T.f.*, *T.d.* and *P.i.* All patients underwent an anti-infectious therapy including scaling and root planing with antibiotics.

**Results:** Smokers and non-smokers benefited significantly from the adjunctive antibiotic periodontal therapy. A significant improvement of the clinical condition was found in both groups after treatment. However, clinical improvement in smokers was less than in non-smokers. Although, the anti-infectious therapy combined with antibiotics reduced *A.a.* and *P.i.* significantly in smokers and non-smokers, *T.f.*, *P.g.* and *T.d.* remained positive in smokers and only *T.f.* in non-smokers during the study.

**Conclusion:** The long term results of this study showed that smoking is negatively associated with periodontal health of patients with aggressive periodontitis especially with the outcome of periodontal pathogens.

### Effects of psychosocial factors on initial outcomes of nonsurgical periodontal treatment in aggressive periodontitis

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As part of a larger study investigating prognostic factors in aggressive periodontitis (AgP), we examined the relation between psychosocial factors and initial periodontal clinical outcomes following a course of non-surgical treatment. 63 patients with a clinical diagnosis of AgP were recruited to the study. Psychosocial factors were assessed by questionnaire using a life events inventory and an inventory of daily hassles. Patients received a standardised course of nonsurgical treatment including OHI and RSD over four visits, and clinical outcomes reassessed after a further 10 weeks. Clinical outcomes included % change in probing depth and CAL, % change in bleeding and plaque scores. Effects of life events and effects of daily hassles were analysed separately by dichotomising between high and low score groups. There was no difference between clinical variables or smoking between the groups at baseline. No correlation was found between any clinical outcomes and life events or their

impacts. However, subjects with low daily hassles scores showed an overall greater reduction in plaque scores post treatment when compared with the subjects with high daily hassles scores. ( $47.31 \pm 37\%$  residual plaque postoperatively for high hassles group,  $32.97 \pm 20.68\%$  for low hassles group;  $P < 0.05$ , Mann–Whitney *U*-test). The results suggest that psychosocial factors, specifically daily hassles, can impair patient responses to oral hygiene instruction during non surgical periodontal treatment.

### Oral, social and behaviour factors influencing patient plaque control

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**Objectives:** This retrospective study investigated if oral, social and behavioural factors were associated with plaque control performance following instruction by dental hygienist students.

**Methods:** Four hundred records were randomly selected. Data were included if at least one plaque score was recorded at baseline and/or 3 months, 6 months and > 12 months. Subjects were grouped by age, gender, employment status, dexterity limitation, smoking, periodontal status, numbers of teeth and numbers of teeth with restorations, orthodontic fixed appliances and removable prostheses. 224 records contained suitable information and were analysed. Analysis of variance was used to assess the effects of the independent variables on plaque scores decreases over 3, 6 and > 12 months.

**Results:** Smoking was significantly associated with less plaque reduction over 3 months (mean 20.8% for smokers and mean 27.8% for non-smokers) (F ratio 4.48;  $P = 0.036$ ). This difference between smokers and non-smokers was not significant over 6 and 12 months. Patients with dexterity limitation had significantly less plaque reduction (mean 11.2%) over 6 months compared to others (mean 33.2%) (F ratio 7.78;  $P = 0.006$ ).

**Conclusion:** Smoking habits were associated with poorer plaque score reduction over 3 months. Patients with dexterity limitation also had poorer plaque score reduction over 6 months. The other factors investigated did not seem to influence plaque control performance either in the short or longer term.

### Factors influencing the clinical outcome of non-surgical periodontal treatment: a multilevel approach

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**Aim:** The response to periodontal treatment varies between subjects as well as within the dentition, and therefore multilevel statistical approaches have been advocated to analyse the influence of subject, tooth and site related variables. The aim of this study was to apply multilevel analysis to identify factors with potential influence for the outcome of non-surgical periodontal therapy.

**Materials and methods:** Forty one chronic periodontitis patients, enrolled in a prospective clinical study, were examined before treatment, 3 and 6 month from baseline. The outcome variables were pocket closure (PPD  $\leq 4$ mm), 6 months PPD and RAL change. A regression multilevel model (logistic for 'pocket closure') was built and estimated for each outcome variable.

**Result:** The logistic regression model built explained 42% of the observed variation ( $R^2 = 0.424$ ). The factors significant for the odds ratio of closing a pocket at 6 month were: at patient level – smoking, at tooth level – molar associated pocket and at site level – plaque and infrabony defect. Initial PPD was included in the null model and was significant. For final PPD, 8% of variability was attributed to patient-level parameters, 9% at tooth- and 83% at

site-level. Same factors were found significant for both continuous outcome variables (PPD, RAL).

**Conclusion:** The outcome of non surgical periodontal therapy is influenced by factors related to the patient as well to tooth and site level. The multilevel analysis allowed modelling the variance at the different levels.

### Baseline intrabony defect angle as predictor in regenerative periodontal surgery with GTR/deproteinized bovine bone mineral or access flaps alone

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The aim of this study was to assess whether an association exists between baseline defect angle and treatment outcomes when regenerative periodontal surgery with GTR/deproteinized bovine bone mineral or access flaps alone are used in the treatment of intrabony defects. Baseline and 12-month radiographs were collected from 122 patients as part of a multicenter trial. All patients had at least one intrabony defect of 33 mm in depth. The treatment consisted of papilla preservation flaps to access the defect. GTR/deproteinized bovine bone were applied in the test subjects and omitted in the controls. 120 pairs of radiographs were obtained of which 110 pairs were measurable. Clinical and radiographic outcomes were significantly superior in the test group when compared with the controls 1 year after treatment. Multivariate analysis indicated that treatment provided, and baseline radiographic depth of the defect significantly influenced the radiographic bone fill 1 year after treatment. The percentage of resolution of the defect was influenced by the treatment, and the baseline plaque score. CAL gain was influenced by the treatment and the baseline PPD. The baseline radiographic defect angle did not show a significant impact on the clinical and radiographic outcomes. The baseline defect angle of an intrabony defect treated with GTR/deproteinized bovine bone mineral failed to show a significant impact in the observed changes in clinical and radiographic outcomes 1 year after treatment.

### Angular osseous defects: evaluation of the long-term effectiveness of the periodontal treatment by using subtraction radiography

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**Purpose:** Explore the significance of factors concerning the angular defect morphology for the long-term periodontal treatment effectiveness.

**Methods:** Sixty single rooted teeth in 60 patients severe proximal angular osseous defects were studied. Treatment included phase I, surgical pocket reduction (without osseous surgery), maintenance

care for 8 years with 75% mean compliance rate. Initial and final radiographic measurements (linear, area) were compared by the EMAGO program. The correlation between factors concerning the initial angular defect and osseous changes, imaged radiographically at 8 years was studied.

**Statistical analysis:** Parametric student's *t*-test, Wilcoxon test for pair differences, sign test, one way analysis of variance, non-parametric Spearman rank correlation coefficient and logistic regression analysis.

**Results:** The degree of osseous defect angle and the initial clinical attachment level were significantly correlated with linear (defect base, osseous crest) and area osseous changes. The initial osseous support was significantly correlated with linear osseous defect base changes. The interproximal distance was highly correlated with linear osseous crest changes and area osseous changes. Patient's compliance with maintenance care program affected the linear osseous changes. The findings revealed that the severity and morphology of the initial angular osseous defect affect the long-term periodontal treatment effectiveness, as assessed by osseous changes.

### The infrabony defect and its determinants

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**Aim:** Assessment of the defect width of infrabony defects and evaluation if the defect width is a function of defect depth.

**Methods:** Complete sets of intraoral radiographs of patients with severe periodontitis that exhibited at least one infrabony defect were digitised. The following parameters were measured: depth (INFRA) and width (DW) of the infrabony defect, defect angle, and width of the interdental spaces.

**Results:** Fifty one patients ranging from 21 to 73 years of age ( $48.5 \pm 13.4$ ) contributed a total of 1,272 teeth with 135 infrabony defects (10.6%). 17 infrabony defects were located at sites without a neighbouring tooth. Infrabony defects were statistically more prevalent in the mandible ( $n = 82$ ) than in the maxilla ( $P = 0.013$ ) as well as at mesial ( $n = 92$ ) than at distal sites ( $P < 0.001$ ). At infrabony defects the width of interdental spaces at the most coronal extension of the alveolar crest could be measured only at sites with neighbouring tooth:  $2.67 \pm 0.78$  mm (1.19–5.70 mm). Analysis failed to reveal a statistically significant difference between DW at sites with ( $2.64 \pm 0.82$  mm) or without ( $2.76 \pm 0.70$  mm) neighbouring tooth. Multilevel analysis revealed narrow defect angles to be related to deep infrabony defects. Width of the interdental space and distal location were related to wide defects.

**Conclusions:** The DW depended on INFRA and was not different at sites with or without neighbouring tooth. Even in severe periodontitis infrabony defects are found only at a minority of teeth.

## Oral session: Periodontal therapy

### Quadrant root planing vs. same-day full-mouth root planing with and without chlorhexidine

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The aim of study was to test the hypothesis that one-stage full-mouth disinfection (FMD) resulted in greater improvement

compared to same-day full-mouth scaling and root planing (FM-SRP) and quadrant scaling and root planing (Q-SRP). 28 patients with chronic periodontitis were randomised into three groups. The control group (Q-SRP) was scaled and root planed quadrant per quadrant at one-week intervals for four consecutive sessions. The two other groups received a one-stage full-mouth scaling and root planing (within 24 h) with (FMD) and without (FM-SRP) the adjunctive use of CHX. At baseline and after 1, 2, 4 and 8 months

## Oral session: Periodontal therapy

clinical parameters were recorded and microbiological samples were taken. The number of A.a.c, P.g, P.i., P.m., D.p., F.n., C.g and C.r. and total bacteria were quantified with Real-time PCR. The FM-SRP group showed significant differences in PD reduction after 2 months compared to the Q-SRP group ( $P = 0.045$ ). This could also be shown for medium pockets (4–6 mm) of multi-rooted teeth ( $P = 0.025$ ). Significant improvements in the other clinical indices could not be revealed in any group. All three treatment modalities resulted in significant reduction of the total bacteria. There were significant reductions in P.i. in FM-SRP patients compared to the other two groups ( $P \leq 0.003$ ). In conclusion, FMD doesn't show advantages in comparison to the other groups. All three treatment modalities revealed clinical improvements and led to a significant reduction of bacteria.

### Clinical comparison of photodynamic therapy and its effectiveness in initial periodontal therapy

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**Introduction:** This randomized controlled clinical trial evaluates the clinical efficacy of bactericidal potential and biostimulative effect of Photodynamic therapy (PDT) in the treatment of periodontitis.

**Materials and methods:** Fifty eight individuals with chronic periodontitis, showing a minimum of at least three active periodontal pockets 5 mm or deeper, bleeding on probing (BoP), and the presence of *Porphyromonas gingivalis* (Pg) were included to this trial. The patients were randomly assigned into two groups, a control group receiving subgingival ultrasonic treatment and a test group with additional photodynamic therapy. The initial clinical values of PI, gingival index (GI), BoP, probing depth (PD), and clinical attachment level (CAL) were registered and compared to their changes at day 90. Clinical microbial sampling was performed at baseline, on days 10, 42, and 90. Therefore Pg, Pi, Bf, Td and Aa were quantified by polymerase chain reaction.

**Results:** After treatment test and control groups showed a significant reduction on all (clinical and microbial) values. When comparing among both groups only BoP showed a significant difference. All other (clinical and microbial) parameters did not show any differences.

**Discussion:** With the reduction of BoP, it seems that PDT has the potential to reduce periodontal inflammation. In terms of eradicating bacterial growth PDT doesn't justify the additional effort when compared to conventional treatment procedures.

### Effects of non-surgical periodontal therapy on patient perception of pain and quality of life a pilot randomized controlled clinical trial

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**Aims:** The aim of this study was to compare the patient's perception of the impact of subgingival instrumentation on pain and quality of life.

**Materials and methods:** Fifty nine patients with periodontitis were treated with a piezoceramic device (Group A  $n = 30$ ) or hand instrumentation (Group B  $n = 29$ ). The impact of quality on life was assessed using the 16-item UK oral health related quality-of-life

measure (OHQoL-UK®). Pain perception was assessed using the short form McGill pain questionnaire (SF-MPQ) with visual analogue scales (VAS). Questionnaires were completed at baseline, treatment, 1, 4 and 8 weeks. Clinical parameters were assessed at baseline and 8 weeks.

**Results:** No differences in clinical outcomes were observed between groups. VAS scores for sensitivity showed a small increase in group B and decreased in group A at 8 weeks. Group differences were statistically significant at 1, 4 and 8 weeks ( $P < 0.025$ ) favouring group A. OHQoL-UK® function scores changed from oral health having no effect to a level of positive effect. At 8 weeks function scores were better for group A than group B ( $P = 0.038$ ).

**Conclusions:** An improvement of quality of life perception was observed in periodontitis patients following non-surgical periodontal therapy delivered with a piezoceramic device.

### The effect of Er:YAG laser on root coverage using coronally positioned flap. A split-mouth study

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**Background:** The aim of present split-mouth study was to assess the efficacy of Er:YAG laser to improve the results of a coronally positioned flap (CPF) procedure.

**Methods:** Fifteen patients with 30 gingival recessions were included. In each patient, one site was randomly assigned to the test group and the contralateral site to the control group. In both groups, a trapezoidal full- and partial-thickness flap was elevated, coronally displaced, and sutured to cover the treated root surface. Er:YAG laser was used in test group to modify the exposed root surface and to prepare the connective tissue receptor site. Recession (R), probing depth (PD), clinical attachment level (CAL) and width of keratinized gingiva (KG) were recorded at baseline and at 3 and 6 months post-surgery.

**Results:** The average baseline R was  $3.38 \text{ mm} \pm 0.84 \text{ mm}$  (mean  $\pm$  SD) for the test group, and  $3.26 \text{ mm} \pm 0.92 \text{ mm}$  for the control group. The mean root coverage after 6 months was  $3.18 \text{ mm} \pm 0.55 \text{ mm}$  for the test group and  $2.71 \text{ mm} \pm 0.41 \text{ mm}$  for the control group. The mean CAL gain after 6 months was  $3.47 \text{ mm} \pm 0.85 \text{ mm}$  for the test group and  $3.19 \text{ mm} \pm 0.69 \text{ mm}$  for the control group. Both differences were statistically significant ( $P < 0.05$ ). No changes of PD and KT were found.

**Conclusions:** This study suggests that Er:YAG laser seem to improve the clinical outcomes of gingival recession treated by means of CPF. Further studies with a larger number of teeth and higher statistical power are needed to support this conclusion.

### Non-surgical periodontal therapy of shallow gingival recession defects: evaluation of the restorative capacity of marginal gingiva after 12 months

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**Background and Aim:** No controlled clinical trials have evaluated the capacity of non-surgical periodontal treatment to promote root coverage. In the present study the restorative capacity of marginal gingiva at shallow recession sites was explored after two different non-surgical treatments.

**Materials and methods:** Twenty four pairs of Class I buccal recessions, up to 2 mm deep, were selected in 24 patients (FMPS and FMBS <20%). Using a split-mouth design, recessions were randomly treated with scaling, root planing and polishing (test group) or polishing alone (control group). Clinical treatment outcome was evaluated 12 months postoperatively.

**Results:** Mean gingival thickness amounted to  $1.00 \pm 0.34$  mm in the test group and to  $0.97 \pm 0.31$  mm in the control sites. Mean recession depth decreased from  $1.64 \pm 0.37$  mm to  $0.78 \pm 0.60$  mm at test sites and from  $1.43 \pm 0.42$  mm to  $1.34 \pm 0.45$  mm in the control group over 12-month period. In the test group 21 of 24 defects (87.5%) displayed coronal shift of gingival margin and 6 (25%) achieved complete root coverage, whereas in the control group most sites (62.5%) remained unchanged. Difference between two groups was significant ( $P < 0.0001$ ).

**Conclusions:** The removal of microbial toxins by polishing prevents further progression of gingival recessions, the reduction of root convexity by scaling and root planing promotes the coronal migration of gingival margin. Gingival thickness would seem to influence the amount of root coverage.

### Ten-year longitudinal study of gingival recession in dentists.

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**Aim:** The aim of the present investigation was to assess the prevalence and development/progression of gingival recession in a population sample with a high standard of oral hygiene and wide knowledge on the role of traumatic tooth-brushing in the aetiology of gingival recession.

**Methods:** Forty dental students in their final year course at the University of Barcelona Dental School were examined for gingival recession in 1994 and 10 years later by the same examiner. A questionnaire collected information concerning tooth brushing habits. Clinical parameters recorded on every recession were: recession depth, probing depth, width of keratinized gingiva and bleeding on probing. Full-mouth plaque index was recorded using the modified Quigley and Hein index.

**Results:** A total of 210 recessions were found in the initial examination and 299 in the second. One hundred and four recessions detected in the initial examination disappeared 10 years later, 106 persisted and 193 new recessions appeared in the second examination. Statistical differences were found in several clinical parameters between these groups of recessions. All the subjects had a significant increase in the plaque index in the second examination despite very few changes in the oral hygiene habits.

**Conclusions:** Some recessions can recover with time after correction of traumatic tooth-brushing. Recessions that persisted after 10 years showed more recession depth than the other groups of recessions.

### A clinical and histological study evaluating rootcoverage in conjunction with an enamel matrix protein derivative

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The purpose of this study was: (i) to clinically evaluate the treatment of Miller class I and II recessions with a coronally

positioned flap, connective tissue graft (CT) and application of an enamel matrix protein derivative (EMD) and, (ii) to histologically evaluate the treatment of Miller class I recession with coronally positioned flap and application of EMD. A total of 52 teeth in 13 patients were treated with coronally positioned flap, CT and application of EMD (Emdogain®, Straumann, Switzerland). At 7 years following therapy, mean root coverage averaged 90%. 100% root coverage was achieved in 83% of the teeth. A 32 year old female was referred for rootcoverage surgery prior to orthodontic therapy. The orthodontic treatment plan included bilateral first premolar extraction in the maxilla. Rootcoverage was performed with a coronally positioned flap and application EMD. 4 months after surgery the tooth was removed and submitted to histologic analysis. The histological analysis demonstrated formation of new cementum, periodontal ligament and bone on the previously denuded rootsurface. In conclusion, this study has shown that: (i) the application of EMD in combination with rootcoverage surgery can result in periodontal regeneration and, (ii) treatment of Miller class I and II recessions with coronally positioned flap, connective tissue graft (CT) and application of EMD may result in an up to 7 years stable root coverage.

### Comparative clinical study between two bilaminar procedures for the treatment of gingival recessions: a 4-year study

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**Aim:** This prospective 4-year study compares two bilaminar techniques: connective tissue graft (CTG) and coronally advanced flap (CAF) with or without vertical releasing incisions (V or nV) in achieving root coverage.

**Materials and methods:** Thirty non-smoking patients with a Miller Class I or II gingival recession were selected for treatment using a bilaminar technique. The patients were randomly assigned to two groups of 15 patients each and treated by means of CTG + CAF/V (group A) or CTG + CAF/nV (group B). The parameters scored at baseline (T0), at 1 and 4 years postsurgery (T1 and T2) were: recession depth (R), pocket depth, clinical attachment level (CAL) and keratinised tissue width (KT). Aesthetic assessments were made at T1 and T2 by a clinician and by the patients through interviews.

**Results:** At T0 mean R-values were 3.87 mm (group A) and 3.67 mm (group B). Mean root coverage (MRC) went from 89.9% (T1) to 87.7% (T2) in group A while MRC changed from 93.5% (T1) to 94.8% (T2) in group B. From T0 to T1/T2 in both groups R reduction, CAL gain and KT increase were statistically significant ( $P < 0.0001$ ) without differences between the groups. Aesthetic results showed significant differences between the groups such as better tissue blending (T1  $P = 0.0008$ , T2  $P = 0.0481$ ) and absence of scar formation ( $P < 0.0001$ ) in group B.

**Conclusions:** Both techniques produced similar and stable clinical results over a 4-year study time. However, aesthetic assessments showed better outcomes in group B.

# Oral session: Bacterial, genetic and other risk factors

## Comparison study of prevalences and serologic profiles of *A. actinomycetemcomitans* in patients with periodontitis in Latin-America and Spain

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**Aim:** To characterize and compare (serotypes), strains of *A. actinomycetemcomitans* (Aa), isolated from different countries, using culture and PCR technology.

**Materials and methods:** This was a cross-sectional epidemiological microbiological study of a non-treated population, showing signs of periodontitis in three countries (Chile, Colombia and Spain). Three centres were involved, with a total 105 patients. Each study subject was examined in one visit. Samples of selected patients were both cultivated for 2 days and amplified using PCR technology for serotyping. Taq-man probe and pairs of primer for serotypes a, b, c, d, e and f of Aa were used. Groups were statistically compared by ANOVA and post-hoc test. Demographic data was compared by means of chi-square test. Contingency tables were constructed to compare PCR serotyping of Aa strains.

**Results:** Prevalence of Aa was of 19.4%, 17.1% and 22.2% in Chilean, Colombian and Spanish patients respectively ( $P > 0.05$ ). Percentage of Aa on positive sites were 2.8%, 0.2% and 0.2% in Chile, Colombia and Spain respectively ( $P = 0.74$ ). For Chilean patients positive for Aa, 7.7% were strain a, 38.5% b, 30.8% c and 23.1% strain e, no patient was positive for strains d or f. In the Colombian group, 100% belonged to strain c. For Spanish positive patients, 50% of Aa were type a, 25% b and 25% c.

**Conclusion:** No differences were detected in prevalence of Aa among the three populations, but evident differences were found in serotype distribution.

## Prevalence of periodontal pathogens in Croatian generalized chronic and aggressive periodontitis patients

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**Aim:** To investigate the prevalence of *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *T. forsythensis* and *T. denticola* in generalized chronic and aggressive periodontitis patients.

**Materials and methods:** Seventy-four patients were divided in two groups: (i) generalized chronic periodontitis (CP,  $n = 39$ ) and (ii) generalized aggressive periodontitis (GAgP,  $n = 34$ ). One examiner (D.B.) using standard periodontal probe recorded at four sites per tooth bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment level (CAL) values. Four subgingival plaque samples were collected from the deepest pockets in each quadrant. Microbiological testing was done utilizing the multiplex PCR of 16S rDNA followed by a simultaneous reverse hybridization to identify five periodontal pathogens in a single run.

**Results:** The average age for CP was 48.18 and for GAgP 32.97. At sampling sites PPD for the CP group was 7.73 mm, CAL was 8.49, for the GAgP group 7.74 mm and 8.36 mm, respectively. Our results show that the isolation frequency of pathogens in the CP group A.a 23%, P.g. 87.17%, P.i. 51.28%, T.f. 92.3% and T.d.

71.79%, and for the GAgP A.a 40%, P.g. 71.42%, P.i. 48.57%, T.f. 85.71% and T.d. 77.14%.

**Conclusions:** We could not find any differences between the groups except for A.a. (40% in GAgP vs. 23% in CP). It seems that the main pathogens in our population for the CP and GAgP groups are the red complex bacteria with T.f. being the most frequent.

## The role of the subgingival biofilm in pregnancy gingivitis: microbiological patterns

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**Background:** The increase in gingivitis in pregnant women independent of a change in clinically detectable plaque has been related to a rise in steroid hormone levels, however, there is some difference of opinion between authors. The aim of the study was to assess the differences in the microbiological patterns between pregnant and non-pregnant women, specifically focussing on *Prevotella intermedia*, in relation to the clinical parameters.

**Materials and methods:** The study group consisted of 20 pregnant women and 20 non-pregnant women who served as matched controls. None of the subjects had periodontal disease. Clinical examinations and microbiological samples were conducted in the first, second and third trimester and also 3 months post-partum in the pregnant women, and twice in the non-pregnant women at a 6 month interval.

**Results:** Although the plaque levels remained unchanged, the pregnant women exhibited higher levels of gingivitis than non-gravidae. The pregnant subjects exhibited higher statistically significant proportions of *Actinobacillus actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi) and total counts of colony forming units (CFU) in the first and second trimester, Aa and Pg in the third trimester and only Pi in the post-partum samples.

**Conclusions:** The elevated hormonal levels characteristic of pregnancy appear capable of influencing the subgingival biofilm and increase the proportions of some periodontopathogens.

## Effects of cigarette smoking on gingival inflammation via inducible nitric oxide synthase pathway

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Periodontal disease pathogenesis is not fully understood yet and has been associated with smoking. Previous *in vitro* data indicated that nicotine enhanced inducible nitric oxide synthase (iNOS) gene expression. We have previously shown increased iNOS levels in periodontitis and significant reduction after periodontal treatment. Purpose of the study was to evaluate impact of smoking on iNOS activity in gingivitis and to compare enzyme activity between smokers and non-smokers. 40 subjects were signed for the study. Groups were consisted of smokers group with gingivitis, non-smokers with gingivitis, periodontally healthy smokers and periodontally healthy non-smokers ( $n = 10$ , for all groups). Full-mouth clinical indices were recorded and biopsies from papillary regions were obtained. Immunohistochemical staining was performed for evaluating iNOS expression. The immunoreactive cells



were semiquantitatively assessed. Inflamed periodontal tissues demonstrated strong iNOS expression in smokers with gingivitis whereas immunostaining appeared weak in non-smokers with gingivitis. Plasma cells, fibroblasts and macrophages within connective tissue displayed strong immunostaining. Immunostaining decreased in endothelial cells and lymphocytes. iNOS expression intensity and number of inflammatory cells showing iNOS expression was found lower in healthy non-smokers group compared to that in healthy smokers. Chronic smoking stimulates the expression of iNOS in the periodontal tissues.

### Genetic variation in radiographic alveolar bone height. A twin study

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**Background:** Previous studies suggested a heritable component in periodontitis. The twin model enables the estimation of genetic contribution to complex diseases like periodontitis.

**Aim:** To estimate the genetic variance for alveolar bone height in periodontitis by means of the twin method.

**Methods:** European Caucasian descent adult twins participated. Five monozygotic (MZ) and five dizygotic (DZ) mean age 54 and 52 respectively. At least one individual of each pair was diagnosed with periodontitis. Number of teeth was registered and the percentage of sites  $\geq 50\%$  bone loss was assessed on periapical radiographs. Genetic variance and heritability were estimated by descriptive statistics (mean values and Kendall's correlation).

**Results:** Means of number of teeth and percentage of sites with  $\geq 50\%$  bone loss were  $24.2 \pm 3.3$  and  $4.6 \pm 6.1$  in MZ and  $22.5 \pm 5.5$  and  $7.1 \pm 9.4$  in DZ. Mean difference in number of teeth and bone loss within twin pairs were  $3.2 \pm 2.3$  and  $6.4 \pm 5.9$  in MZ and  $5 \pm 4.4$  and  $4.2 \pm 1.5$  in DZ. Number of teeth showed positive correlation in MZ ( $r = 0.4$ ) with 20% estimated heritability (DZ  $r = 0.3$ ). No positive correlation was found in severity of disease for either group.

**Conclusion:** Approximately 20% of the variance in number of teeth is genetically determined in this population. However, with regard to periodontitis a larger sample with more severe periodontal breakdown is needed to further explore the biological and behavioural nature of periodontitis.

### Are the MCP-1 -2518 G and RANTES -403 A alleles a risk factor for periodontitis?

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**Objective:** The goal of this study was to assess the possible association between RANTES (Regulated on Activation, Normal T cell Expressed and Secreted) and MCP-1 (Monocyte Chemoattractant Protein-1) polymorphisms and susceptibility to periodontitis in Turkish patients.

**Materials and methods:** The MCP-1 and RANTES gene polymorphisms were investigated in samples of 51 with chronic periodontitis (CP) and 48 periodontally healthy subjects. Genomic DNA was analysed for polymorphisms in RANTES -403G/A and MCP-1 -2518A/G by polymerase chain reaction amplification followed by restriction enzyme digestion and gel electrophoresis, taking into consideration age, gender and smoking status. Data were analysed by chi square test, ANOVA, and by calculating odds ratio (OR) and 95% confidence intervals (CI).

**Results:** The genotype distribution and allele frequencies in this study were in Hardy-Weinberg equilibrium and were not different in CP and healthy group. MCP-1 -2518G allele frequencies were

### Oral session: Bacterial, genetic and other risk factors

25.5% and 20% in CP and healthy group, respectively [OR = 0.8, (95% CI = 0.4–1.89),  $P > 0.05$ ]. RANTES -403A allele frequencies were 31.9% and 28.7% in CP and healthy group, respectively [OR = 1, (95% CI = 0.2–4.46),  $P > 0.05$ ]. When we were analysed genotype-phenotype relationship, no significant associations were observed between sex, age, smoking and polymorphisms ( $P > 0.05$ ).

**Conclusions:** These results show that a polymorphism in MCP-1 and RANTES gene is not associated with chronic periodontitis in Turkish population.

### FC-mediated phagocytosis is enhanced in fcγriia 131h/h periodontitis patients

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**Background:** Receptors for the Fc part of IgG (FcγRIIa) on polymorphonuclear leukocytes (PMNs) are important mediators of phagocytosis. Of this receptor various genotypes are described, e.g. 131H/H, R/R and H/R. The FcγRIIa-131H/H genotype is reported to bind IgG opsonized antigens better than the R/R variant. Data also shows that the H/H genotype is associated with a higher level of periodontitis-associated bone loss than the R/R genotype.

**Aim:** In order to find out whether the PMNs of the H/H patients have a higher capacity of phagocytosis through the FcγRIIa than PMNs of R/R patients, we studied phagocytosis by PMNs of H/H and R/R patients.

**Methods:** The PMNs from 7 H/H and 4 R/R patients were incubated with whole [WS] or heat inactivated [HIS] serum-opsonized *Actinobacillus actinomycetemcomitans* (A.a). Phagocytosis was measured by flow cytometry.

**Results:** The PMNs of H/H and R/R patients phagocytosed A.a similarly when it was opsonized with WS (74% and 62% of the PMNs showed phagocytosis respectively;  $P = 0.412$ ). However, when HIS was used for opsonization, twice as many of the H/H PMNs showed phagocytosis compared the R/R PMNs (35% vs. 17% respectively;  $P = 0.038$ ).

**Conclusions:** The results show that the polymorphism in the FcγRIIa gene has a functional consequence. We speculate that an increased phagocytic activity of the H/H-PMNs may lead to a higher release of granular enzymes which could result in a more severe periodontal breakdown as seen in patients of this genotype.

### Effects of acute psychological stress on immune response in chronic gingivitis

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Several studies have shown psychological stress to be associated with gingival immune response to plaque. In these studies immune responses were measured in the context of an experimental gingivitis design. Less is known about stress effects on immune responses under conditions of chronic gingivitis. In the present study we therefore analysed such effects. Seven male and six female students volunteered for participation. All students suffered from clinically validated plaque-associated gingivitis at all sampling sites when included into the study. They were observed for an additional period of at least 5 weeks to confirm they suffered from chronic gingivitis. Afterwards they were either subjected to a standardized psychological laboratory stressor (public speech) or to a control condition on two consecutive days in a cross-over design. Gingival crevicular fluid (GCF) samples were taken at sites of inflammation prior to stress vs. control condition and 0, 45 and 90 min

## Oral session: Clinical tips and cases – aesthetics

afterwards. Acute stress induced a significant increase of GCF Interleukin-8. In contrast to results from experimental gingivitis studies, however, no stress effects on Interleukin-1 $\beta$  were observed. These results are the first to show that psychological stress may

affect Interleukin-8 concentrations at chronic gingivitis sites. They further indicate that psychological stress effects in experimental and chronic gingivitis might differ at least with respect to Interleukin-1 $\beta$ .

# Oral session: Clinical tips and cases – aesthetics

## Causal relationship among factors in coronally advanced flap. The Bayesian network analysis

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**Aim:** The aim of this study is to explore possible causal relationship among patient-, tooth-, and site-related variables in coronally advanced flap for root coverage procedure using the Bayesian Network analysis.

**Materials and methods:** Sixty patients, 15 males and 45 females, each showing maxillary buccal recessions ( $\geq 2$  mm) identified as Miller's class I were consecutively enrolled. All the defects were treated by coronally advanced flap procedure. From all patients, age, gender, smoking habits were recorded. In addition, the following clinical data were measured and calculated: recession depth, width of keratinized tissue, probing depth, distance between incisal margin and cemento-enamel junction (CEJ), root sensitivity, distance between gingival margin and CEJ immediately after surgery (GM<sub>1</sub>). A structural learning of Bayesian Networks was performed.

**Results:** The main findings reveal that GM<sub>1</sub> was affected by the baseline recession depth, deeper recessions being associated with more apical GM<sub>1</sub>. Moreover, complete root coverage appeared to be influenced by GM<sub>1</sub>, more coronal GM<sub>1</sub> levels being associated with greater probability of complete root coverage.

**Conclusion:** The utilization of a structural learning of Bayesian Networks seems to facilitate the understanding of the results and to indicate further possible relationship between the considered variables.

## Morph-metric study of interproximal unit in esthetic region to correlate anatomical variables effecting the aspect of soft-tissue embrasure space.

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Presence or absence of a normal papilla is crucial to avoid unpleasant aesthetic defects of major concern to periodontists, restorative dentists and to patients. In 1992 Tarnow first proposed a study to correlate a specific anatomical feature with the presence or absence of the interproximal papilla. The aim of our study was to correlate different anatomical variables influencing the harmonical and eutrophic presence of the papilla in anterior region. We analysed 120 interdental spaces among four anteriors of 40 patients comparing data collected both clinically and radio-graphically using a special metric device fixed to a Rin centrator with a known measure as reference value to detect the distance contact point-bone crest and the inter-radicular distance. The other two variables were the papilla vestibular mesio-distal base length and the distance contact point-tip of the papilla. All the measurements

were recorded digitally using measuring software (Scion image). We propose a new classification system based on the first-look aesthetic papilla's aspect in a general smile descriptive assessment. Our data suggest that interradicular distance may have a considerable role in terms of presence or absence of papilla and therefore joint consideration of both variables must be advisable for decision-making process of a combined prosthetic-periodontal rehabilitation therapy in esthetic region. We also intended to give a simple and repeatable protocol for therapy planning in such cases.

## Decision-making in root coverage surgical techniques: an evidence-based approach

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Root recession is a regular concern of our patients, afraid of loosing their teeth. In many cases, an aggressive brushing technique is involved. In front of this periodontal defect, we need to control or eliminate, if possible, the aetiology, often multiple. A modification of the brushing technique is usually needed. The level of the attachment apparatus of the neighbouring teeth and the thickness of the periodontal tissues will be considered in the surgical decision. Theoretically, the aim of a root coverage is to regenerate all the compounds of the periodontium. Clinically, for miller's class i and ii recession, the maximum of coverage will be researched, with the gingival margin at the cemento-enamel junction, a ppd of 2 mm, no bleeding on probing and the best cosmetic integration. Simple decision-trees and meta-analysis of the scientific literature, based on evidences, are a good help in choosing among several surgical protocols.

## Autologous fibrin clot (platelet rich fibrin) for the treatment of multiple class2 recession type defect

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The aim of this study is to determine the efficiency of a coronally advanced flap on multiple recessions root coverage with or without the adjunction of an autologous fibrin clot. A controlled and randomized split mouth study was performed on 20 patients with multiple class I and II Miller's recession defects in the maxilla. All patients were in good general health and smokers were included. Full mouth plaque index had to be  $< 20\%$ . Both surgeries (test and control) were performed during the same session by one practitioner. The choice of the side of treatment was randomly determined. A blood sample was taken just before anaesthesia. Recession defects were scaled and no root conditioning was used. Incisions and flap management were done according to the Zucchelli et al. (2000) protocol. The flap gingival margin was covering completely the recessions with or without the fibrin clot

and maintained in a coronal position with suspended sutures around the contact point. Clinical parameters were obtained at baseline, 1, 3 and 6 months after surgery. We will illustrate our preliminary statistical data and clinical results at 3 months. One month after surgery, root coverage was >95% in the control site and from 50 to 60% on the test site, while gingiva thickness seemed to be increased in the test site.

### Register of labial transference (RLT) in the treatment of gummy smile. Clinical protocol

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**Background:** When it comes to raise a treatment of a gummy smile, three main facts need to be evaluated: the quantity of visible gingiva on the preceding and back parts of the maxilla, the length of the clinical dental crown and the incisal and occlusal planes. All this, settled on a context of labial dynamics, which should be transferred to the study models, and will permit designing the gingival edge and the clinical dental crown shape, aesthetically more appropriate to the particular labial dynamics of each patient. The aim of this communication is to present, throughout a series of cases, a clinical protocol for the surgical treatment of a gummy smile, starting on a RLT.

**Methods:** Three gummy smile cases are presented, with a clinical coronal length inferior to 75% its wideness, in which incisal abrasions that could justify the appearance of small teeth are not evident. The cases are surgically solved, starting on the application of RLT. The medical history, toxic habits, gingival index, periodontal morphotype, periodontal sounding, bone sounding and labial dynamics are evaluated.

**Results:** Clinical results manifest that the application of the clinical protocol for the surgical treatment of a gummy smile starting on a RLT, drives to a more natural and aesthetical relations between gingiva and teeth, on a labial dynamics framework.

**Conclusions:** Register of labial transference permits predicting pretreatment, on a reliable way, the final aesthetic result obtained throughout coronal enlargement procedures.

### Osseodistraction after attachment loss in the esthetic zone – a case report

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The interproximal bone height determines esthetics in between teeth and implants. When the inflammation of periodontally compromised upper incisors has been stopped, the alveolar process in between both canines, together with residual teeth, will be raised by osseodistraction. The soft tissues at the gingival margin will not be reflected. Thus further bone resorption, caused by surgical procedures at the level of the bone crest can be avoided. Residual papillae and microvascularisation will be preserved. New devices for osseodistraction allow vertical and horizontal control of the frontal segment of the alveolar process after osteotomy. By increasing the height of the alveolar process, this special treatment for Bone Augmentation at the basis of the alveolar process will also shift the interproximal tissues coronally. There is even a gain of attachment around adjacent teeth, next to the osteotomy line. An

atraumatic removal of the residual roots and tissue-protecting insertion of implants (Immediate Implant Placement) will allow the prosthodontic reconstruction on an esthetical point of view. A comparison of the results will demonstrate that osseodistraction is not only an effective tool to gain vertical bone height but also for the management of periodontal soft tissues, when residual roots will be kept within the osteotomy segment during osseodistraction. No surgery or further soft tissue management is necessary at the level of the gingival margin when interproximal tissues can be preserved after periodontal disease.

### Surgical reconstruction of the interdental papillae

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The loss of interdental papillae can occur after periodontal treatment or with the development of periodontal disease. In the front teeth, the loss of papillae may cause an aesthetic disharmony as well as phonetic and sensitivity problems. At this moment in time the reconstruction of interdental papillae is still a challenge. The aim of this communication is to present a technique to create new papillae. This technique consists of a first incision in the free gingival margin to obtain small flaps, a second incision to elevate a full thickness flap. Then, after a meticulous cleansing of bone and roots, we suture the flap filling the interdental spaces with the small flaps of the first incision. Several clinical cases, the technique step by step as well as literature review are presented in this paper.

### The effect of using bone grafting materials in the extraction socket to avoid ridge resorption (preliminary results)

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The aim of this study is to evaluate the efficacy of Demineralized Freezed Dried Bone (Allobatan) and Deproteinized Bovine Bone (Bio-Oss) in the extraction socket after tooth extraction to avoid alveolar ridge resorption. A total of 12 patients undergo bone augmentation in the extraction socket of the upper jaws using either Allobatan or Bio-Oss particles and three patients as control without having any of bone grafting materials. Clinically is observed intra oral using a pocket probe directly after bone augmentation, 3 and 5 months to measure the height (cervico-incisal) and width (bucco-lingual) of the alveolar ridges of each socket. Radiograph has been made simultaneously and 3, 5 months to measure the height using a caliper from the top of the ridge to the demarcation line which is made through apexes of neighbouring teeth. Biopsies were taken after 3 months from buccal and 5 months from vertical for further histomorphometric. Radiographically shows radio-opacity in all areas grafted evidenced calcification in those areas. Clinically demonstrates both height and width of grafted areas are comparable with the adjacent teeth. Biopsies taken 3 months after bone grafting reveal new bone formation surrounding grafting materials and further examination for evaluation. In summarizing, both bone grafting materials augmented in the extraction sockets will be advantageous to avoid ridge resorption for preservation the esthetic of alveolar process in width and height.

# Oral session: Periodontal connective tissues

## Osteogenic effect of interleukin-11 and its synergism with ascorbic acid in human periodontal ligament cells

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**Background:** The HPDL cells are the only able in the masticatory apparatus to differentiate into osteoblasts. Our aim was to determine if IL-11 a cytokine with osteogenic ability, alone or in combination with Ascorbic acid (A.A), essential for bone and connective tissue formation, can stimulates osteoblastic differentiation of HPDL cells.

**Materials and methods:** Confluence HPDL cells stimulated with concentrations of IL-11 alone or with A.A. in presence or absence of inhibitor of collagen formation (ICF) were examined for the expression of IL-11 receptor (IL-11r) and osteoblastic markers (Runx2, OCN and BSP) using RT-PCR. Total protein (TP) content, cell proliferation and Alkaline Phosphatase (ALP) activity were measured with colorimetric assays and ALP staining.

**Results:** The IL-11r and osteoblastic markers were found in cells stimulated with IL-11, together with A.A this expression was enhanced as also happened with the ALP activity, moreover ALP was upregulated by IL-11 + A.A in a similar level of its downregulation by IFC. Furthermore we found an inverse relation of IL-11 with cell proliferation and TP content.

**Conclusion:** The mechanism that IL-11 undergoes to induce the osteogenic process is stimulating the cells in early maturation to differentiate into osteoblasts, decreasing proliferation and increasing synthesis of collagen and ALP activity, which is synergistically enhanced by A.A. IL-11 can be recognized as an osteopromotive cytokine for bone regeneration after periodontal therapy.

## Differentiation of human dental follicle cells into cementoblasts: inductive role of BMPs and EMD

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Tooth root development is regulated by epithelio-mesenchymal interactions between Hertwig's epithelial root sheath (HERS), dental papilla and dental follicle (DF) cells. HERS cells synthesize Bone Morphogenetic Proteins (BMPs) and amelogenin which are the main compounds of an Enamel Matrix Derivative (EMD). Although previous data suggest that EMD and BMPs might induce DF progenitors to differentiate towards cementoblasts during periodontal development, the mechanisms involved remain unknown. We tested the effects of EMD, BMP-2 and -7 on the proliferation and differentiation of human dental follicle cells (HDFC). HDFC were isolated from whole DF surrounding third molars and stimulated by rhBMP-2 and -7 or EMD and/or rhNoggin. Immunofluorescence and immunohistochemistry were used to localize Stro-1, BMP receptors and cementoblast markers. Expression of Cementum Attachment Protein (CAP) was analysed by Western-blot. HDFC submitted to EMD demonstrated increased proliferation and differentiation rates dependent on the presence of BMP-2 and -7. EMD and BMP-2 and -7 significantly increased CAP expression, suggesting a specific effect of these compounds to commit HDFC towards the cementoblast phenotype. RhNoggin partially inhibited the effects of EMD implying that they also exert BMP-independent effects on HDFC. Our

results point out the potentiality of using HDFC or other mesenchymal precursors as cementoblast progenitors, offering new perspectives in periodontal regeneration.

## Expression of S100A8 and S100A9 in human periodontal ligament cells

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**Background:** Previous reports showed that members of S100 calcium-binding protein family, S100A8 and S100A9, were detected in gingival crevicular fluid (GCF). Their heterodimer with antimicrobial activity, calprotectin, was also detected in GCF in accordance with local inflammatory process. Human periodontal ligament (HPDL) cells secreted S100A8 that acted as an autocrine chemotactic factor. The function of these S100 proteins in PDL is still unclear. The aim of the present study is to clarify their function in periodontal tissue using cultured HPDL cells.

**Materials and methods:** The HPDL cells were obtained from healthy human teeth. The cells were cultured with or without supplements inducing osteoblastic differentiation. The cells were also treated with LPS from *E. coli*. The expression of S100A8 and S100A9 was detected by RT-PCR.

**Results:** The expression of S100A8 was found in HPDL cells, whereas S100A9 was not. Its expression was down-regulated after the induction of osteoblastic differentiation. Inflammatory stimulus using LPS resulted in up-regulation of S100A8 expression.

**Discussion/Conclusion:** These results suggest that S100A8 is expressed in PDL and works as an inhibitor against mineralization. Such function may contribute to keep PDL constant between alveolar bone and cementum. It is also suggested that S100A8 in PDL is up-regulated in periodontal inflammation. The roles of S100A8 in pathogenesis of periodontitis should be further investigated.

## Effect of platelet-rich plasma (PRP) on gingival (GF) and periodontal ligament fibroblast (PDL) repair in an *in vitro* wound healing model

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Platelet-rich plasma (PRP) contains several growth factors and is widely used to promote healing. The aim of this study was to evaluate the effect of PRP on GF and PDL cell healing on a special wound model designed by the workgroup. A wound with a 5 mm of diameter has been performed on PDL cells and GF cell cultures. The cell wells were divided into five groups. The control group received only DMEM, where the test groups received 5% PRP with 1/3 thrombin; 1% PRP with 1/3 thrombin; 5% PRP with 1/2 thrombin and 1% PRP with 1/2 thrombin respectively. All of the groups were stained with erythrosine and basic fuchsin on days 2, 5, 7, 9 and 11. Digital screenings were performed on each time stop and a grid with 304 equal squares has been placed on each wound circle. The squares occupied by the cells have been scored from 1 to 4 and all the sample pictures were evaluated by three observers. The results were interpreted by means of % surface area. It has been found out that 1% PRP with 1/3 thrombin group have closed up the wound circle with a significant difference ( $P < 0.01$ ) and

GF respond is better than PDL cell respond ( $P < 0.01$ ). The present data suggest the possibility that PRP might effectively promote wound healing at sites of injury in periodontal disease and the developed wound model may be utilized in future investigations of the biological basis of periodontal wound healing.

### **A synthetic glycosaminoglycan mimetic (RGTA) restructures the tissues destroyed by periodontitis in hamsters**

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A new class of candidate therapeutic agents, the RGTA family, may have capacity to induce programs of tissues repair reminiscent of developmental processes. We induced periodontitis in hamsters and, starting eight weeks later, administered injections of RG 1503 (1.5 mg/kg/w) or saline for 8 weeks. Mandibles were processed for immunohistochemistry and morphometry. Three different compartments of periodontium were evaluated. The amount of alveolar bone increased around the first molar, and the interradicular bone was rebuilt. In the gingiva, the collagen network was repaired in the treated group and the elastic network was rebuilt. The continuity and the thickness of the basement membrane were restored. Apoptotic cell numbers were decreased in the pocket epithelium, and more so in the infiltrated connective tissue. The root cementum was thickened and the number of proliferating cells in the periodontal ligament was increased close to the cementum. RG1503 treatment induces potent anabolic reactions in all compartments of the periodontium. These effects may be related to the already documented inhibition of proteolytic enzymes in the gingiva and to the recruitment of progenitors that restructure gingival tissues, cementum and rebuild lost bone. The specific properties of RGTA observed in this model of periodontitis which shares numerous similarities with the human disease, suggest that RGTA may be an effective treatment for human periodontitis.

### **Apoptosis in healthy and diseased periodontal tissues of experimentally induced diabetic rats**

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**Objectives:** The aim of this study was to evaluate the apoptotic behaviour of periodontal tissues in the presence or absence of experimentally induced diseases; diabetes and periodontitis.

**Methods:** Sprague Dawley rats were used in this study. Experimental periodontitis was created by placing silk ligature around cervices of first mandibular molars. Experimental diabetes was induced by streptozotocin (STZ). Following the induction of the both experimental diseases, the animals were divided into four groups; (1) healthy group (H), (2) diabetes group (D), (3) periodontitis group (P), (4) diabetes and periodontitis group (DP). Apoptotic cells were determined by immunohistochemical method.

**Results:** In the H, D, P and DP groups, the mean apoptotic index scores were  $1.00 \pm 0.47$ ,  $0.88 \pm 0.64$ ,  $2.09 \pm 1.14$  and  $3.14 \pm 1.07$  for the periodontal ligament, whereas  $1.70 \pm 0.95$ ,  $0.88 \pm 0.83$ ,  $2.73 \pm 1.10$  and  $3.29 \pm 0.49$  for alveolar bone, respectively. The differences between the group P and group H, and between the groups D and DP were statistically significant ( $P < 0.05$ ).

**Conclusions:** The results of this study showed that experimental periodontitis alone up-regulated apoptosis in the periodontal tissues, experimental diabetes alone suppressed apoptosis,

whereas experimental diabetes together with experimental periodontitis up-regulated it. We suggest that diabetes mellitus may contribute to tissue destruction in periodontitis by modulating periodontal tissue quality.

### **Evaluation of telomerase activity in gingival fibroblasts of cyclosporin a treated patients**

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Gingival overgrowth (GO) is a common side-effect following administration of cyclosporin A (CaA). Some case reports have reported that squamous cell carcinomas could arise in GO induced by CsA and phenytoin. It is also known that human telomerase is activated in about 90% of cancers and it is mainly composed of hTR, hTERT and TPI. The aim of this study was to investigate the potential role of telomerase activity in the pathogenesis of CsA-induced gingival overgrowth. A total of nine patients medicated by Cyclosporine-A, four with and five without gingival overgrowth, were included in the study. Gingival tissues were obtained during gingivectomy or flap procedures and gingival fibroblasts were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 000 U/mL penicillin, 10 mg/mL streptomycin, 2 mM L-glutamine and 10% heat-inactivated fetal bovine serum at 37 °C under a humidified 95% air 5% CO<sub>2</sub> atmosphere. A quantitative detection of hTERT mRNA was performed with the commercially available LightCycler Telo TAGGG hTERT Quantification Kit using real-time online PCR. hTERT mRNA expression was found positive in one patient while hTERT mRNA expression was negative for the others. Even the results indicate that there could be a relationship between CsA induced GO and positive telomerase activity, detailed studies should be performed to clarify and confirm the present findings.

### **The effect of age on the development of gingivitis. histometrical and immunohistochemical findings**

R. Mengel, D. Züschner\* and L. Flores-de-Jacoby  
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The aim of the study was to evaluate the difference in the histometrical and immunohistochemical reaction of the gingiva in experimental gingivitis of periodontally healthy young and aging individuals. These persons were divided into two groups aged from 18 to 30 years ( $n = 11$ ) and from 46 to 77 ( $n = 10$ ). After establishing a high level of oral hygiene, clinical data were recorded and a biopsy was taken. After establishing an experimental gingivitis the clinical examination was repeated and a new biopsy was taken. All the biopsies were examined histometrically and immunohistochemically. Antibodies were used to demonstrate the existence of various cells in the connective tissue (CD 3, CD 4, CD 8, CD 20, CD 45 RA, CD 45 RO, CD 68, elastase, mast cells, tryptase). In comparison of the two age groups, there was a greater number in all cells examined in both the healthy and diseased tissue of the younger group (except for helper-T-cells and mast cells). In both groups, a large proportion of mast cells were detected in both the healthy and the diseased tissue. In the connective tissue below the epithelium in the older group, the number of suppressor-T-cells ( $P = 0.048$ ) and activated lymphocytes ( $P = 0.001$ ) in the diseased tissue was significantly smaller. In conclusion, the study showed that there is a greater number of cells in both the healthy and diseased tissue in the younger test persons. Both age groups demonstrated a large proportion of mast cells in healthy and diseased tissue.

# Oral session: Implant therapy – outcomes

## The survival rate of endosseous dental implants performed in previously failed implant sites

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The survival rate of endosseous dental implants is considered high (1st year survival exceeding 95% is common). However, some systemic environmental and local factors might modify this rate.

**Purpose:** The purpose of this study is to examine the effect of previously failed implant sites, on the success of new dental implants placed in these same sites. 34 patients with 55 repeated dental implants were included in this study. Age ranged between 23–86 Year (mean  $57 \pm 10.6$ ). All implants were FDA approved, with rough surfaces topography and either internal or external connection. Patients were followed for a period of 9–60 months (mean  $27.0 \pm 1.9$ ). Slightly more than half of these implants (53%) were placed submerged, while 47% were non-submerged. Implants length and diameter ranged between 10–15 mm (mean  $12.1 \pm 1.4$ ) and 3.25–5 mm (mean  $4.0 \pm 0.5$ ), respectively. Nine of these implants failed during the first year, while non have failed since, which amounts to an overall survival rate of 83.6%. Implants that were placed submerged had slightly lower survival rate (79.2%) compared to these placed non-submerged (88.4%), however these differences were not statistically sig. ( $P > 0.05$ ).

**In conclusion:** Dental implants placed in previously failed implant sites have lower survival rate than previously reported for fresh sites. This would tend to suggest that when replacing a failed implant the use of an adjacent rather than the original implant site, might increase the survival rate of these implants.

## Implant failure predictors in the posterior maxilla: a retrospective study of 274 consecutive implants

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**Objective:** We aimed to assess the implant failure rate placed in the posterior maxilla and to identify failure predictors at least 6 months after final restoration.

**Material and methods:** We retrospectively collected data from all subjects who underwent implant placement in the posterior maxilla. The sinus elevation procedures that were used included: the 1-step antrostomy, the 2-step antrostomy and the osteotome sinus floor elevation. We defined implant failure as the presence of clinical mobility, peri-implant radiographic radiolucency and the presence of pain, infection and neuropathies. Multivariable logistic regression was conducted with implant failure as the dependent variable.

**Results:** The overall implant failure rate in the posterior maxilla was 4.9%. Multivariable logistic regression showed an effect of smoking and surgical complications on implant failure. In the covariate adjusted models we included gender, diabetes, smoking, implant length and diameter, restoration design, membrane use, sinus technique and complications. The odds ratio of implant failure in smokers and subjects with surgical complications was 6.5 ( $P = 0.025$ ) and 8.2 ( $P = 0.004$ ) respectively. The use of sinus elevation techniques was not associated with an increase in the risk of implant failure ( $P = 0.702$ ).

**Conclusion:** Implant failure in the posterior maxilla is more common in smokers and subjects with surgical complications. Sinus elevation procedures do not increase the risk of implant failure.

## Clinical comparison of implants placed in fresh sockets and adjacent healed sites: preliminary results of a prospective study in humans

P. Casentini\*, F. Greco and M. Chiapasco

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**Aim of the study:** To compare clinical performance of implants placed in fresh sockets versus implants placed in adjacent healed sites.

**Materials and methods:** Forty-six straumann implants were placed in 17 patients: 25 implants (group 1) were inserted in extraction sockets and 21 in adjacent healed sites (group 2). Seven patients (19 implants) received an immediate fixed temporisation, while 10 patients (27 implants) were treated by traditional loading. Evaluated parameters at 12 and 24 months were: (a) peri-implant bone resorption and clinical parameters (PI, PD, BOP); (b) survival and success rates of implants. Statistical analysis was conducted for each parameter.

**Results:** A significantly higher ( $P < 0.05$ ) bone resorption was recorded in group 2 at 12 months follow-up and a higher probing depth was recorded for group 1 implants at 12 and 24 months. Differences in these parameters can be explained with deeper positioning of immediate implants. Peri-implant tissues health was considered very satisfactory and cumulative survival rate of implants was 100% in both groups. No statistically significant differences were recorded between implants with immediate temporisation and implants with delayed loading.

**Conclusion:** Preliminary results suggest that immediate implants and inserted in adjacent healed sites have very similar clinical and radiographic performance allowing successful rehabilitation. Immediate temporisation does not seem to affect implant survival rate.

## Variables affecting bone loss around internal and external hex MIS implants. a long-term retrospective analysis

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Hadassah Med Center, Israel, Priv. Clinic, Holland

**Aim:** A retrospective analysis was held to analyse the effect of formerly documented variables on bone loss around MIS implants, and to compare the bone loss around internal and external abutment connections.

**Materials and methods:** Patients from two clinics who were treated with either internal or external hex MIS implants were recalled for a clinical and radiographic follow-up. Patients' records with more than 30 months of follow-up period were examined to include data about their health condition, habits, implants and prosthetic appliances. Bone loss was calculated out of obtained radiographs.

**Results:** Forty-five patients with 180 internal hex, and 34 patients with 74 external hex implants were examined, exhibiting an overall 95% and 97% success rate respectively. The mean bone loss around the internal and the external hex implants was  $3.01 \pm 1.57$  and  $2.35 \pm 0.87$  mm ( $P = 0.03$ ), respectively, with a corresponding mean number of exposed threads of 0.89 and 1.39 ( $P = 0.07$ ). In the internal hex group, smoking, and in the external hex group, type of prosthesis, were found to be significantly correlated with the bone loss. In both groups, neither time nor any of the other suspected variables significantly affected bone loss.

**Conclusion:** This study confirms that both types of MIS implants exhibit success rate of more than 95%, and suggest that the configuration of the implant neck, smoking habits and type of prosthesis may influence the amount of bone loss (Supported by MIS).

### 10-year longitudinal study of patients treated for generalized aggressive periodontitis and osseointegrated implants

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The aim of the study of patients with aggressive periodontal disease and periodontally healthy patients was a comparison of teeth and implants, and assessment of the implant success rate. 10 partially edentulous patients provided with a total of 38 implants were enrolled in the study. Oral rehabilitation was undertaken in five patients treated for generalized aggressive periodontitis (GAP) and five periodontally healthy patients. The examinations of the teeth and implants were carried out within the framework of a 3-month recall schedule over a 10-year period. At each session, clinical and microbiological parameters were recorded. Additionally X-rays were taken. At the implants and teeth, a slight increase in PD and a continuous loss of AL was recorded in GAP patients. The microorganisms reflected healthy conditions throughout the observation period and revealed no significant differences between the patient groups or between implants and teeth. The bone loss at implants and teeth was significant higher in GAP patients than in periodontally healthy patients. The implant success rate was 96.77% in GAP patients and 100% in periodontally healthy patients. In conclusion, the results showed that oral rehabilitation can be performed with implants in GAP patients. However, slight attachment loss and bone loss were registered at the implants and teeth in the patients with aggressive periodontitis.

### A prospective, 5-year study of tooth-implant and implant-implant supported fixed partial dentures

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**Aim:** To evaluate the biological and technical outcome of implants and teeth supporting tooth-implant (T-I) and implant-implant (I-I) FPDs.

**Material:** Sixteen patients with a mean age of 62 years, a Kennedy class I dentition and natural teeth or implants as antagonists were treated with 47 Astra Tech Implants supporting FPDs. A randomised split-mouth design was used. Each patient was treated with one T-I and one I-I FPD. Thus, three groups were obtained: The implants in the T-I FPDs (test-implants), the implants in the I-I FPD (control implants) and the teeth in the T-I FPDs (control teeth). All FPDs were followed for at least 5-years. Descriptive statistics and Wilcoxon paired test was used.

**Results:** Two of the sixteen T-I FPDs failed during the 5-years follow-up period and none of the I-I FPDs. The median (range) change in marginal bone level during the 5-years was: test implants 0.4 mm (0–3.2 mm); control implants 0.3 mm (0–4.3 mm) and control teeth 0.2 mm (0–0.9 mm). No significant difference was found between test and control implants, but significantly more bone was lost at the test implants compared to the control teeth. Technical complications: loss transversal screw: 5%/yr, loss occlusal screw/retention: 2.5 %/yr for implants and 1.25%/yr for control teeth.

**Conclusion:** During a 5-year observation period more bone was lost at implants than at the teeth supporting T-I FPDs. There was no difference in bone loss between implants supporting T-I FPDs and I-I FPDs.

### Changes in soft tissue dimensions following second-stage surgery – 1 year results

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**Objective:** The aim of this study was to compare the increase of keratinized mucosa using three different techniques for second-stage-surgery.

**Materials and methods:** Thirty patients receiving prosthetic rehabilitation of the maxilla with dental implants (1–8) were selected for this study. The patients were divided into three groups (10 patients each) based on preoperative anatomical considerations. Second stage surgery was performed using either the roll flap (group RF) or the apically repositioned flap (group ARF) or an apically repositioned flap combined with a connective tissue graft (group ARFCT). The height and thickness of the keratinized mucosa was measured preoperatively, postoperatively, at 2 weeks, 3 and 12 months after surgery.

**Results:** After 1 year the average gain of thickness and height of keratinised mucosa was 1.2 mm and 4.8 mm in the ARF group, 2.5 mm and 1.2 mm in the RF group as well as 2.8 mm and 4.3 mm in the ARFCT group, respectively. ARF and ARFCT increased the amount of keratinised mucosa significantly more compared to RF while gain of volume was significantly increased by RF and ARFCT compared to ARF.

**Conclusion:** From these results it can be concluded that in case of missing volume a roll flap should be performed while a lack of keratinized mucosa indicates the apically repositioned flap. When there is the necessity to increase keratinised mucosa as well as volume an apically repositioned flap combined with a free connective tissue graft can be recommended.

### Outcome of dental implants in immunosuppressed patients: a 10 years study

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**Objective:** To review the outcome of dental implants placed in immunosuppressed patients.

**Material and methods:** Between April 1995 and October 2005, 19 patients were treated with 86 dental implants. All they were immunosuppressed individuals: three patients with heart transplant, three liver transplant, nine renal transplant and three with psoriatic arthritis. Patients mean age was 50 years (36–61), 12 women (63.2%). Crestal bone was high enough to place implants without bone surgery. No patients had evidence of organ rejection. Data gathered included blood analysis, X-Ray, CT, operative reports, complications and prosthesis. All patients were followed for a mean time of 5.7 years (range 1–11).

**Results:** Charts were available for 17 patients (89.5%). Implants were placed in ambulatory surgery; patients were discharged without complications. A patient with psoriatic arthritis presented severe pain in the implant zone the second week. Patient's exam was normal, the implant left in place and pain disappeared in 2 weeks. Nine patients showed infectious gum problems, four were solved medically and five needed surgical treatments. Two implants (2.3%) were lost in early osseointegration period (6. 9 week). Prosthesis was loaded in a mean time of 10 weeks (6–12). The mean treatment time was 107 days (3–5 m).

**Conclusions:** Immunosuppressed patients show a higher morbidity and mortality from infectious disease, despite that, implant treatment doesn't have higher risk. Global success rate is similar to the normal patients.

# Oral session: Clinical tips & cases: Tissue regeneration

## Calcification of a novel collagen barrier in dogs

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*Private Practice*

**Introduction:** A novel glycation technique, mimicking a natural collagen cross-linking, was used to produce a porcine type I collagen barrier. The purpose of this study was to compare the safety, efficacy and degradation of 2 porcine collagen barriers.

**Materials and methods:** Two months following extraction of mandibular premolars in 10 beagle dogs, two buccal L-shape defects were randomly assigned to four groups: Ossix-Plus + Bio-Oss (OP + BO), Bio-Gide + Bio-Oss (BG + BO), Bio-Oss (BO) or sham operated (ShO). Dogs were sacrificed after 8, 16, and 24 weeks and the mandibles prepared for histomorphometric analysis.

**Results:** At 8, 16 and 24 weeks the amount of new bone and restoration of original ridge morphology were OP + BO = BG + BO > BO > ShO. Both membranes showed early degradation at 8 weeks, and moderate to marked at 16 and 24 weeks respectively. One out of four BG barriers was completely absent at 8 and 16 weeks. Increased calcification of OP and augmentation of lingual bone volume were seen in all specimens advancing with time into areas not in direct bone contact and was completed at 24 weeks.

**Conclusions:** Both membranes exhibited barrier function with bone formation and ridge restoration. OP was partially degraded at 6 months, whereas BG showed variation in degradation kinetics. OP membrane calcification supporting bone augmentation was observed in all specimens. This suggests that glycation technology may produce an Osseo-conductive collagen matrix, in addition to its degradable barrier function.

## Vertical ridge augmentation around implants by e-PTFE titanium-reinforced membrane and bovine bone matrix: 10 consecutive cases. 12 – 42 months study

L. Canullo\* and V. A. Malagnino

*Univ. Chieti, Italy*

This retrospective study evaluates the long-term survival (12–42 months) of 10 vertically augmented ridges in which 24 Defcon implants were inserted. Deproteinized bovine bone (Bio-Oss) was used as the only augmentation filler-material and was covered with an titanium reinforced e-PTFE membrane (Gore-Tex). Clinical evaluations showed defect heights between –9 and –2 mm (average –5.1 mm; SD = 2.1). Bone height gain was between +3 and +9 mm (average 5.3 mm; SD = 1.7). Differences were statistically significant for a mean value of > 4 mm ( $P < 0.005$ ). For three augmented areas bone samples were retrieved for histological and histomorphometric examination. They show significant new bone formation, and remodelling of the deproteinized bovine bone material. The histologies, the radiographic data, and the clinical stability show that all implants were successfully osseointegrated. In addition, the radiographic and clinical analysis indicates that the generated bone crest levels are stable. This clinical study suggests that vertical ridge augmentation with an e-PTFE membrane and deproteinized bovine bone is predictable and can lead to long-term success comparable to the results reported with autogenous bone.

## The totally submerged connective tissue graft. a periodontal plastic surgical technique to prevent exposure of non resorbable membranes at GBR

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*Private practice*

**Introduction:** Exposure of non-resorbable membranes during healing is one of the most frequent complications of guided bone regeneration procedures (GBR). When a non-resorbable membrane is exposed, it is quickly colonized by bacteria, which may produce an infection, jeopardizing the GBR.

**Objective:** To show a technique of periodontal plastic surgery that prevents the exposure of non-resorbable membranes at GBR.

**Material and methods:** Description of the technique: When a non-resorbable membrane is positioned, covering a bone graft and the alveolar ridge, a connective tissue (CT) graft obtained from the contralateral side of the palate, is placed over the membrane, along its supracrestal part. The CT graft must be partially submerged under the palatal mucosa and sutured to the palatal flap. Then the buccal flap is sutured to the palatal flap, leaving the CT graft totally submerged. Care must be taken not to suture the graft to the buccal flap, in such a way that if during healing, the buccal flap has a recession or a dehiscence, the CT graft is exposed, isolating the membrane from the oral environment.

**Results:** The surgical technique will be presented, as well as cases treated successfully under this approach, showing that if this technique were not used, membrane exposure would have occurred.

**Conclusion:** The totally submerged connective tissue graft is a valuable approach to prevent exposure of non-resorbable membrane when a guided bone regeneration procedure is carried out.

## Emdogain and autogenous bone graft in the treatment of deep-wide intrabony defects

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**Initial background:** Potential limitations of EMD may be due to their gel-like consistency. Thus, it has been suggested to combine EMD and bone replacement grafts in the treatment of deep-wide intrabony defects. However, the association of autogenous bone graft and EMD has not been yet clinically evaluated in humans.

**Aims of the report:** The aim of this 6-months retrospective trial is to evaluate the clinical effect of the association of autogenous bone graft and EMD in the treatment of deep-wide intrabony defects.

**Materials and methods:** Five patients with severe chronic or aggressive periodontitis were enrolled in the study for a total of nine deep-wide intrabony defects. The criteria needed for inclusion were: presence of at least one intrabony defect with a probing depth > 6 mm, on maxillary or mandibular molars, with a deep-wide, 1 or 2 wall intrabony components of  $\geq 4$  mm, and a radiographic angle  $\geq 30^\circ$ .

**Results:** At baseline, the mean CAL is  $9.78 \pm 1.3$  mm and the mean PD is  $8.44 \pm 1.13$  mm. At 6 months, the results of this pilot study demonstrate a PD reduction of  $5.33 \pm 1.11$  mm, a  $5.33 \pm 1.22$  mm of CAG and the absence of gingival recessions.



**Discussion and conclusions:** This association may have lead to a synergistic effect. The clinical results of this pilot study are encouraging and need to be supported by a prospective clinical study. The future direction in the development of EMD must be a new carrier with supportive capacity.

### Prospective study comparing 3 different crestal sinus grafting techniques: 2-year results

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The aim of this prospective study was to compare three different crestal sinus grafting techniques as a predictable solution to vertical bone defects situations in the maxilla. All clinical cases were treated using delayed implant placement. Seven months after sinus grafting, 89 implants (Zimmer dental) were placed in 41 patients, divided in three groups related to the grafting technique: group A = 50 implants using particles of deproteinized bovine bone material with a resorbable membrane, group B = 18 implants) using Block bovine material associated with particles and membrane, and group C = 39 implants with a new innovative crestal bone reflecting trap (CBRT) technique associated with bone particles. The decision criteria depend on residual ridge morphology. Rx control the same day, 3, 7, 12 and 24 months later enabled us to analyse the bone loss, crestal and apical bone remodelling, and implant survival rate. 2 years later, no statistically significant difference in the three groups (cumulative implant survival rate 97.75%): peri-implant and apical bone loss from the group A and C (14.6%) were similar and less for the group B (5.9%), despite a significant difference concerning bone graft success rate: A = 96%, B = 87.5%, C = 100%. Crestal approach is a successful solution to bone deficiency situations. The CBRT technique presents many advantages: preserving the crestal cortical bone, playing the role of a biological membrane and protecting the biomaterial inside the sinus cavity.

### Parietal bone as a source for maxillary grafts in implants rehabilitations (About 3 clinical cases)

A. Gaudin\*, G. Brunel and E. Solyom  
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Bone augmentation for implant dentistry has become a necessary procedure for a number of edentulous patients. The purpose of this lecture is through three clinical cases, to describe the procedure for harvesting the bone graft from the cranium, to present advantages and disadvantages, to evaluate the histological results of the parietal graft and finally to analyse and discuss the use in onlay or inlay bone graft. Simultaneous or delayed implant placement can be performed. **Material and methods:** A literature review with the main electronic databases investigates on the stability of the reconstruction. (Resorbition, implants survival rates, type of graft: inlay or onlay.) Biopsies of the augmented regions of the clinical cases were performed after 6-month healing period (for the delayed implantation). A survey was made to evaluate the postoperative effects (pain, esthetic aspects at the donor site, fatigue) on 150 patients.

**Results:** The micro architecture of parietal bone and his cellular properties explain his resistance to resorbition (Tulasne 2005.) histological and histomorphometric findings demonstrates a living bone that shows feature characteristic of mature and compact osseous tissue. The side effects are very few. Nevertheless very rare (11/13 000) neurological complications can happen (Kline and Wolfe 1995).

**Discuss:** The parietal bone is reliable for onlay grafts in severe atrophy of edentulous ridge but does not represent superiority to other techniques for inlay grafts.

### Data of 500 internal sinus lift procedures with different grafting materials and the use of platelet rich plasma

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**Introduction:** PRP has an effect on bone enhancing procedures with autologues bone. Non-invasive procedures in bone grafting techniques try to reduce autologues bone harvesting and add different grafting materials to enhance the volume of the graft. This study analysis the effect of PRP to different grafting materials and their mixtures in internal sinus lift procedures.

**Materials and methods:** Internal sinus lift procedures were proceeded after measuring of dental ct-scans in residual bone heights (six groups from 1 to 12.5 mm) Biogran, Cerasorb, DFDBA, Bio Oss always mixed with 30-40 vol% autologues bone harvested as local bone chips and by the bone trap.

**Results:** Different concentrations of PRP have been achieved: 1200 blood bank, 573% labfuge, 450% PCCS System, 472% Smart PRP. Additional bone heights in no 1 (res. bone 1 mm) were 11.8 mm to no 6 (res. bone 8 mm) 3.8 mm. The loading times had a com spread of 11 weeks for DFDBA, 19 weeks for Biogran, 26 weeks for Cerasorb, 28 weeks for Bio Oss. Survival Rates: 98.2% in groups 3-6 (res. bone height 5-11 mm), 84% in group 2(3-4.5 mm), 79% in group1 (1-2.5 mm).

**Conclusion:** The internal sinus lift procedure seems to be a save and predicable method to enhance bone management in the upper posterior arch. The results of this study show an effect of PRP to the different grafting materials in combination with autologous bone. Grafting materials can be added to autologous bone in combination with activated PRP to enhance bone-grafting procedures.

### Bone tissue engineering in periodontology – a new surgical approach by endoscopically assisted micro surgery – augmentation with DBM and RHBMP-2

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**Aims:** In the past complications like infections of the wound margins and recessions of the gingiva could be observed after periodontal flap operations. A newly developed operation technique is meant to minimize these complications.

**Material and methods:** This new technique protects the papillae and enables the surgeon to move them coronally. Therefore the gingiva has to be cut vertically followed by a bridge flap which is detached from the bone and if necessary displaced coronally. Endoscopically assisted the root surfaces and the bone are cleaned and planed. Afterwards the bony defects can be treated with a suitable augmentation material. Demineralized human bone graft is used for bony defects with two and more walls while additionally rhBMP-2 is inserted to create new bone growth in defects without existing bone walls. The newly developed operation technique will be demonstrated in a short video film.

**Results:** Clinical results of this new operation technique are: no recession of the papillae, no loss of attachment and seldom superficial infection of the wound margin. In all cases of coronal movement of the papillae a clear attachment gain with bony underlay is recognizable.

**Conclusion:** The newly developed bridge flap technique in comparison to the usual flap technique promises advantages in wound healing and therefore better postoperative results.

# Oral session: Bone regeneration

## The experimental use of a polymer of polylactic and polyglycolic acid (fisiograft®) in rabbit calvarial bone defects for bone regeneration

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*Master Prog. of Period., U. Complutense, Madrid*

**Aim of the Study:** The aim of the Study is to evaluate the influence of a polymer of polylactic and polyglycolic acid, Fisiograft® (FG) used as grafting material for bone regeneration.

**Material and methods:** Experimental animals: Thirty-six adult New Zealand white rabbits were, divided in three groups of twelve animals each to be sacrificed in 7, 14, and 30 days for histological evaluation.

**Experimental modalities:** Two round defects of 9 mm in diameter were performed in the parietal bone. Four experimental modalities were considered, and randomly assigned to the corresponding defects: (a) FG sites (FGS) (b) FG and autogenous bone sites (FG-ABS) (c) Autogenous bone sites (ABS) (d) Untreated sites (US) Tested material FG is a synthetic co-polymer of polylactic and polyglycolic acid. For the present study, FG powder presentation was used.

**Results:** Sixty-four samples were subjected to histological analysis. In the 7, 14 and 30 days groups, the % of new bone formation ranked 3.9–11.6, 2.0–4.4, and 3.2–12.4 for the US, 7.8–13.3, 1.4–4.2, and 5.2–16.9 for the FGS, 9.1–17.3, 12.9–19.2, and 13.4–29.2 for the FG-ABS, and 11.0–39.8, 8.7–26.6, 9.3–26.0 for the ABS.

**Conclusions:** The results suggest that the use of FG alone or in combination autogenous bone in this animal model did not promote a significant new bone formation when compared to autogenous bone alone.

## Evaluation of two different composite bone graft mixture for sinus augmentation

P. Galindo\*, G. Ávila and F. Mesa et al.

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**Background:** Placing of implants in the posterior maxilla often poses a challenge to the clinician because of the bone loss caused by tooth losses at these sites. Sinus lift is a highly suitable therapeutic option to overcome this problem.

**Objectives:** The present study compares two graft materials used for sinus augmentation to evaluate its clinical and biologic benefits

**Material & methods:** Five patients, who underwent a bilateral sinus augmentation, were recruited. Two different grafts per patient were used in each sinus lift: Cortical bone overlying the sinus membrane in combination with bovine bone (Bio-OssR) and with synthetic resorbable bone substitute (NovaboneR). Biopsy samples of the graft were taken 6 months later, at the time of implant placement. 6 months later the fixations were loaded with FPD. Periimplant bone was evaluated 12 months after loading. A comparative radiographic analysis was made using the program Trophy 6.0.

**Results:** The histological study showed that the grafts present a high biocompatibility, but demonstrated little growth in case of Bio-Oss. Novabone samples revealed a better remodeling, with some particles included in new normal bone with active osteoblasts producing osteoid matrix. All implants placed after sinus

augmentation are functional after a 12-months period of loading. No bone loss associated with the grafts was observed.

**Conclusions:** Sinus augmentation technique using a composite graft is a predictable technique with adequate long-term results.

## Histological evaluation of peri-implant bone regeneration after application of calcium phosphate bone cement to buccal dehiscence defects in dogs

C. Hayashi\*, S. Oda and I. Ishikawa et al.

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**Background:** The objective of this study was to evaluate histologically the effects of calcium phosphate bone cement applied in surgically created bone dehiscence defect on the fixture in six adult beagle dogs.

**Methods:** Bilateral all mandibular premolars were extracted in six beagle dogs. After 12 weeks of healing, buccal dehiscence defects were created on the buccal side of the drilled holes for implants, then dental implants were implemented. Application of injectable calcium phosphate cement (Norian PDC®) in one side (test) or no graft in the contralateral side (control) were performed. At 24 weeks postsurgery, the animals were sacrificed and block biopsies were prepared for histological section and analysis.

**Results:** In the experimental sites, Norian PDC® remained intact in the defect at 24 weeks. Newly formed bone was observed between the implant and the residual Norian PDC®. In the control sites, a limited amount of bone was observed and the length of osseointegration was always less than that seen in the experimental site. In the histometric analysis, placement of Norian PDC® resulted in an approximate 3-fold increase in osseointegration ( $P < 0.02$ ), a 4-5 fold increase in bone area ( $P < 0.015$ ), and a 2-fold increase in bone height.

**Conclusions:** The results indicate that Norian PDC® would be effective to improve peri-implant tissue healing, especially in case of dehiscence defect adjacent to the dental implants.

## Comparison between brushite set-cement granules and Bio-Oss for bone augmentation in rabbit calvaria

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Bio-Oss granulate has been extensively used in bone regeneration as an osteoconductive graft. Nevertheless, its low resorption rate compromises the quality of the regenerated bone on the long term. Brushite is a biocompatible osteoconductive calcium phosphate with very good *in vivo* resorption rate. In this study we compare the use of Bio-Oss with a novel granulate that consists of a brushite based set-cement in a rabbit bone osteoconduction model. Defects were prepared in the calvaria of 16 adult New Zealand rabbits and they were implanted with: (1) Bio-Oss (2) brushite set-cement granulates (3) left empty (negative control). After 4 weeks of the intervention, the animals were sacrificed, biopsies were taken, and the following parameters were analysed: bone augmentation, bone mineral density and graft resorption. Statistical analyses were performed with student's *t* test, with a significance level of 5% for comparison of data. Mean bone mineral densities of the experimental groups (1, 2) were similar but higher than the negative controls (3) The graft resorption and bone augmentation were significantly higher

in the defects treated with the brushite based set-cement (2) The lowest bone augmentation was formed in the negative control defects (3) In our study, the use of novel brushite set-cement granules produces better results than Bio-Oss in bone augmentation procedures.

### Vertical ridge augmentation with xenogenous bone blocks – a histomorphometrical study in dogs

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The aim of the present study was to histologically evaluate healing following vertical ridge augmentation using newly developed xenogenous deproteinized bone blocks (XB) or autogeneous bone blocks (AB) in dogs. Standardized vertical-typed jaw defects were surgically created in edentulous ridges in the lower jaws of six foxhounds. Two bone blocks (6 × 10 × 15 mm) were fixed on each mandibular side with both a titanium implant and an osteosynthetic screw. Three groups were tested: [A] XB alone, [B] XB covered with a chemically cross-linked collagen membrane (CM), and [C] AB in inlay-technique, harvested from the opposite side. Three months later, the screws replaced by secondary dental implants. After a final healing period the animals were sacrificed. During the primary healing period, three out of 12 jaw sides had to be excluded from the study due to severe inflammatory reactions (2 x B, 1 x C). In general, histological analysis revealed that XB, used alone or in combination with CM, exhibited osteoconductive properties on a level equivalent to AB, resulting in a high grade of ossification of the bone blocks. However, all specimens revealed obvious signs of bone/graft resorption, which appeared to be more progressive on the vestibular sides. Within the limits of the present study it was concluded that I. XB revealed osteoconductive properties and graft resorption on a level equivalent to AB, and II. The combination of XB + CM did not seem to improve the outcome of therapy additionally.

### Vertical bone regeneration and graft resorption with a novel brushite cement granulate

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Biocompatible Brushite (DCPD) based cement decomposes in physiological environments and is resorbed by the body. The regenerating potential of DCPD cements has been studied when they are applied in the form of a setting paste, but recently, its use as granules has gained interest. The preparation of granules at low temperature allows the encapsulation of active agents added during the setting reaction. Moreover, the high surface/volume ratio of the granules can increase their resorption rate and is appropriate for drug delivery applications. We have synthesized and characterized a novel DCPD cement that sets using glycolic acid. The diametral tensile strength of this cement was  $2.9 \pm 0.7$  MPa and the mineral composition consisted of DCPD (83%) and beta-tricalcium phosphate (17%). Granules were made up from the set cement and used as bone grafting material on seven New Zealand adult rabbits in a pilot study. Bilateral circular cranial defects 10-mm-diameter 1 mm-deep, were prepared in each rabbit. The defects were surrounded with titanium rings (4 mm high) allowing only true vertical bone regeneration. Test defects were filled with the novel material and the contralaterals were left empty as negative controls. After 4 weeks of the implantation, a considerable cement resorption and significant improvement in the bone mineral density (201%) and bone neoformation (327%) was observed in

the grafted samples with no signs of immunological or inflammatory reactions.

### Adding PRP to Bio-Oss improves bone regeneration in osteoporotic rabbits

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In periodontology and implantology, bone regeneration treatments are often needed in patients with reduced bone healing capacity such as osteoporotic subjects. To correct an osseous defect or to obtain greater bone thickness, biomaterials including bone growth stimulating factors are often used. Among these therapies the use of the platelet rich plasma (PRP) is common in dentistry. The aim of the present study is to evaluate the effectiveness of adding PRP to alloplastic hydroxyapatite (Bio-Oss) in the vertical bone regeneration of osteoporotic rabbits. Two groups of sixteen New Zealand white adult osteoporotic rabbits were included in this randomised, blind, prospective pilot study. Two identical 10-mm-diameter grooves were created in each rabbit cranium with a trephine burr in order to fix and stabilize a titanium ring 4 mm high, and 10 mm diameter, creating a defect limited by the cortical bone floor and the titanium rings' walls. Inside the defect only true vertical bone regeneration occurs emerging from the defect floor. The defects were grafted with: (a) a mixture of PRP with Bio-Oss (b) PRP alone (c) Bio-Oss alone and (d) left empty for control. The rabbits were evaluated for histological and histomorphometric analysis at 4 weeks of the intervention. It was observed that the combination of PRP + Bio-Oss achieved a significant higher percentage of osseous regeneration than Bio-Oss alone.

### Histomorphometric studies on bone regeneration using PRP

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Regenerative medicine techniques found a wide ground of application in oral implantology. The purpose of this study was to observe the performance of an organic bone matrix coated with the cell binding peptide P-15 (PepGen P-15) in combination with DFDBA, PepGen P-15 Flow or PRP. A histomorphometric analysis was performed on 12 patients for reconstruction of 15 maxillary sinuses.

**Materials and method:** The patient age range was 23–65 and smokers were included. Three mixtures were compared: mixture 1: 50% PepGen P-15 + 50% DFDBA; mixture 2: PepGen P-15 + PepGen P-15 Flow and mixture 3: PepGen P-15 s + PRP. In each mixture, 10–15% autogenous bone was included. A total of 15 crestal biopsies were taken after a 4 or 5 month healing time, stained with Stevenel's blue and Van Gieson's picro fuchsin and analysed histomorphometrically.

**Results:** In all cases, the histology demonstrated that when PepGen P-15 granules are mixed with DFDBA or PepGen P-15 Flow, the granules are interconnected by bridges of newly formed vital bone. No inflammatory or foreign body response was observed. In contrast, interconnected bone bridges were not observed in grafts containing PepGen P-15 plus PRP in the absence of a spacer material.

**Conclusions:** Optimal bone formation requires PepGen P-15 combined with a spacer material (DFDBA or a hydro gel). PepGen P-15 combined with PRP did not substantially speed the bone formation process in the absence of a spacer material.

# Oral session: Implant therapy

## Registration of implant sites on a 3D CT image using a novel tactile bone surface mapping and registration technique

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Three-dimensional visualization of a virtual implant and a co-located jaw enables the selection of the most suitable implant site, direction, and dimension. The study investigated a novel computerized navigated implant system which is based on a miniature intra-oral stent (ILS) that extracts tactile information, maps the jaw surface, and performs registration onto a reconstructed 3D jaw image.

**Materials and methods:** CT scans were taken from six edentulous fabricated human jaw replica models. A reference ILS frame was firmly attached to the models. ILS tactile sensors comprised of a micro-needle array were attached to the reference frame. Upon activation, the needle beam was inserted into the mucosa until jaw cortex was reached. Following sounding, a computerized surface-matching algorithm was applied to co-locate and register the CT image and the measured jaw outline. Least squared estimation test and Pearson chi-square test were used to analyse and validate image registration accuracies and extract the cross-correlation between those and the metric values respectively.

**Results:** Mean registration error for all measurements was  $0.4 \text{ mm} \pm 0.2 \text{ mm}$ . A significant correlation was found between metric values for post-convergence and registration accuracies ( $R_v = 0.223$ ,  $P < 0.004$ ).

**Conclusions:** The ILS system can accurately map jaw surfaces by performing tactile bone sounding. A corresponding computerized surface matching procedure presents accurate and repeatable image registration.

## Computer-aided quantification of mandibular osseous graft for sinus augmentation

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**Background:** The quantity of intra-oral osseous transplants necessary for sinus augmentation has not been determined. A pre-surgical measurement methodology has not been established.

**Objective** To quantify the osseous volume that can be harvested to bilaterally augment a patient's maxillary sinuses.

**Methods** Computerized axial tomography (CAT) scan images from symphysis & rami ( $n = 40$ ) were mapped by computer-aided design software program (AutoCAD®). Safety margins of 5 mm were outlined. After importing the CAT scan images into the AutoCAD® file, each 1 mm cut was mapped by a polyline. The volume was calculated by adding the consecutive cuts. A 0.5 mL graduated cylinder was used for intra-surgical measurements ( $n = 12$ ). Measurements of the augmented sinuses were made to calculate the gain in volume at 6 months.

**Results** The mean symphysis volume excluding the lingual cortex was 1.42 cc (SD = 0.56; range = 0.54–2.71). The mean ramus volume was 0.79 cc (SD = 0.20; range = 0.42–1.22). Intra-surgically, the ramus volume averaged 2.30 cc per site (SD = 0.43; range = 1.80–3.00) and the symphysis 2.10 cc (SD = 0.38; range = 1.70–2.80). This would yield a total of 4.60 cc (rami) + 2.10 cc (symphysis) = 6.70 cc. The osseous gain in sinus volume at 6 months averaged 1.88 cc (SD = 0.45; range = 1.31–2.60).

**Conclusions** The volume of a  $4 \times 10$  implant is 1.25 cc. Bilateral sinus augmentation requires approximately 5.00 cc of bone. Plausibly, an average of 6.70 cc of bone, can be harvested from mandibular symphysis and rami.

## Histological evaluation of bovine-derived bone xenograft in alveolar ridge augmentation prior to implant placement

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This study was designed to evaluate the histological changes at 8 and 12 months after the insertion of bovine bone xenografts (Bio-Oss® Geistlich, Pharma A G, Wolhusen, Switzerland) in future implant sites of the anterior maxilla. A total of 14 sites in 13 patients were examined. Bone cores were trephined out at the time of implant placement at 8 months and 3 mm punch biopsies were taken of the grafted sites at 12 months during second stage implant surgery. Ground sections were prepared from biopsies taken of the grafted sites and examined histologically in order to assess new bone growth as well as degradation of bovine bone xenografts. The data were analysed using a Wilcoxon/ Kruskal-Wallis Rank Sums test. The medians (interquartile ranges) of area measurements for bovine bone xenografts in specimens as a percentage of the total area of specimen were  $13.93 \text{ mm}^2$  (11.83) at 8 months and  $8.37 \text{ mm}^2$  (12.50) at 12 months ( $P \leq 0.001$ ). The median (interquartile range) for area of new bone in specimens at 8 months as a percentage of total specimen area was  $17.60 \text{ mm}^2$  (22.65) compared to  $35.64 \text{ mm}^2$  (30.74) at 12 months ( $P \leq 0.001$ ). The results of this study suggest there was an increase in area of new bone in specimens between 8 and 12 months and a decrease in graft material over the same period. Although appreciable amounts of graft material persisted at 12 months, all implants were integrated and successfully restored.

## Vertical ridge augmentation using composite bovine bone mineral, fibrin sealer and collagen membrane: clinical and histological findings

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The deficiency of crestal bone height may result in the need for supracrestal bone regeneration in order to have a proper three-dimensional implant placement. In this study a protocol for the treatment of reduced alveolar ridges was evaluated. In 16 patients, 24 endosseous implants presenting supracrestal bone defects were placed. Vertical ridge augmentation was performed by the combination of a composite bovine xenograft (Bio-Oss Collagen®) stabilized with a fibrin seal (Tissucol®) and covered by a porcine collagen membrane (BioGide®). After implant placement, the mean height of supracrestal defects measured  $3.06 \pm 1.70 \text{ mm}$ . Healing at the surgical zones was uneventful in all cases. Second-stage surgery was performed after 4 to 6 months. At the re-entry, a hard bone-like tissue was detectable at the defect sites. Histologic examination performed on one site confirmed the presence of newly formed bone and revealed residual embedded particles of bovine bone mineral. A final mean gain of  $2.58 \pm 1.79 \text{ mm}$  in the vertical defects was reported, corresponding to  $83.96 \pm 24.59\%$  of regenerated tissue. The present study reported the possibility of obtaining supracrestal bone regeneration around osseointegrated

implants using a mixture of bovine bone mineral and fibrin glue covered by a collagen membrane. The surgical procedure proposed was able to stabilize the blood clot, maintaining the space for bone regeneration and minimizing the risk for membrane exposure.

### Ridge augmentation with periodontal regeneration

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**Background:** Intrabony defects are treated by GTR. Horizontal defects are the least predictable in regenerative treatment. Various techniques exist to augment alveolar ridges before implant placement. The aim of our study was to develop a one-step surgical protocol for ridge augmentation and regeneration of intrabony and horizontal defects of adjacent teeth.

**Methods:** Eight patients with inadequate alveolar ridge around teeth presenting CAL  $\geq 5$  mm were included. Intrabony and horizontal components of the defects were filled with natural bone mineral (NBM) and covered with barrier membrane. Sub epithelial connective tissue graft (SCTG) was sutured to the oral flap. Core biopsies were retrieved 8–20 months later. Periodontal probing depth (PPD), gingival recession (GR), clinical attachment level (CAL), depth of intrabony defects (INTRA) and horizontal bone loss (SUPRA) were measured at baseline and re-entry.

**Results:** Mean PPD reduction was  $4.2 \pm 1.9$  mm, mean CAL gain was  $4.8 \pm 2.0$  mm. Intrabony components filled in all cases, SUPRA levels were reduced by  $3.2 \pm 1.8$  mm, GR by  $0.4 \pm 1.8$  mm. The majority of NBM particles were surrounded by new bone and osteoid tissue. Histomorphometry showed a mean new bone formation of  $23.6 \pm 7.1\%$  and a mean graft area of  $17.3 \pm 3.9\%$ .

**Conclusions:** Our findings indicate that grafting with NBM + GTR + SCTG is a novel technique for simultaneous ridge augmentation and periodontal regeneration of adjacent teeth.

### Ribose-crosslinked collagen membranes are more effective than ePTFE membranes in supporting bone augmentation in humans: a morphometric study

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The efficacy of absorbable collagen membranes in GBR depends on their resistance to collagenolysis. Ribose is a novel non-toxic agent for cross-linking collagen matrices and increasing their resistance to degradation. This study was aimed to quantify the capacity of ribose-crosslinked collagen membranes - Ossix<sup>®</sup> to support new bone formation in a lateral ridge augmentation model in humans in comparison to that of ePTFE Gor-Tex<sup>™</sup> membranes. 28 patients (14 in each group) were treated randomly with either Ossix or Gor-Tex membranes. BioOss<sup>™</sup> served as a filler material. Impressions of preop and postop alveolar ridges (AR) were obtained at an interval of 6 months. These were cast in acrylic. An acrylic stent supported by the residual dentition was glued to each cast. Reference marks on the stent were used to obtain 1 mm thick bucco-lingual sections of preop and postop casts at identical sites. The sections served to determine changes in the AR height, width and volume. The morphometric analysis indicated that: (1) the preop and postop AR heights remained unchanged in both groups; (2) the mean preop AR width did not differ between the 2 groups; (3) there was a significant increase in the postop AR width and bone volume in the Ossix group compared to the preop AR in this group and to the postop AR width in the Gor-Tex group ( $P < 0.05$ ). The results indicate for the first time that the ribose-

crosslinked collagen membranes were more effective than ePTFE ones in supporting GBR in humans.

### The effect of platelet-rich plasma (PRP) on the bone healing around cap coated and non-coated oral implants in trabecular bone

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Platelet rich plasma (PRP), a autogenous volume of high platelet concentration and a proven source of growth factors, has been suggested for use in conjunction with dental implant placement. The effect of local application of autologous PRP on bone healing combined with titanium (Ti) implants with two different surface configurations was investigated. PRP fractions were obtained from venous blood sample of six goats and applied via gel preparation and subsequent installation in the implant site, or via dipping of the implant in PRP liquid before insertion. A total of 36 implants (18 non-coated and 18 CaP-coated) were placed into the goat femoral condyles (trabecular bone). The animals were sacrificed at 6 weeks after implantation and implants with surrounding tissue were processed for light microscopical evaluation. Besides histological description, histomorphometrical variables were also evaluated (the bone-implant contact and the bone mass adjacent to the implant). Significantly more interfacial bone contact was observed for all the three groups of CaP-coated implants and the Ti/liquid group (non-coated implant with PRP liquid) compared to the other two non-coated Ti groups (with PRP gel or without PRP). The additional use of PRP did not offer any significant effect on the bone response to the Ca-P coated implants, while PRP in a liquid form showed a significant effect on bone apposition to roughened titanium implants during the early post-implantation healing phase.

### Trans-gingival implant design for immediate loading: a prospective human study

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Immediate loading of dental implants has gained considerable attention. Two-piece implants designs were utilized for this purpose. The aim of the present prospective study was to examine the efficacy and success of transgingival implants design (3I Osseotite TG<sup>®</sup>) in an immediate loading protocol. 15 consecutively treated subjects received 38 dental implants that were immediately restored (ISIL). Solid abutments were connected and a prefabricated metal reinforced acrylic restoration cemented. Implants survival rate, radiographic bone loss (using computerized measuring software), and implant's stability (Periotest<sup>®</sup>) were examined quarterly at baseline until 12 months. Five implants failed during the healing period, which amounts to an overall 86.8% survival rate. Mean bone height (BC to implant) was  $1.16 \pm 0.5$  &  $2.52 \pm 0.7$  mm 0 & 12 M respectively (BL =  $1.36 \pm 0.2$  mm;  $P < 0.05$ ). There was a positive ( $r = 0.435$ ) and sig. ( $P < 0.05$ ) correlation between initial bone height and eventual bone loss. To the contrary, we could not find any correlation between implant's stability, length, diameter or bone width and BL in the first year. Pocket depth at 12 M, was sig. shallower in single crown versus bridge restoration ( $2.17 \pm 0.1$  vs.  $3.2 \pm 0.2$  mm;  $P = 0.01$ ). Finally, the overall time for this procedure was markedly reduced compared with previous experience with two-piece implants design.

**In conclusion:** A simplified ISIL protocol using non-submerged implant design was proven predictable and time consuming.

# Oral session: Periodontal regeneration

## Orthodontic movement into infrabony defects augmented with a composite bovine xenograft: clinical and radiological results

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Periodontal disease may create infrabony pockets adjacent to pathologically migrated teeth. In these situations an interdisciplinary approach can be useful. Aim of the present work is to evaluate the modifications caused by orthodontic movement into infrabony pockets filled with bone grafts. Ten adult periodontal patients, presenting pathologic migration of a maxillary central incisor with the presence of a bony defect, were treated. At baseline, PPD and CAL were measured and, on standardized intraoral radiograms, the vertical (RVBD) and the horizontal (RHBD) dimensions of the infrabony defects were assessed. The flaps were elevated according to the modified papilla preservation technique and the defects were filled with a composite bovine xenograft (BioOss Collagen®). After two weeks the orthodontic movement started in order to move the teeth into the defects. 6 months after the end of the orthodontic therapy the final assessment of PPD, CAL, RVBD and RHBD was recorded. At baseline mean PPD and CAL were 7.2 and 8.95 mm. Mean final PPD was 3.1 mm, with a CAL gain of 5.05 mm. The radiological resolution of the original defects' dimension was 72.63% in the vertical and 73.68% in the horizontal component. Plaque control was optimal at all sites and no bleeding on probing was reported. From a clinical and radiological point of view all patients benefited from the treatment performed, obtaining pockets closure and healthy periodontal conditions together with aesthetic improvement.

## Enamel matrix protein derivative alone or in combination with a bioactive glass in the treatment of wide intrabony periodontal defects

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This study aims to assess the clinical and radiographic outcome of wide intrabony periodontal defects treated by EMD alone or in combination with a bioactive glass (BG). Twenty-three patients who received non-surgical periodontal therapy and had radiographical interproximal defects with an associated probing depth (PD) of  $\geq 6$  and an intrabony component of  $\geq 4$  mm, were included in this controlled parallel design study. Each of the patient was treated with either EMD alone or EMD + BG. Clinical and radiographic measurements for a total of 40 defects (20 in each group) were performed at baseline and 8 months following therapy. No adverse event related to the use of EMD was noted. In both groups clinical and radiographical parameters were improved at 8 months when compared with baseline ( $P < 0.001$ ). The groups EMD + BG and EMD alone, presented a mean PD reduction of 5.55 mm and 4.98 mm, recession of 0.63 mm and 0.94 mm, attachment gain of 4.87 mm and 4.03 mm, clinical bone gain of 2.48 mm and 2.13 mm and radiographic bone gain of 2.68 mm and 2.15 mm, respectively. Intergroup comparison revealed also significant differences for all of the parameters, yielding a more favourable outcome towards the combined approach. Within the limits of the study, both treatments

performed either with EMD alone or EMD + BG resulted in significant clinical and radiographical improvements, but combined treatment seems to enhance the results in the treatment of wide intrabony defects.

## Treatment of intrabony defects with emdogain alone, emdogain + cerasorb and modified widman flap surgery.comparison of 1-year outcomes

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**Aim:** This controlled clinical trial was designed to compare different treatments of deep intraosseous defects up to 12 months' follow-up: EMD alone, EMD +  $\beta$ -tricalcium-phosphate (Cerasorb), MWF surgery.

**Materials and methods:** Fifty-six patients were paralleled for clinical parameters and randomly assigned to treatment. They displayed one angular defect each with an intrabony component  $\geq 3$  mm, PPD and PAL  $\geq 7$  mm and PI  $< 1$ . Nineteen defects were treated respectively with EMD + Cerasorb or EMD alone using micro-surgical approach and 18 defects with MWF. PI, Gi, BOP, PPD, PAL and REC were measured before and 12 months after treatment.

**Results:** Significant PPD decreases and PAL gains were proved in all treatment-groups after 1 year. Combined treatment produced  $4.1 \pm 1.2$  mm mean PPD reduction and  $4.0 \pm 1.0$  mm PAL gain ( $P < 0.001$ ). Treatment using EMD alone yielded  $3.9 \pm 1.3$  mm PPD decrease and  $3.7 \pm 1.0$  mm PAL gain ( $P < 0.001$ ). Outcome parameters were not significantly different between the EMD groups, recessions increased by  $0.7 \pm 1.3$  mm. Following MWF, gained attachment was significantly smaller ( $2.1 \pm 1.4$  mm;  $P < 0.001$ ). PPD reduction was not significantly different ( $3.8 \pm 1.8$  mm), while recessions increased by  $1.5 \pm 0.7$  mm ( $P = 0.042$  vs. EMD).

**Conclusions:** Limited to this study: Additional use of the bone replacement graft material Cerasorb is not supported by our results. The clinical use of EMD clearly appears to be a more effective therapy modality than MWF surgery in the treatment of intrabony defects.

## Enamel matrix proteins combined with bone grafts in smoker and non-smoker aggressive periodontitis patients

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The purpose of this study was to assess the effect of smoking on regenerative periodontal treatment in generalized aggressive periodontitis (GAP) patients. A total of 20 GAP patients, 10 smokers (S) and 10 non-smokers (NS) were included in this study. In every patient, enamel matrix protein derivatives (EMD) and bovine derived xenograft (BDX) (EMD + BDX) and EMD and bioactive glass (BG) (EMD + BG) combinations were used in either maxilla or mandible. Prior to surgery and at 8 months, plaque and sulcus bleeding indices, probing depth (PD), relative attachment and probing bone levels were measured. At 8 months, the mean PD reductions in S in the EMD+BDX and EMD+BG groups were  $3.17 \pm 1.07$  mm and  $3.16 \pm 1.30$  mm ( $P < 0.05$ ), whereas

in NS these reductions were  $3.69 \pm 1.18$  mm and  $3.45 \pm 0.96$  mm ( $P < 0.01$ ), respectively. The changes in relative attachment levels in S in the EMD + BDX and EMD + BG groups were  $1.90 \pm 0.61$  mm and  $1.80 \pm 0.69$  mm ( $P < 0.05$ ), whereas in NS these changes were  $2.13 \pm 0.64$  mm and  $1.89 \pm 0.72$  mm ( $P < 0.01$ ), respectively. Evaluation of the hard tissue findings revealed that the mean clinical bone gain in S and NS was  $0.95 \pm 0.33$  mm ( $P < 0.05$ ) and  $1.05 \pm 0.31$  mm ( $P < 0.01$ ) in the EMD + BDX group whereas it was  $0.92 \pm 0.31$  mm ( $P < 0.05$ ) and  $1.07 \pm 0.25$  mm ( $P < 0.01$ ) in the EMD + BG group. Intergroup differences for all clinical parameters were found to be insignificant in S and NS. Within the limits of this study, similar clinical improvements were obtained in both S and NS.

### Regenerative periodontal surgery with enamel matrix derivate. Baseline radiographic defect angle as a prognostic indicator

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**Aims:** The aim of this study was investigate whether an association exists between baseline radiographic defect angle and treatment outcome when enamel matrix derivate (EMD) is used in periodontal regenerative surgery.

**Materials and methods:** The study was realized on 30 patients who had pair test sites and control sites, with pockets  $\geq 6$  mm and infra-osseous defects with depth  $\geq 3$  mm measured by probing and X-ray evaluation radiographic defect angle was  $\leq 22^\circ$  than when it was  $\geq 36^\circ$ .

**Results:** The average values of the clinical attachment level (CAL) in the test sites were 1.8 mm at 6 months and 2.1 mm after 12 months, for the group with EMDOGAIN®, 1.1 mm at 6 months and 1.2 mm at 12 months for the control group. The radiographic gain was ascertained after 12 months.

**Conclusions:** This study showed that there was a significant association between baseline radiographic defect angle and CAL gain of  $\geq 4$  mm after regenerative surgery with EMD is used in narrow ( $\leq 22^\circ$ ) intrabony defects suggests that the baseline radiographic defect angle might be used as a prognostic indicators of treatment outcome.

### Guided tissue regeneration in combination with deproteinized bovine bone. 6-year results from a randomized controlled clinical trial

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The present study reports the results of GTR in combination with Bio-Oss in the treatment of human intrabony defects, 1- and 6 years after surgery. 60 patients with an intrabony defect with PPD  $\geq 7$  mm and radiographic evidence of an intraosseous component  $\geq 4$  mm were treated either with a bioabsorbable membrane (M), or with a bioabsorbable membrane in combination with Bio-Oss impregnated either with sterile saline (BO-) or with gentamicin sulphate 2% (BO+), or with flap surgery alone (F). Statistically significant clinical improvements were observed in all four groups after 1 year, but treatment modality did not seem to influence the amount of residual PPD and PAL gain (M: 2.9 mm, BO-: 2.5 mm, BO+: 3.8 mm, F: 1.5 mm). These clinical improvements remained basically stable in the 3 GTR groups, but not in group F. At the 6-year examination (42 patients), PAL gain was 2.4 mm, 2.3 mm, 4.1 mm in M, BO-, BO+ groups, respectively; a PAL loss of 1.2 mm compared to baseline was observed in

group F. Smoking, frequency of dental visits and plaque on the treated site at the 1- and 6-year examination did not seem to influence the amount of PAL gain after 6 years or the occurrence of PAL loss  $\geq 1$  mm or  $\geq 2$  mm from 1- to 6 years. In the three GTR groups, 12 teeth lost  $\geq 1$  mm and 6 teeth lost  $\geq 2$  mm of the PAL gain obtained after treatment, and three teeth were lost at the 6-year examination. It can be concluded that the results of GTR in combination with Bio-Oss are basically stable on a long-term basis.

### Regenerative periodontal therapy with tutodent chips and tutodent membrane

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The purpose of the present study was to compare clinically the treatment of intrabony defects with a combination of a bovine derived xenograft (Tutodent Chips, Tutogen, Germany) and a bioresorbable collagen membrane (Tutodent, Tutogen, Germany) (test) to access flap surgery (control). 22 patients each of whom displayed one intrabony defect received either the test or the control therapy. The results were evaluated at 6 months following therapy. Healing was uneventful in all patients. At 6 months after therapy the test group showed a reduction in mean probing depth (PD) from  $8.5 \pm 1.4$  mm to  $2.7 \pm 1.5$  mm ( $P < 0.001$ ) and a change in mean clinical attachment level (CAL) from  $9.7 \pm 1.6$  mm to  $5.6 \pm 1.4$  mm ( $P < 0.0001$ ). In the control group mean PD was reduced from  $8.2 \pm 1.2$  mm to  $4.5 \pm 1.9$  mm ( $P < 0.001$ ) and mean CAL changed from  $9.8 \pm 1.8$  mm to  $8.0 \pm 1.9$  mm ( $P < 0.01$ ). The test treatment resulted in statistically higher PD reductions ( $P < 0.05$ ) and CAL gains ( $P < 0.001$ ) than the control one. It was concluded that the combination of a bovine derived xenograft and a bioresorbable collagen membrane appears to be a suitable alternative for treating intrabony periodontal defects.

### Ostim® for the treatment of intrabony periodontal defects: a randomized controlled clinical study

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**Objective:** The aim of the present randomized controlled clinical study was to compare the clinical outcomes of papilla preservation flap surgery with or without the application of a novel nanocrystalline hydroxyapatite bone substitute.

**Material and methods:** Twenty-four intrabony periodontal defects in 12 adult patients with chronic periodontitis were included. In a split-mouth design defects ( $\geq 4$  mm) were randomly assigned to receive Ostim® (Heraeus Kulzer, Hanau, Germany) or open flap debridement (OFD). At baseline and during surgery probing pocket depths (PPD) and vertical bone level (BL) were measured from a customized acrylic stent. Six months following surgery PPD and BL (by vertical bone sounding) were assessed again.

**Results:** A statistical significant improvement over time for PPD and BL was detected in both groups. The test group (Ostim®) exhibited statistically significant greater changes compared to the control sites (OFD) in probing depth reduction:  $8.3 \pm 1.2$  to  $4.0 \pm 1.1$  mm vs.  $7.9 \pm 1.2$  to  $5.1 \pm 1.2$  mm ( $P < 0.05$ ) and vertical bone gain:  $4.0 \pm 1.0$  vs.  $2.8 \pm 1.4$  mm ( $P < 0.05$ ).

**Conclusions:** Treatment with a novel nanocrystalline hydroxyapatite compared to open flap debridement led to significant more favourable clinical improvements in intrabony periodontal defects.

# POSTERS

## Aetiology and pathogenesis

1

### Salivary interleukin 6 and tumour necrosis factor alpha in periodontal disease

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Periodontal disease is a chronic inflammatory disease related closely to the bacteria, host responses and genetics. However, its clear ethiopathogenesis still remains unclear. Cytokines have been known to have tremendous influence on oral diseases as well as periodontal disease. The aim of this study was to evaluate salivary interleukin 6 and tumour necrosis factor  $\alpha$  levels in patients with periodontal disease as it is known that cytokines are the key mediators of the host response to antigens. Study group consisted of 27 participants (18 males and 9 females), age range 28–62 yrs, mean 37.2 yrs. The control group consisted of 37 controls, age range 27–65 yrs; mean 39.3 yrs. Enzyme-linked immunoassay was used to determine the concentration of IL-6 and TNF- $\alpha$  in the whole saliva. Statistical analysis was done by use of Student t test and values below 0.05 were considered as significant. Levels of salivary IL-6 were significantly increased in patients with periodontal disease when compared to the control group ( $P = 0.041$ ). Levels of salivary TNF- $\alpha$  did not differ between patients and controls ( $P = 0.88$ ). We might conclude that salivary IL-6 plays more important role in periodontal disease than TNF- $\alpha$  and their action is not synergistic as it was previously taught.

2

### Development of coronal periodontum in dental eruption

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The periodontum establishes a functional unit that experiences modifications with age. These modifications are given in the process of teeth eruption, until periodontum reaches the morphology and proper structure of the adult.

**Aim:** To examine the dimensional changes occur in different structures of coronal periodontum, so much to level of soft as hard tissues during the eruption of 1.1 teeth.

**Material and methods:** One hundred and four children (6 to 11 years) with the upper central incisor teeth permanent whole or partially erupted. Variables: probing gingival sulcus, gingival band width, clinical crown length, distance between CEJ and bone crest, biologic width dimension, thickness of connective attachment, gingival overlapping the enamel surface, and length of anatomical crown using the parallel profile radiograph technique (Alpiste, 1999).

**Results:** Depending on the age, is observed a statistically significant decrease of the dimension of the gingival band width ( $P < 0.016$ ), in the depth of the sulcus ( $P < 0.001$ ) and in the gingival overlapping the enamel surface ( $P < 0.001$ ). It increases in a statistically significant way the dimension of the clinical crown ( $P < 0.001$ ). Statistically significant changes perceive neither for

the biological space nor for the distance among CEJ and bone crest.

**Conclusions:** Changes are produced in the level of soft tissues. The dimension of the biological space does not show a clear trend to change with the age and it might be determined before the tooth begins the eruption.

3

### IL-1 $\beta$ production in CD14-positive cells is affected by FCG receptor genotype

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**Background:** Functional bi-allelic polymorphism of immunoglobulin G (IgG) Fc $\gamma$  receptor IIa (Fc $\gamma$ RIIa-R/H131) has been shown as a genetic risk factor for periodontitis. Fc $\gamma$ RIIa is most expressed on mononuclear cells, and is also considered important to regulate production of inflammatory cytokine such as IL-1 $\beta$ . The aim of this study is to examine whether IL-1 $\beta$  production in mononuclear cell upon IgG-Fc $\gamma$ R cross-linking is affected by Fc $\gamma$ RIIa genotypes.

**Methods:** Peripheral blood mononuclear cells (PBMCs) were obtained from volunteers who had undergone the Fc $\gamma$ RIIa. PBMCs were stimulated with heat aggregated human IgG1 and IgG2, and then stained with phycoerythrin-conjugated anti-CD14 monoclonal antibodies. The expressions of CD32 on CD14 positive cells and the intercellular expressions of IL-1 $\beta$  within CD14-positive cells were assessed by flow cytometry.

**Results:** We found an increase in the percentage of IL-1 $\beta$  producing CD14-positive cells in a dose dependent manner. The percentage of IL-1 $\beta$  producing CD14-positive cells was increased with an incubation time. CD32 expression level on CD14-positive cells was not different among Fc $\gamma$ RIIa genotypes. There was a difference in levels of IL-1 $\beta$  producing-cells between the Fc $\gamma$ RIIa-H/H131 and -R/H131, Fc $\gamma$ RIIa-H/H131 and -R/R131 genotypes.

**Conclusions:** These results support the concept that Fc $\gamma$ RIIa genotype may affect IL-1 $\beta$  production in mononuclear cells, possibly leading to inter-individual differences in susceptibility to periodontitis.

4

### *Actinobacillus actinomycetemcomitans*, *porphyromonas* and pigmented *prevotella* in subgingival microflora of children with primary and mixed dentition

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Although associated with periodontal diseases in adults, little is known about the presence of *Actinobacillus actinomycetemcomitans*, *Porphyromonas* and pigmented *Prevotella* species in children. The purpose of this study was to examine subgingival microflora of 100 children aged 3–11 years with primary ( $n = 50$ )



and mixed dentition ( $n = 50$ ) for the presence of these bacteria determining their periodontal health. The subgingival samples were investigated using continuous anaerobic culture techniques and the bacteria identification has been verified by enzyme profiles and biochemical characteristics. *Prevotella intermedia* and *Prevotella nigrescens* have been differentiated by Polymerase Chain Reaction (PCR) technique using species specific 16 S rRNA primers. *A. actinomycetemcomitans* and *Porphyromonas* species were not detected. The frequency of *P. nigrescens* was increased with age (Mann–Whitney U-test,  $P < 0.05$ ) and this was statistically higher at mixed dentition than at the primary dentition (Chi-square test,  $P < 0.05$ ). Bleeding on probing, associated with gingivitis, was correlated only with *P. intermedia* among the other *Prevotella* species studied. Thus, we concluded that *P. intermedia*, which is associated with periodontal diseases in adults should be considered as indicator bacterium for children, as well.

This study was supported by the Research Fund of the University of Istanbul. Project number: T-1027/19022001.

5

### Subgingival microbiota in aggressive and chronic periodontitis Colombian subjects

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**Background:** Control of microorganisms in dental biofilm is one of the goals of periodontal therapy, so it is important to establish the subgingival microbiota of Colombian patients.

**Methods:** Subgingival samples of 128 Colombian subjects with localized aggressive (LAP) ( $n = 20$ ), generalized aggressive (GAP) ( $n = 40$ ) and chronic periodontitis (CP) ( $n = 68$ ) were obtained, and periodontopathic and some opportunistic organisms were cultured and identified.

**Results:** Frequencies of most prevalent pathogens identified were: *Fusobacterium* spp (75.8%), *P. gingivalis* (64.1%), *P. intermedia/nigrescens* (53.9%), *D. pneumosintes* (34.4%), *A. actinomycetemcomitans* (32.8%) and Gram-negative enteric rods (32%). 42% of patients with GAP, 30% with LAP and 28% with CP resulted positive for *A. actinomycetemcomitans*. *P. gingivalis* was found in 85% of GAP patients, 50% of LAP and 55.9% of CP ( $P \leq 0.05$ ). *P. micros* was recovered from 41% of CP patients, 35% of LAP and 17% of GAP ( $P \leq 0.05$ ). Gram-negative enteric rods were prevalent in all clinical periodontal conditions evaluated.

**Conclusions:** Microbial profile of Colombian LAP was found different from North American and European reports that have shown a high prevalence of *A. actinomycetemcomitans*. Ethnical differences may account in part to the diverse subgingival microbial profiles showed among Colombian subjects with periodontitis. More studies of microbiology and prevalence of aggressive and chronic periodontitis are needed in South American populations.

6

### Cyclosporin-induced gingival overgrowth reveals elevated gene expression for MMP-1, MMP-10 and TIMP-1

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**Aims:** In humans, it has been shown that treatment with the immunosuppressant drug cyclosporin A (CsA) leads to increased levels of fibrillar extracellular matrix (ECM) type-I collagen in the

connective tissue of CsA-induced gingival overgrowth (GO). CsA also up-regulates gene expression of type-III collagen and proteoglycans (PG). In this context it still remains unclear whether this increase is associated with alterations of molecules involved in the turnover of collagens and PG.

**Material and methods:** The present study explores the status of MMP-1 and -10 and their tissue inhibitor TIMP-1 on the gene expression as well as on the protein level on frozen sections derived from GO and normal tissue.

**Results:** *In situ* hybridization (ISH) revealed elevated levels of MMP-1 gene expression in the epithelial compartment of GO compared with normal tissue. This elevation also applied to MMP-10, while MMP-10 gene transcription appeared generally stronger, rather than that observed for MMP-1. For TIMP-1, transcription levels were weak in normal and strong in GO tissue. These differences detected in ISH were corroborated by quantitative RT-PCR. Detection of the protein by indirect immunofluorescence showed that normal gingival tissue was devoid of all three proteins, while they were detectable in GO tissue, with emphasis on TIMP-1.

**Conclusions:** Analysis of our data indicates that in addition to MMP-1 and -10, particularly TIMP-1 may contribute to the pathogenesis of CsA-induced GO.

7

### Effect of antimicrobial agents on early and mature biofilm *in vitro*

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The aim of the study was to assess the effect of antimicrobial agents on an *in vitro* dental biofilm model using culture and confocal laser scanning microscopy (CLSM). Saliva-coated hydroxyapatite discs were incubated with oral bacteria including *A. naeslundii* (OMZ 745), *F. nucleatum* (ATCC 25586), *S. oralis* (OMZ 607), and *V. dispar* (OMZ 493) in modified fluid medium (mFUM) in anaerobic conditions for 12 h (early biofilm) or 72.5 h (mature biofilm). Biofilms were exposed for 1 min to 0.2% triclosan (TRI), 0.2% chlorhexidine (CHX), 0.1% isopropyl methylphenol (IPMP), 0.05% cetylpyridinium chloride (CPC), or 10% ethanol (control) at various time intervals. Bacteria were harvested and the total number of CFUs was determined by culture. Specimens were observed by CLSM following live/dead fluorescence staining. 3D images were reconstructed using Imaris 4.2 software. For early biofilm, TRI ( $1.9 \times 10^9$  CFU/mL), CHX ( $1.2 \times 10^9$ ), and IPMP ( $1.2 \times 10^9$ ) resulted in a significant decrease in CFUs compared to controls ( $4.7 \times 10^9$ ). For mature biofilm, a significant reduction was also observed with TRI ( $3.9 \times 10^{12}$ ) and CHX ( $3.2 \times 10^{12}$ ) compared to controls ( $9.7 \times 10^{13}$ ) but to a lesser extent with IPMP ( $9.1 \times 10^{12}$ ). CPC had little effect on both, early and mature biofilms. The effect of antimicrobials on bacterial vitality was confirmed by CLSM observations. 3D analysis revealed that killing occurred mostly in the superficial layers and that live bacteria were still present in the deepest parts of biofilms.

8

### Differences in MMP-1 and MMP-8 gene and protein expression patterns between chronic and aggressive periodontitis

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In periodontitis patients, an imbalance between activated matrix metalloproteinases (MMPs) and their inhibitors leads to break-

down of extracellular matrix. MMP-1 and MMP-8 are implicated in the destruction of extracellular matrix in periodontal disease. The purpose of this study was the exploration of the expression patterns of MMP-1 and MMP-8 mRNA and mature protein levels in human gingival tissues from patients with chronic and aggressive periodontitis. Gingival samples were harvested from nine subjects: three with healthy and intact periodontium (C) requiring surgical crown lengthening, three with chronic (CP) and three with aggressive periodontitis (AP). Patients initially were subjected to phase-I periodontal therapy and periodontal surgery. Gingival specimens were harvested during periodontal surgery. Gene expression was determined by using reverse polymerase chain reaction assay (RT-PCR). Expression of MMP-1 and MMP-8 at mature protein level was revealed by using Western blot-analysis. AP subjects presented significantly higher MMP-1 and -8 mRNA and mature protein levels (Fishers' exact  $P$ -value = 0.036). MMP-1 and -8 mRNA and mature protein levels in gingival specimens from CP or C patients were so low that they were barely detectable. These results revealed that MMP-1 and -8 mRNA and mature protein levels remained high after phase-I periodontal treatment only for the AP group. This finding might indicate a different response of the AP cases to phase-I periodontal treatment.

9

### Expression of high mobility group box 1 (HMGB1) in gingival epithelial cells

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**Aims:** High mobility group box 1 (HMGB1), originally described as a DNA-binding protein, can also be released extracellularly and functions as a late mediator of inflammatory responses. However, its role in the periodontal field remains unknown. The purpose of this study was to demonstrate HMGB1 expression in gingival tissue of periodontitis, and to identify the role of HMGB1 in the pathogenesis of periodontal disease progression.

**Materials & methods:** In gingival tissue of the patients with periodontitis, the protein expressions of HMGB1 and its receptor, RAGE, were assessed using immunohistochemical staining. The human gingival carcinoma cell line (Ca9-22) was exposed to TNF $\alpha$  and HMGB1. The level of HMGB1 in supernatant of Ca9-22 was confirmed by Western immunoblotting. The signalling pathway involved in the release of HMGB1 were examined using the specific and potent MAPKs inhibitor. The levels of IL-8 and IL-6 were quantified by ELISA.

**Results:** Immunostaining revealed the expressions of HMGB1 and RAGE in the gingival epithelial tissue with chronic periodontitis. TNF $\alpha$  triggered the release of HMGB1 from Ca9-22 cells in a dose- and time-course dependent manner. The specific inhibitor of p38 but not ERK1/2 or JNKs significantly reduced HMGB1 release. Moreover, HMGB1 increased the secretions of IL-8 and IL-6 from Ca9-22.

**Discussion/conclusion:** These results suggest that extracellular HMGB1 may be a key player in the chronic periodontitis.

10

### Implication of secretory phospholipase A<sub>2</sub> (SPLA<sub>2</sub>) in periodontal disease

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Secretory phospholipase A<sub>2</sub> (SPLA<sub>2</sub>) participates in inflammatory conditions, as it catalyses the release of arachidonic acid,

initiating the production of eicosanoid mediators, namely prostaglandins and leukotriens. SPLA<sub>2</sub> may be secreted either constitutionally or as an acute-phase reactant upon stimulation by proinflammatory cytokines (IL-1 $\alpha$ , IL-1 $\beta$ , TNF $\alpha$ ) and endotoxins (LPS). In this study we have investigated the SPLA<sub>2</sub> activity in gingival crevicular fluid (GCF) in periodontal patients. GCF samples were harvested from 15 subjects with moderate to severe adult periodontitis and 15 healthy volunteers. All participants were in good health and none of them had received any periodontal treatment or antibiotic therapy in the previous 6 months. Clinical measurements were recorded after obtaining GCF samples. SPLA<sub>2</sub> activity was investigated using the Schädlich et al method. It was found to be calcium dependent and was estimated in patients fourfold higher. GCF was subject to chromatographic separation (HPLC). The SDS electrophoresis revealed two bands of 15.6 and 14 kDa. These results suggest that the increased SPLA<sub>2</sub> activity in the GCF of periodontal patients and, therefore, the higher levels of inflammatory mediators (prostaglandins, leukotriens) in conjunction with the direct action of SPLA<sub>2</sub> as an acute phase protein, may aggravate the periodontal tissue damage. The different SPLA<sub>2</sub> activity bands could represent two molecules with different roles in the inflammatory process.

11

### Antioxidant capacity and periodontal condition in pregnant women

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Pregnancy is a physiological state of oxidative stress arising from increased oxygen requirement and placental mitochondrial activity. Evidence suggests pregnancy-oxidative stress, periodontitis-preterm birth, periodontitis-oxidative stress links. To analyse antioxidant (AO) defence-periodontal status relation in pregnant, serum and gingival crevicular fluid (GCF) total antioxidant capacity (TAOC) and superoxide dismutase (SOD) concentrations were compared with those of non-pregnant chronic periodontitis (CP) patients. 33 pregnant with CP who were followed in 1., 3. trimesters (PCP1, PCP3), 18 pregnant with gingivitis (PG1, PG3), 27 CP and 25 control women were studied. After measuring probing depth, clinical attachment level, gingival bleeding, gingival and plaque indices, and samplings (1., 3. trimesters in pregnant), TAOC and SOD were measured by automated and spectrophotometric tests. Clinical parameters were higher in pregnant and CP groups than were in controls, in PCP3 than in CP group and increased from 1. to 3. trimester in pregnant (except plaque index) ( $P < 0.05$ ). Serum and GCF TAOC and SOD were lower in pregnant, the lowest in PCP3, and the highest in controls and reduced from 1. to 3. trimester in pregnant ( $P < 0.05$ ). The findings showed reduced systemic and local AO defence in CP, greater AO reduction and worse periodontal status in pregnant, suggesting oxidative stress an important pathogenic mechanism of periodontal problems-preterm birth link in pregnancy.

12

### Salivary detection of *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia* and *Porphyromonas gingivalis* from Portuguese adolescents with PCR

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A number of forms of periodontitis affecting adolescents are likely to be influenced by periodontal microflora and competency of the host response with saliva as the principal way of transmission. The aim of this study was to assess the salivary presence of *Actinobacillus actinomycetemcomitans* (A.a.), *Prevotella intermedia* (P.i.) and *Porphyromonas gingivalis* (P.g.) from systemically healthy 12 years old adolescents, using Multiplex PCR. 169 adolescents, 107 (63.3%) females and 62 (36.7%) males, contributed with 10 mL of saliva stimulated by paraffin wax. Samples were sent to the laboratory, vortexed at the maximal setting and 1 mL of each, transferred to an eppendorf tube. After centrifugation, the supernatant was discarded and 50 µL of TE 1x (Tris-EDTA) were added to resuspend the pellet. Samples were frozen at -20 °C until PCR analysis. Of the 169 adolescents, 51 (47.7%) and 83 (77.6%) of females, harboured respectively A.a. and P. i. 36 (58.1%) of males were colonized by A.a. and 48 (77.4%) by P. i. The simultaneous occurrence of A.a. and P. i. was detected from 50 (46.7%) of females and 35 (56.4%) of males. P.g. was not found in any sample. These results suggest a high percentage of colonization by A. a. and P. i. among these adolescents which can represent a risk factor for periodontitis development and demonstrated the absence of P. g., a later colonizer.

13

### Synergistic action of periodontal pathogens on the production of interleukin-1 $\alpha$

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**Objectives:** To investigate the interaction of periodontal pathogens in stimulating IL-1 $\alpha$  production by monocytes.

**Methods:** Bacterial supernatants from cultures of *P. gingivalis*, *F. nucleatum*, *C. rectus*, *A. actinomycetemcomitans*, *P. intermedia* and *T. denticola* were collected for cell stimulations. *E. coli* LPS was used at 100 ng/mL as a positive control. The single supernatants or mixtures were added (diluted 1:250 in media) to Monomac-6 cell cultures ( $1 \times 10^6$  cells per mL) and incubated for 6 hours at 37 °C. The production of IL-1 $\alpha$  was quantified by enzyme-linked immunoassays.

**Results:** Supernatants from four species, Aa, Fn, Cr and Pi, produced marked up-regulation of IL-1 $\alpha$  levels (e.g. controls  $6.29 \pm 0.079$  pg/mL, Aa  $5.66 \pm 91.4$  pg/mL). In contrast *P. gingivalis* induced only small increases in IL-1 $\alpha$  and *T. denticola* had no effects when used on its own. However, *T. denticola* had significant synergistic effects when used in combination with other pathogens. In particular, there was a dramatic increase in IL-1 $\alpha$  production with *T. denticola* mixed with *P. gingivalis* or *P. intermedia* (e.g. Pg only  $52.28 \pm 0.23$  pg/mL; Pg + Td  $579.8 \pm 164.1$  pg/mL). When combined with other species *T. denticola* induced relatively small changes.

**Conclusions:** This study confirms that some periodontal pathogens may act to significantly enhance the immune response triggered by other bacterial species. This serves to emphasize the complex nature of periodontal disease as a disease with involving polymicrobial infection.

14

### Evaluation of major cigarette smoke components acrolein and acetaldehyde in saliva.

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**Background:** The aim of this research was to evaluate the concentration of acrolein and acetaldehyde, major components of cigarette smoke, in the saliva of subjects recruited according to age and number of cigarettes smoked per day.

**Materials and methods:** Two saliva samples, one taken before and one after cigarette smoking, were collected from 186 subjects divided into groups according to age and number of cigarette smoked. Samples were analysed by capillary electrophoresis.

**Results:** Base levels of acrolein and acetaldehyde were found to be significantly higher in the saliva of smokers of all age cohorts. In addition, the concentration of these aldehydes in saliva was found to increase with the number of cigarettes smoked per day and with age.

**Discussion:** The authors developed a capillary electrophoretic technique, recently published in Electrophoresis. Some authors suggest that the higher concentration of acetaldehyde in the saliva of smokers is due to an increase in its production caused by a variation in the oral bacterial microflora. According to others, smoke may inhibit the enzyme ALDH, and acrolein itself could be responsible for this inhibition. The increase in concentrations of acrolein and acetaldehyde in saliva with increasing age and number of cigarettes smoked suggests that the antioxidant power and enzyme activity of saliva are altered as a function of these factors. Smoke saliva-interaction may deplete saliva efficacy in protecting oral tissues.

15

### The effectiveness of TACE inhibitor; new option for treating periodontal inflammation

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TUMOR NECROSIS FACTOR- $\alpha$ : TNF- $\alpha$  is a proinflammatory cytokine, plays a pivotal role in the inflammatory reaction. Its precursor is cleaved by a metalloprotease named TNF- $\alpha$ -converting enzyme (TACE) to generate the mature TNF- $\alpha$ .

**Objective:** Currently, intense effort underway to regulate the effective treatment of periodontitis remains challenging due to the complexity of this disease. To achieve this goal, we aimed to generate a potent inflammatory drug, TACE inhibitor to overcome this heterogeneity disease where TNF- $\alpha$  is thought to be pathologically indicated.

**Results:** TACE-immunopositively localize mainly in macrophages and gingival fibroblast in inflamed-gingival tissues. By means of immunofluorescence assay, TACE immunolocalize on cultured human gingival fibroblast (HGF) as well as on human monocytic cell line (HL-60). The TACE inhibitor eliminates kinetics of LPS-induced TNF- $\alpha$  secretion beginning from 100-nanomolar concentrations by ELISA. The inhibitor of TACE prevents the cleavage of HGF-bound TNF- $\alpha$  in a dose-dependent manner with an IC50 of  $1.0 \times 10^{-7}$  M.

**Conclusion:** TACE inhibitor may be effective to inhibit TNF- $\alpha$  production on inflamed gingival tissue. TACE inhibitor appears to be an attractive target for treating human periodontitis.

16

### Semiquantitative analysis of the tight junction associated claudin 1, 4, 7 in healthy human gingiva

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**Objectives:** To quantify the expression of three identified Claudin (CLDN) tight junction proteins in healthy gingival epithelium.

**Patients and methods:** The expression of CLDN1, CLDN4 and CLDN7 were analysed in histologically evaluated, healthy gingival samples obtained from five young patients prior to surgical removal of impacted mandibular third molar. The expression of Claudin tight junction protein was quantified in different epithelial cell layers. The digital images captured from the stained samples were optimized and converted by Adobe Photoshop software to greyscale TIFF files. The semiquantitative analysis of Claudin expression was performed by using ImageQuant software (Molecular Dynamics). The background was eliminated by a threshold set up. On the images, we set up five randomly selected polygonal areas with equal size in each cell layer. We determined the baseline intensities of polygons and subtracted from the actual pixels intensities obtained from specific staining. The statistical significance was determined by ANOVA probe and Tukeys post-hoc test.

**Results:** The expression of CLDN1, CLDN4 and CLDN7 significantly elevated in spinous cell layer of gingival epithelium.

**Conclusion:** The multiple claudins family members play role in paracellular transport in human epithelial tissues. Our results suggesting that different claudins may play differential role to maintain epithelial integrity in human gingiva.

17

### Advanced glycation endproducts (age) in periodontal disease

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**Background:** Advanced glycation endproducts (AGE), formed by non-enzymatic glycation of protein, causes extensive tissue damage through activation of inflammatory processes and alteration of protein structure. Although the pathology of diabetes-associated periodontal disease has been attributed to AGE accumulation, there exists a paucity of information. In this regard the aim of the study was to investigate the role of AGE in the pathogenesis of periodontal disease in diabetic versus non-diabetic patients.

**Method:** Gingival biopsies were obtained from nine untreated chronic periodontitis patients (six diabetic; three non-diabetic patients). Biopsies from four periodontally healthy patients were obtained as controls. The biopsies were digested with collagenase and AGE levels were quantified using the characteristic excitation: emission fluorescence spectra of 325/390 nm.

**Results:** Quantification of the fluorescence emitted showed no observable difference between diabetic compared to non-diabetic patients. Gingival biopsies from control patients, however, demonstrated significantly lower fluorescence per microgram of protein compared to chronic periodontitis patients ( $P < 0.02$ ).

**Conclusion:** We have shown that gingival biopsies digested with collagenase demonstrate a fluorescent profile characteristic of AGE. Further, our observations lead to the hypothesis that AGE is a risk factor for periodontal disease that is not specifically associated with diabetes.

18

### RANKL and OPG mRNA expression in cyclosporin - a treated patients with chronic periodontitis

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**Aim:** The relative expression of the osteoclastogenesis inducing factor RANKL (receptor activator of NF-kappa B ligand) and its decoy receptor osteoprotegerin (OPG) is the main determinant of bone resorption in health and disease. Cyclosporin-A (CsA) is thought to regulate RANKL and OPG expression. The aim of this study was to investigate RANKL and OPG gene expression

pattern in gingival tissues of CsA treated patients with chronic periodontitis (CsA-CP) and compare this expression with that of chronic periodontitis (CP) and periodontally healthy subjects.

**Methods:** In this pilot study, gingival tissue biopsies were obtained from CsA-CP, CP and healthy subjects. Five subjects were included in each group. mRNA levels of RANKL and OPG were quantified by real time quantitative RT-PCR, and normalized against 18S RNA.

**Results:** RANKL expression markedly increased in CsA-CP and CP patients, which was very low in healthy subjects. OPG was reduced by 99% in CP, and only 67% in CsA-CP patients compared to healthy subjects ( $P < 0.05$ ). The RANKL/OPG ratio was also higher in CP and CsA-CP patients than that of healthy subjects. CsA-CP patients had lower RANKL/OPG ratio compared to CP patients (Ratios: CsA  $0.05 \pm 0.027$ ; CP  $0.25 \pm 0.11$ ).

**Conclusion:** This preliminary data suggests that CsA therapy decreases the RANKL/OPG ratio in gingival tissues, by enhancing OPG expression in CsA-CP patients.

19

### Recombinant RgpA confirms protection against *P. gingivalis* induced experimental periodontitis

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**Background:** Gingipains, a group of cysteine proteases produced by *Porphyromonas gingivalis* (*P. gingivalis*) are thought to be one of the bacteria's major virulence factors. As such, they are considered to be candidate antigens for vaccine development.

**Aims:** To investigate the effect of RgpA and Kgp gingipains on alveolar bone loss (ABL) and to evaluate the efficacy of immunization with recombinant RgpA in preventing ABL.

**Materials and methods:** The mouse oral infection model (Baker et al., 1994) was used. In order to test the effect of gingipains, five groups of Balb/c mice were infected with *P. gingivalis* wild-type, RgpA<sup>-/-</sup>, Kgp<sup>-/-</sup>, RgpA<sup>-/-</sup> + Kgp<sup>-/-</sup> or vehicle. The efficacy of immunization was investigated by immunization of orally infected mice with 30 kd recombinant peptide derived from RgpA, 60 kd recombinant RgpA, heat-killed *P. gingivalis* or none.

**Results:** The wild-type, as well as the Kgp<sup>-/-</sup> mutant bacteria, induced significant ABL compared to controls. However, the RgpA<sup>-/-</sup> (and the double) mutant did not, and the ABL was not different from non-infected mice. Both recombinant vaccine preparations were able to significantly reduce the ABL, compared to the non-immunized group, and were as effective as a whole-bacteria vaccine.

**Conclusions:** RgpA, but not Kgp, is a major virulent factor affecting the mouse ABL after oral infection with *P. gingivalis*. Immunization with recombinant peptides derivatives from RgpA may act as antigens for the prevention of periodontal disease.

20

### Expression of e-selectine in gingival tissues in aggressive and chronic periodontitis before and after phase I periodontal treatment

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Cell adhesion molecules (CAMs) are cell surface proteins involved in the binding of cells, usually leukocytes, to each other, to endothelial cells, or to extracellular matrix. Recent studies have indicated that selectins (E, L, P) are implicated in cell trafficking, an important aspect of inflammation-related process. Either of these selections can bind carbohydrate molecules found on the leukocyte, resulting in an increase in the time the leukocyte remains

associated with the endothelium. This appears microscopically as an increase in number of leukocytes attached to the luminal surface of the endothelium, or increased rolling. This study compared e-selectin expression in gingival samples from Aggressive Periodontitis (AP) and Chronic Periodontitis (CP) to findings in controls. Gingival specimens were collected from individuals before and after phase I periodontal treatment. Gingival tissue samples were immunohistochemically analysed for e-selectin expression in three groups. There was no significant differences among groups and there was no significant differences between AP and CP groups before and after phase I periodontal treatment. These findings suggested that inflammation markers in periodontal disease can help the prognosis and monitoring of periodontal illness. Specific signals produced in response to wounding and infection control the expression and activation of certain of this adhesion molecule.

21

### Neutrophil functions before and after periodontal treatment in generalized aggressive periodontitis

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The objective of this study was to evaluate clinical parameters, phagocytosis and oxidative burst functions of neutrophils in generalized aggressive periodontitis (GAP) and chronic periodontitis (CP) before and after initial periodontal therapy. 10 GAP, 10 CP patients and 10 healthy individuals were included. At 0 and 49 days peripheral blood analyses including phagocytosis and oxidative burst functions have been performed by flow cytometric method. At the same days plaque index, bleeding on probing, probing depth and relative attachment level were measured. Significant clinical improvements were observed in two patient groups ( $P < 0.01$ ) compared to their respective baseline values only with the exception of relative attachment level changes in CP. Intergroup comparisons revealed no significant differences. Regarding to the immunological evaluations % phagocytosis and oxidative burst functions demonstrated no significant differences between the three groups. The differences between % phagocytosis and oxidative burst functions in intergroup comparison did not reveal statistical significance. The mean fluorescence intensity considered as the degree of phagocytosis was found to be much higher in GAP patients than in CP and healthy group before the initial periodontal treatment but reached almost similar levels after the treatment. It can be concluded that the investigated neutrophil functions of GAP can be considered as in expected normal ranges.

22

### Distribution of human lactoferrin in the root, GCF and saliva, and its roles in the pathogenesis of periodontitis

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Human lactoferrin (hLf) is a multifunctional glycoprotein, with anti-inflammatory and anti-tumour effects. hLf inhibits interaction between LPS and CD14; thus, hLf released from neutrophils may affect the inflammatory responses. This study examined the distribution of hLf in the root, GCF and saliva, and its roles in the pathogenesis of periodontitis. 19 teeth not salvageable by periodontal treatment were extracted with informed consent of the patients. Five extracted teeth were incubated with anti-hLf and Alexa 532-conjugated secondary antibody; subsequently, fluorescence was measured. Grinded samples were obtained from remaining 14 roots from the area with calculus and without, and dissolved in PBS and hLf and endotoxin levels in the supernatant were measured. Additionally, hLf levels in GCF, saliva, and

supra- and sub-gingival plaque were determined. Furthermore, LDH activity and cytokine levels in culture supernatants of human monocytic cells, THP-1, were measured when cultured with hLf and LPS from *E. coli* for 24 h. Significantly higher hLf and endotoxin levels were detected in the area with calculus than without. hLf levels were higher in the GCF than in the saliva. LDH activity and TNF- $\alpha$  levels were increased following LPS stimulation; however, decreased when LPS and hLf were added together. These findings suggest that hLf released from neutrophils is bound to LPS in periodontal pockets, which may affect cytokine releases and chemotactic responses of monocytic cells.

23

### Gingival crevicular fluid levels of IL-1B, TNF-A, IL-6 and IFN-G in patients with aggressive and chronic periodontitis patients

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**Background:** The cytokines Interleukin-1 $\beta$  (IL-1 $\beta$ ), Tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), Interleukin-6 (IL-6), Interferon gamma (IFN- $\gamma$ ) are inflammation-related cytokines associated bone resorption in periodontal disease. IL-1 $\beta$ , TNF- $\alpha$ , IL-6 and IFN- $\gamma$  have regulatory effects on osteoclastogenesis and the most aggressive forms of inflammatory bone loss. The aims of this study were to investigate the levels of IL-1 $\beta$ , TNF- $\alpha$ , IL-6, IFN- $\gamma$  in gingival crevicular fluid (GCF) samples from patients with chronic periodontitis (CP) and aggressive periodontitis (AgP) and to clarify the correlation of GCF levels of this markers with the severity of periodontitis.

**Methods:** Ten patients with CP, 22 patients with AgP, 8 healthy control (C) subjects were selected for the study. The GCF was collected by using sterile filter papers. Assays for GCF IL-1 $\beta$ , TNF- $\alpha$ , IL-6, IFN- $\gamma$  were carried out by an ELISA method. The groups compared with each other by utilizing Mann-Whitney U-test.

**Results:** The GCF level of TNF- $\alpha$  in GAgP group and C group were statistically significant higher than the LAgP group ( $P < 0.05$ ). A significant increase of IL-6 was observed in GAgP and LAgP groups compare to C group ( $P < 0.05$ ).

**Conclusions:** These results suggest that IL-6 has a major effect on progressive bone loss in aggressive forms of periodontitis. In addition, the higher GCF level of IL-6 in GAgP and LAgP can be demonstrate that IL-6 is associated with the severity and extension of the soft and hard tissue destruction.

24

### Gingival crevicular fluid levels of IL-2, IL-5, IL-6, IL-10 and IL-12 in the patients with aggressive and chronic periodontitis

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**Background:** The immune response to infection is regulated by the balance between Th1 and Th2 cytokines. The aims of this study were to investigate the expression of IL-2, IL-5, IL-6, IL-10 and IL-12 in gingival crevicular fluid (GCF) samples from patients with chronic periodontitis (CP), and aggressive periodontitis (AgP) and to examine the possible correlations between the GCF levels of IL-2, IL-5, IL-6, IL-10 and IL-12.

**Methods:** Ten patients with chronic periodontitis (CP), 14 generalized aggressive periodontitis (GAgP), 8 patients with localized aggressive periodontitis (LAgP) and 8 healthy control (C) subjects were selected for the study. The GCF was collected by using sterile filter papers. Assays for GCF IL-2, IL-5, IL-6, IL-10 and IL-12 were carried out by an enzyme-linked immunosorbent

assay (ELISA) method. The groups were compared with each other by utilizing Mann-Whitney U-test.

**Results:** The GCF level of IL-6 in GAgP and LAgP groups was statistically higher than in the C group. A significant increase of IL-6 level was observed in only AgP groups. The level of IL-10 in control group was statistically higher than CP group.

**Conclusions:** These data suggest that the amount of IL-6 and IL-10 is associated with periodontal status. The expression of IL-6 may reflect inflammation in gingival tissues and IL-6 may have a profound effect on progressive bone resorption in AgP. The absence or presence of IL-10 may play an important role in identification of the diseased or healthy tissues.

25

### Gingival crevicular fluid levels of VEGF in patients with aggressive and chronic periodontitis

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**Background:** Vascular endothelial growth factor (VEGF) is a cytokine produced by gingival fibroblasts and responsible for vascular hyperpermeability and angiogenesis in inflammation and wound healing. VEGF plays an important role in the pathogenesis of periodontitis by causing the inflammatory cells infiltration, edema and swelling. And VEGF promotes the progression of periodontal disease. The aims of this study were to investigate the level of VEGF in gingival crevicular fluid (GCF) samples from patients with chronic periodontitis (CP) and aggressive periodontitis (AgP) and to compare the GCF levels of this marker between periodontally healthy and diseased groups.

**Methods:** Ten patients with (CP), 14 patients with generalized aggressive periodontitis (GAgP), eight patients with localized aggressive periodontitis (LAgP), and eight healthy control (C) subjects were selected. The gingival crevicular fluid was collected by using sterile filter papers. Assay for VEGF was carried out by an enzyme-linked immunosorbent assay (ELISA) method.

**Results:** The GCF level of VEGF in GAgP, LAgP and CP groups was statistically higher than the C group. And VEGF level was higher in the CP group than the AgP groups.

**Conclusion:** These data's suggest that VEGF could have an important role in periodontal diseases. The higher level of VEGF in CP group than the GAgP and LAgP groups may be related to intermittent destruction in CP. VEGF might be associated with the etiology of periodontitis in its early stages.

26

### Effects of fibroblasts on BMP-induced osteoblastic commitment

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The aim of this study was to investigate the hypothesis that gingival connective tissue might directly inhibit BMP-induced bone formation by production of soluble factors. Conditioned media derived from rat periodontal ligament (PDL), skin and gingival fibroblast cultures were tested for their ability to inhibit the BMP-induced osteoblastic commitment of the C3H10T1/2 (10T1/2) mesenchymal stem cell line. 10T1/2 cells were stimulated with conditioned media,  $10^{-9}$  M of retinoic acid, and 100 ng/mL BMP-2, and tested for induction of osteoblastic commitment as measured by alkaline phosphatase activity (ALP) after 72 hours. Conditioned medium from gingival and skin fibroblast cultures markedly inhibited BMP-induced ALP expression ( $17.7 \pm 2.06\%$  and  $40.2 \pm 2.1\%$  reduction from controls respectively,  $P < 0.05$ ). In contrast, conditioned medium from PDL cells strongly enhanced the effects of BMP-2 on ALP expression in 10T1/2 cells ( $274.4 \pm 0.75\%$

increase over controls,  $P < 0.05$ ). These results demonstrate that gingival and skin fibroblasts inhibited BMP induced osteoblast commitment by release of soluble factors. However, PDL cells potentiated BMP activity, consistent either with endogenous BMP production in PDL cells, or due to other potentiating factors present. Results from this pilot study are consistent with the hypothesis that fibroblasts produce BMP-inhibitors such as noggin, which may limit the potential for coronal bone growth during regenerative therapies.

27

### The relationship between apoptotic cell death and gingival crevicular fluid neopterin levels in periodontitis

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Neopterin is an early biomarker of cellular immunity and produced by human macrophages after induction by interferon-gamma (IFN- $\gamma$ ). Apoptosis is activated by various signals, such as the one caused by tumor necrosis factor (TNF). Combination of IFN- $\gamma$  and TNF- $\alpha$  were found be related to the formation and release of nitric oxide (NO). Neopterin was reported to be a possible activator for inducible nitric oxide synthase enzyme (iNOS), thus it has potential to direct apoptosis. The aim of this study was to investigate the relationship between apoptotic cell death and neopterin in patients with both aggressive (AgP) and chronic (CP) periodontitis. Clinical indices were taken from 20 AgP, 15 CP patients and 10 periodontally healthy (PH) subjects. Apoptosis in tissue samples was determined in situ hybridization method. iNOS and TNF- $\alpha$  were assessed by immunohistochemistry. The gingival crevicular fluid (GCF) neopterin levels were determined by ELISA. Clinical parameters were found significantly elevated in the AgP and CP groups, compared to PH subjects. The difference of GCF neopterin levels was significant between periodontitis and PH groups. iNOS and apoptotic cell amount in the periodontitis groups were found statistically greater than PH group. It may be concluded that the neopterin-NOS-NO pathway and apoptosis might be involved in the periodontal disease process.

28

### Role of IL-17 in adult periodontitis

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**Background:** IL-17 is a proinflammatory cytokine that modulates the inflammatory process in periodontitis. Our purpose is to examine IL-17 distribution in the gingival tissues and analyse its concentration in healthy and diseased human gingival crevicular fluid (GCF) samples.

**Materials and methods:** Gingival tissue and fluid samples were collected from ten healthy controls (age range 20–37 years) and ten adult periodontitis patients (age range 31–45 years) from periodontally affected sites (mean probing depth  $\geq 5$  mm). GCF was collected using filter paper strips and enzyme-linked immunoabsorbent assay (ELISA) was performed to determine the concentration of IL-17. Localization of IL-17 in tissue specimens was studied by immunohistochemistry.

**Results:** The intensity of IL-17 staining was stronger in periodontitis and the number of IL-17 immunoreactive cells was higher in periodontitis samples compared to healthy controls. ELISA results showed that the cytokine concentration was higher in periodontitis affected group than the controls ( $P < 0.01$ ).

**Conclusion:** IL-17 was increased in periodontitis. Considering its role to stimulate the production of tumour necrosis factor alpha and interleukin 1 beta, it may play a key role in the pathomechanism of the periodontitis.

29

### The relationship between p38 map kinase and gingival crevicular fluid interleukin-1 $\beta$ levels in periodontitis

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p38 is a member of the mitogen-activated protein (MAP) kinase family and is a critical enzyme in the proinflammatory cytokine pathway. Interleukin-1 (IL-1) is an important inflammatory mediator and plays a central role in the destruction of connective tissue matrices in periodontal diseases. It is well established that IL-1 activation of the MAP kinase pathway. The aim of this study was to evaluate the relationship between p38 in gingival tissues and gingival crevicular fluid (GCF) IL-1 $\beta$  levels of patients with both chronic (CP) and aggressive periodontitis (AgP). Clinical indices were taken from 20 AgP, 15 CP patients and 10 periodontally healthy (PH) subjects. The presence of p38 in gingival tissue samples was determined by immunohistochemistry and the level of IL-1  $\beta$  in GCF was assessed by ELISA. Clinical parameters were found significantly elevated in the AgP and CP groups, compared to PH subjects. The p38 amounts in the AgP and CP groups were statistically greater than in PH group. The GCF IL-1 $\beta$  levels in periodontitis groups were found statistically higher than PH group. In AgP patients, a significant correlation was detected between p38 and the GCF IL-1 $\beta$  levels. This is the first report to evaluate the involvement of p38 in AgP and CP and this might be considered of value in understanding disease mechanisms.

30

### The expression of proliferating cell nuclear antigen in amlodipine induced gingival enlargements

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Gingival overgrowth is a common undesired side-effect in patients taking calcium channel blockers. Amlodipine is a second generation calcium channel blocker used in the management of angina and hypertension. Exact cause of induction of the hyperplasia is unknown. The increase in the proliferation of gingival epithelial cells and fibroblasts of lamina propria may have a role in the development of gingival hyperplasia. The aim of study is to investigate the expression and possible role in amlodipine induced gingival enlargement of proliferating cell nuclear antigen (PCNA) protein that stimulates cell cycle progression. A total of 40 gingival samples were taken from 4 different patient groups;(1) inflammatory gingival hyperplasia (10), (2) amlodipine induced gingival hyperplasia (10), (3) amlodipine not induced gingival hyperplasia (10), (4) gingivitis (10). All samples were immunohistochemically studied for PCNA. The results showed that Proliferating index (PI) of keratinocytes and fibroblasts was higher in inflammatory gingival hyperplasia group and the amlodipine induced hyperplasia group than other two groups. The correlation was found between PI and clinical parameters. The proliferation of keratinocytes and fibroblasts have a role of development gingival hyperplasia which may be stimulated by both inflammation and induction of drug.

31

### Expression of Fc $\gamma$ RIIb in human blood and gingiva with periodontitis

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**Background:** Fc $\gamma$ RIIb is one of IgG receptors and suppress the activation of B lymphocytes through cross-linking with BCR via immune-complex. Fc $\gamma$ RIIb is also has a critical role in antigen presentation. Our previous study demonstrated Fc $\gamma$ RIIb gene to be

a risk factor for periodontitis. In this study, we investigated the expression of Fc $\gamma$ RIIb in peripheral blood leukocytes and gingiva from periodontitis patients.

**Methods:** Peripheral blood leukocytes were obtained from 12 patients with periodontitis and 18 healthy volunteers with Fc $\gamma$ RIIa H/H131 genotypes. Fc $\gamma$ RIIb expression was analysed with flowcytometry using the antibody 41H.16. The same antibody was used for inflamed gingiva obtained from seven patients.

**Results:** Intensity of Fc $\gamma$ RIIb expression was significantly lower on B lymphocytes from periodontitis patients compared with those from healthy controls (Mann–Whitney U-test,  $P < 0.05$ ). Fc $\gamma$ RIIb expression was also confirmed in inflamed gingiva with periodontitis. Intense expression was observed on infiltrated B lymphocytes in the tissue section. Macrophages showed relatively lower intensity.

**Conclusions:** Expression levels of Fc $\gamma$ RIIb on B lymphocytes were lower in patients with periodontitis. This might be a cause of less effective inhibition of overactivation of B lymphocytes in the pathogenesis of periodontitis. Further studies are ongoing to elucidate the genetic background of this observation and the effect of cytokines on the expression of Fc $\gamma$ RIIb.

32

### Interleukin-12 levels in gingival crevicular fluid in periodontal disease

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**Background:** Periodontitis is a chronic inflammatory disease resulting from a complex interaction between specific bacteria and host's immune response. Complex cytokine network which mediates the immune response effects activation of macrophages and differentiation of CD4+ cells toward either Th1 or Th2 phenotypes. There is mounting evidence that an imbalance in Th1/Th2 response is critical in periodontal destruction. Interleukin-12 (IL-12), produced by monocytes, macrophages and neutrophils, is reported to play a major role in differentiation of Th1 cells. The aim of this study was to investigate IL-12 in GCF of periodontitis patients.

**Methods:** A total of 40 patients including 13 chronic periodontitis (CP), 14 gingivitis (G) and 14 healthy controls (C) were enrolled. The clinical periodontal indices were recorded. GCF samples were collected from six maxillary sites per patient and analysed for IL-12 by ELISA.

**Results:** Mean total amount of IL-12 in GCF was found to be 3.880 pg, 2.554 pg, and 2.228 pg for periodontitis, gingivitis and healthy controls respectively. The difference between CP and C group was statistically significant. ( $P = 0.0403$  Kruskal-Wallis) IL-12 levels showed a tendency to increase from periodontal health to periodontal disease.

**Conclusion:** These findings suggest that IL-12 stimulates Th1 response, and provides a link between innate and adaptive immunity, may play an important role in periodontal destruction.

33

### *Porphyromonas gingivalis* dihydroceramides induced apoptosis in huvec depends on caspase activation and disturbed mitochondrial integrity

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*Porphyromonas gingivalis* synthesizes complex lipids which are found in extracts from calculus, diseased gingival tissue, and atherosclerotic plaques. These lipids have been shown to promote inflammatory responses in gingival fibroblasts and peripheral

blood monocytes. We could show that *P. gingivalis* lipids provoke programmed cell death in human umbilical vein endothelial cells (HUVEC). In this study we investigated the pathways of endothelial apoptosis induced by extracted *P. gingivalis* lipids. Freshly isolated HUVEC were stimulated with 1 µg/mL *P. gingivalis* total lipid extract and 0.1 µg/mL purified lipid fractions. The stimulated cells were preincubated with different caspase inhibitors, forskolin (Sigma-Aldrich) and RP-73401 (Roth), or N-acetylcysteine (NAC) (Sigma-Aldrich). Cell death detection ELISA and Cytotoxicity detection kit (Roche), Western Blot analysis of apoptosis inducing factor (AIF), procaspases 3, 9 and caspase 6 were performed. The *P. gingivalis* phosphorylated dihydroceramide lipid fraction induced caspase dependent apoptosis in HUVEC. By measurement of LDH in the supernatant necrosis was excluded. Apoptosis was blocked by reducing oxidative stress with NAC as well as elevated intracellular cAMP after preincubation with forskolin and RP-73401 indicating mitochondrial involvement. Furthermore, AIF was released. These data support the hypothesis that *P. gingivalis* dihydroceramides may be important virulence factors of *P. gingivalis*. Supported by DFG GRK 325.

34

### Prevalence of periodontopathogens in Romanian population with periodontitis

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**Background:** Little recent information exists on periodontitis-associated subgingival microflora of the Romanian population. The occurrence, the interspecies relationships and the relationship with the basic clinical parameters for a group of five periodontal bacteria were determined in a Romanian study population.

**Methods:** Subgingival microbial pool samples were obtained from patients with localized (LagP = 18), generalized (GagP = 37) and chronic periodontitis (CP = 41). PCR was used to detect *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythensis*, *Treponema denticola* and *Prevotella intermedia*. Chi-square and Kruskal-Wallis tests were used to assess the differences between the three diagnostic groups.

**Results:** In all germs, excepted Aa, the detection frequency did not significantly differ between the CP, LAGP and GAGP groups. The detection frequency of Aa significantly differs, continuously increasing between the CP, LAGP and GAGP groups ( $P = 0.0003$ ). The mean proportions of Aa was higher in GAGP and LAGP than in CP respectively ( $P = 0.0001$ ). The mean proportion of Td was higher in LAGP than in CP ( $P = 0.01$ ).

**Conclusions:** Results demonstrate that the five periodontal pathogens analysed are strongly associated with Romanian periodontitis. In particular, Aa and PG are more significantly associated with generalized aggressive periodontitis ( $P < 0.0001$ , 0.027 respectively), while Td are more significantly associated with localized aggressive periodontitis ( $P = 0.006$ ).

35

### Immunohistochemical localization of PDGF-alpha receptors and basicFGF receptors in cyclosporin-a induced gingival overgrowth

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**Background:** Cyclosporine A (CSA) is a potent immunosuppressive drug used to prevent organ transplant rejection. A frequent side effect of CSA use is gingival overgrowth (GO). But, the mechanisms of the GO are unknown although it has been postulated that certain drugs play role in this pathology by modulating levels of various mediators and their receptors. The

aim of this study is to show the expression of certain growth factor receptors (PDGF- $\alpha$ -R and bFGF-R) in gingival biopsies of patients having CSA induced GO.

**Methods:** Specimens of both CSA induced GO and healthy gingiva were obtained during periodontal treatment, either gingivoplasty or crown-lengthening procedures. The samples were kept in paraffin blocks until the immunohistochemical staining. After paraffin sections were obtained, samples were stained with antibodies against PDGF- $\alpha$ -Rs and bFGF-Rs.

**Results:** Epithelium was stained strongly for bFGF-R both in CSA samples and in the control samples while PDGF- $\alpha$ -R staining was strong in control group but not in CSA group. For the connective tissue staining for both growth factors were strong in CSA patients while less staining were observed in control samples.

**Conclusion:** From these results, it can be assumed that CSA use increases the level of PDGF- $\alpha$ -R and bFGF-R in the connective tissue but decreases in the epithelial layer of the gingiva causing the thinning of the gingival epithelium resulting in, comparatively, more edematous and reddish appearance among other GOs.

36

### The role of Th2 cytokines on nitric oxide and arginase production in periodontal tissues of chronic periodontitis patients

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The arginine-nitric oxide (NO) pathway in chronic periodontitis has been shown in previous studies. The presence of NO release at the periodontal tissues may have a role in selecting the type of T-cell response. The aims of this study were: (i) to compare the salivary and gingival tissue arginase and NO production between patients with chronic periodontitis and healthy controls, (ii) to investigate the role of Th2 cytokines on NO and arginase production. Our study included 18 chronic periodontitis patients and 10 healthy controls. Gingival biopsies and unstimulated saliva samples were obtained at baseline, at flap surgery and 2 months postoperatively from the same site. GCF samples were obtained from teeth where gingival biopsies performed. ELISA was employed to determine the amount of Th2 cytokines; IL-10 and IL-4 in GCF. The levels of NO were determined with a nonenzymatic NO kit. Specific arginase activity was determined spectrophotometrically. NO was measured higher in saliva of chronic periodontitis patients compared with that in gingival tissue. Similar amounts of arginase were observed in both gingival tissue and saliva. Periodontal treatment did not have an impact on salivary NO and arginase levels, however, tissue NO levels elevated significantly after therapy, whereas arginase levels dropped dramatically. While IL-10 was observed to inhibit NO production, IL-4 seemed not to down-regulate NO. Th2 cytokines might have a role in NO-arginase involvement in periodontal disease mechanisms.

37

### The effect of some sugar-free chewing-gums on bacterial plaque formation. a comparative *in vivo* investigation

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**Background:** The use of sugar-free chewing gums (SCs) significantly inhibits plaque formation on occlusal surfaces of the teeth, whereas a similar effect cannot be found on smooth surfaces. The aim of this comparative *in vivo* investigation was to assess whether the SCs containing additives such as zinc gluconate, silicon dioxide or lactoperoxidase and glucose-oxidase inhibit plaque formation more effectively than the additive-free SCs.



**Materials and methods:** The study was a double-masked, randomized 4 × 4 Latin square cross-over design, involving 12 volunteers in a 4-day plaque regrowth model. On day 1, subjects received professional prophylaxis, suspended oral hygiene measures, and commenced chewing their allocated SCs. Gum chewing was one piece chewed for 30 minutes 4 times per day. On day 5, subjects were scored for plaque after disclosing from smooth and occlusal surfaces.

**Results:** There were no significant differences in antiplaque activity of the SCs tested, neither for the smooth, nor for the occlusal surfaces ( $P = 0.83$  and  $P = 0.42$ , respectively). Similar results were obtained for the anterior and posterior sites of the smooth surfaces ( $P > 0.05$ ), and for the lower and upper sites of the occlusal surfaces ( $P = 0.45$  and  $P = 0.53$ , respectively).

**Conclusions:** The SCs containing additives has not shown a higher antiplaque activity than the additive-free ones and should be considered neither effective aids for gingivitis prevention nor useful adjuncts to mechanical oral hygiene.

38

### The effects of an amine fluoride/stannous fluoride and an essential oil mouthrinse on supragingival plaque regrowth

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**Background:** The problem associated with side effects of chlorhexidine (CHX) has stimulated the search for alternative antiplaque agents, such as amine fluoride/stannous fluoride (ASF) and essential oils (EO). The aim of this study was to investigate the plaque inhibitory effects of two commercially available mouthrinses containing ASF and EO, respectively.

**Materials and methods:** The study was an observer-masked, randomized 5 × 5 Latin square cross-over design, involving 15 volunteers in a 4-day plaque regrowth model. A 0.12% CHX rinse and a saline solution served as positive and negative controls, respectively. On day 1, subjects received professional prophylaxis, suspended oral hygiene measures, and commenced rinsing with their allocated rinses. On day 5, subjects were scored for disclosed

plaque. The ASF rinse was tested with two dosages: 10 mL and 20 mL (ASF-10 and ASF-20, respectively).

**Results:** The ASF and EO rinses showed a significant inhibition of plaque regrowth in comparison to saline ( $P < 0.0001$ ), but the lowest plaque indices were obtained with the CHX product ( $P < 0.01$ ). There were no significant differences among products containing ASF-10, ASF-20, and EO ( $P > 0.05$ ). There was no correlation between the occurrence of side effects and the use of a particular rinse product ( $P > 0.2$ ).

**Conclusions:** The ASF- and EO-containing mouthrinses exerted effective and quite similar plaque inhibition. The two regimens of use tested for ASF rinse did not differ in antiplaque activity.

39

### Serum IgG antibody to periodontopathogens and its association with bacterial colonization

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**Objectives:** To define serum IgG antibody response to periodontal diseased and healthy group and its relationship with bacterial colonization.

**Methods:** Thirty subjects with advanced chronic periodontitis and 30 healthy controls who were ethically, gender and age-matched were equally selected in numbers from each race. Serum IgG antibody levels to *Pg*, *Tf*, *Pi* and *Aa* were assessed by enzyme-linked immunosorbant assay (ELISA).

**Results:** Serum IgG antibody responses to all the four periodontopathogens were found both in the diseased and healthy subjects. Diseased group had significantly higher antibody titer as compared to healthy controls. Serum antibody responses to *Pg*, *Tf* and *Aa* were higher in the presence of these bacteria.

**Conclusions** Multiple bacterial types may play a role in periodontal disease destruction and a higher systemic antibody response exists reflective of subgingival colonization at diseased sites indicating either predominance in colonization by certain pathogens or either enhanced or poor response to their colonization.

Study supported by Vote F 0131/2003C.

## Posters: Pre-clinical models

40

### Effect of ibuprofen on bone proliferation

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In dentistry, non-steroidal anti-inflammatories (NSAIDs) as ibuprofen are usually used after a bone surgery because these drugs exhibit favourable anti-inflammatory, analgesic and antipyretic properties. There is increasing evidence that NSAIDs drug like ibuprofen, can adversely affect bone repair. Studies *in vitro* have shown that NSAIDs inhibit osteoblast proliferation and stimulate protein synthesis, but either animal tissues or cell lines. The purpose of the present study was to obtain further insight into the effect of ibuprofen on human osteoblast-like cells proliferation.

**Methods:** Osteoblasts were obtained from samples of human bone sections obtained in the course of third molar surgery. Human osteoblast lines were cultured with two different concentration of ibuprofen (5 mM and 25 mM). Osteoblast proliferation was examined by count of cell number after 24 and 48 hours.

**Results:** After 24 hours the experiments demonstrated a decrease in cell proliferation in cultures incubated with 5 mM and 25 mM concentrations of ibuprofen compared with controls ( $P = 0.001$ ,  $P = 0.002$  respectively). The results weren't statistically significant after 48 hours.

**Conclusions:** Our results suggest that ibuprofen reduces osteoblast proliferation. So that in bone healing and alternative analgesic is preferable where an increase in osteoblast number is important. More studies are necessary to determine the effects of NSAIDs on other cell parameters involved in osseointegration like cellular adhesion.

41

### The effect of diclofenac on MG-63 cells proliferation

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Osteosarcoma is the most common primary malignant bone tumour. MG-63 is a osteosarcoma cell line that expresses

**COX-2.** COX-2 inhibitors exert antitumour activity via COX-2-dependent and independent pathway. Meloxicam is a nonsteroidal anti-inflammatory drugs (NSAIDs) which acts as a COX-2 inhibitor. Meloxicam has inhibitory effects on osteosarcoma cell growth and invasiveness. The aim of this study was to investigate the *in vitro* effects of two different concentrations of diclofenac, another NSAID with a selective COX-2 inhibition, on osteosarcoma cell line (MG-63) proliferation.

**Methods:** MG-63 line were incubated with diclofenac at different concentrations: 1-10-100  $\mu\text{M}$ . To study the proliferation included the count of cell number for proliferation, after 24 and 48 hours of treated.

**Results:** A statistically significant difference between test and control cultures was observed after 24 hours of incubation; for concentration 1  $\mu\text{M}$  ( $P < 0.04$ ) and for 10  $\mu\text{M}$  ( $P < 0.02$ ). Cells number in culture incubated with diclofenac at 100  $\mu\text{M}$ , declined after 48 hours significantly too ( $P < 0.01$ ).

**Conclusions:** These results demonstrate that diclofenac reduces osteosarcoma cell line proliferation. New experiments should be realized in order to investigate the anti-carcinogenic potential of diclofenac as is described with other NSAIDs as Meloxicam in MG-63 and other tumoural cell lines. By other hand our results suggest that, care should be taken when administering NSAIDs to patients with skeletal problems.

42

### Types I and III collagen distribution in healthy and regenerated bone and periodontal ligament. histomorphometric study in dogs.

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This study evaluated the distribution of types I and III collagen in healthy and regenerated bone and periodontal ligament. The lower P3 and P4 of five dogs, bilaterally, were used. Buccal furcation lesions were surgically created and chronified. After that, full flaps were elevated and expanded polytetrafluoroethylene membranes were adapted, sutured and recovered by the flaps. Two membranes were removed 2 weeks and the other two 4 weeks after surgery. The dogs were euthanized by 12 weeks following placement of the membranes. P3 and P4, as well as P2 (health control teeth), and their periodontal tissues were removed and histologically processed. The sections were stained with Picrosirius Red for Collagen Quantification (COLQ), comparing, for both bone and periodontal ligament, the native (nearby the regenerated area), the regenerated (formed in the defect area) and the control (healthy) tissues. The results of COLQ for bone showed that the amount of type III collagen was higher (Tukeys's Multiple Comparison,  $P < 0.05$ ) in native bone, which was under higher functional demand during the healing period. For periodontal ligament, COLQ for type I collagen showed statistically significant differences between regenerated and control tissues. The membrane removal with 2 or 4 weeks postoperatively did not influence the collagen composition of bone or periodontal ligament. It was concluded that there are differences in collagen composition among the regenerated, native and control tissues.

43

### Effect of local antimicrobial agents on palatal wound healing by secondary intention. a histomorphometric study in rats

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**Aim:** Harvesting of gingival graft creates excisional wound exposed to direct contact with the antimicrobial agents which

display both bactericidal activity and potential cytotoxicity. The aim of the study was to assess the influence of local antimicrobial agents on wound healing by secondary intention.

**Materials and methods:** Excisional wounds, 5 mm in diameter were made in the center of the palate of Wistar rats. In four experimental groups, six rats each, chlorhexidine digluconate (CHX) 0.1% solution, 1% CHX gel, phenolic compounds solution and amine/stannous fluoride solution and in control group saline solution were applied daily. Wound diameter and epithelialization rate were determined photographically and histologically at 3,7,14, and 21 days post-surgery.

**Results:** The mean diameter of the circumscribed defects and the area not covered by epithelium decreased significantly and positively by the time ( $P < 0.001$ ) in experimental and control groups. At day 14 the highest residual wound area was measured in the saline group (70%) and the smallest in the 1% CHX gel group (33%) with 20% of the specimens treated with 1%CHX gel presenting complete epithelialization of the wound as compared to none in the other tested and control groups.

**Conclusions:** The best epithelialization rate was achieved using 1% CHX gel ( $P = 0.03$ ). Each of the tested anti-plaque agents when applied on excisional wound with epithelial and connective tissue deficiency does not affect negatively the wound closure.

44

### Skeletal phenotype of lysyl oxidase knockout mice

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Lysyl oxidase is essential for the accumulation of cross-linked and insoluble collagen, which provides mechanical integrity, rigidity, and strength in bone tissue. The role of lysyl oxidase in osteogenesis is not well known. However, our previous data suggest a precise regulation of lysyl oxidase in osteoblast differentiation. The aim of our study was to investigate the effects of lysyl oxidase deficiency on skeletal tissue and matrix mineralisation *in vivo*. We analysed E18.5 homozygous and heterozygous lysyl oxidase knockout mice as well as wild type mice (C57 BL/6) by histological means, three-dimensional reconstruction and Environmental Scanning Electron-microscopy (ESEM). Our results demonstrated overall thinner, more fragile craniofacial bones in the homozygous animals. Three-dimensional reconstructions showed major morphological differences in the bony mandible of the mutant mice compared to the Wild type. Quantitative distance measurements showed longer mandibles with thinner cranio-caudal dimensions and decreased bone volumes up to 21%. ESEM analyses revealed in the upper and lower jaws of the homozygous animals less trabeculae which were more massive and with lower connectivity. In addition, thinner and fragmented calvarial bony surfaces were noted. In conclusion, our findings indicate that lysyl oxidase may be crucial for the development of proper craniofacial bone morphology.

45

### Dose-dependent effects of nicotine in human osteoblastic cell cultures

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**Introduction/Aims:** Nicotine appears to play a significant role in the adverse effects of tobacco smoking in the bone tissue. This work describes the effect of nicotine in the proliferation/differentiation behaviour of human osteoblastic bone marrow cells in a concentration range representative of plasma and salivary levels found in the average smoker.

**Materials and methods:** Human bone marrow cells were cultured, for 28 days in the absence and in the presence of nicotine – 10 ng/mL to 1 mg/mL, in experimental conditions that favour the

proliferation and differentiation of osteoblastic cells. Cultures were characterised for cell morphology, cell proliferation/viability, alkaline phosphatase (ALP) activity and matrix mineralization.

**Results:** The exposure to 10 ng/mL nicotine (plasmatic level) did not significantly affect the cell behaviour. The presence of salivary levels, 0.05 to 1 mg/mL, caused dose-dependent effects: an increase in the cell proliferation, ALP activity and matrix mineralization in the range 0.05 to 0.2 mg/mL, an initial inhibitory effect followed by a progressive recovery in the presence of 0.3 mg/mL and a dose-dependent inhibitory effect for concentrations similar and higher than 0.4 mg/mL. The presence of 1 mg/mL resulted in cell death within few days.

**Conclusions:** Nicotine causes dose-dependent effects in the proliferation/differentiation of human osteoblastic cells. Plasma levels do not significantly affect cell behaviour. Salivary levels have mixed effects.

46

#### The effect of cyclosporin-A on alveolar bone of rats subjected to experimental periodontal disease

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**Background:** Cyclosporine-A (CsA), broadly used in organ transplantation, may contribute to pathogenesis of osteoporosis. The aim of this study was to investigate effects of CsA on alveolar bone of rats subjected to experimental periodontal disease using biochemical, radiographic, and histometric analysis.

**Materials and methods:** Forty Wistar rats were divided into four equal groups: Group1 (Control), Group2 (CsA was injected subcutaneously in a daily dose of 10 mg/kg), Group3 (Ligature was placed around the mandibular molars), Group4 (Ligature + CsA). After 60 days; rats were decapitated, serum alkaline phosphatase and calcium levels were measured. Radiographic alveolar bone loss (ABL), histometric ABL, and percentage of new alveolar bone formation (NABF%) were determined on mandibular molars.

**Results:** Significant increase in alkaline phosphatase ( $P < 0.001$ ), significant decrease in calcium ( $P < 0.01$ ) levels were observed in ligated Group3 compared to Group1 whereas, no significant difference was found between ligated and CsA-treated Group 4 and Group 1 ( $P > 0.05$ ). Radiographic and histometric ABL were significantly less ( $P < 0.001$ ), NABF% was significantly greater ( $P < 0.05$ ) in Group4 than in Group3. No significant difference in any of the parameters between Group2 and Group1 was found.

**Conclusions:** The results showed that CsA therapy diminished resorption and induced formation of alveolar bone. It can be concluded that inhibition of immune system by CsA may decrease the periodontal breakdown.

47

#### The morphometric analysis of cyclosporin A induced gingival overgrowth in rats

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**Aim:** Cyclosporin A (CycA) induced gingival overgrowth depends on dose, serum level and drug administration. The objective of this investigation was to examine the effects of CycA on gingival tissue in an 8-week period by using morphometric analysis.

**Materials and methods:** Eighty Sprague-Dawley rats were randomly assigned into two groups of 40 animals each. The test group animals were subcutaneously injected daily 10mg/kg body weight of CycA. The control group animals were injected similarly with saline (0.9%NaCl). In the 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup> weeks, 10 animals in each group were sacrificed. Gingival thickness

measurements (mm) were obtained from the disto-buccal, mid-buccal and mesio-buccal sites of second molar teeth by using stereomicroscopy.

**Results:** In the 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup> weeks, the test group gingival thickness scores were significantly higher than those in the control group ( $P < 0.05$ ). In the 2<sup>nd</sup> week, the difference between groups was not statistically significant ( $P > 0.05$ ).

**Conclusion:** The morphometric analysis results of this study demonstrated that systemic administration of CycA for an 8-week period caused significant gingival overgrowth and this effect was parallel with the duration of medication.

This study was supported partly by Novartis Pharma AG and Selcuk University Scientific Research Fund.

48

#### Effect of cyclosporin-A on Serum Calcium and Alkaline Phosphatase levels in rats with experimental periodontitis

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**Aim:** The aim of this investigation was to evaluate the effect of cyclosporin A (CycA) on serum Calcium (Ca) and Alkaline Phosphatase (ALP) levels in rats with experimental periodontitis. The data presented here were derived from an experimental study in which the effect of CycA on the rat gingival tissue was examined.

**Materials and methods:** In 80 Sprague-Dawley-rats, experimental periodontitis were induced by placing a silk suture around the cervix of the mandibular first molars. The animals were randomly distributed into test and control groups of forty animals in each. Test group animals were injected subcutaneously in a daily dose of CycA 10 mg/kg body weight, while the controls received subcutaneous saline injection throughout the study period. Serum Ca (mg/dL) and ALP (UL/mL) levels were determined at the time periods, 2, 4, 6 and 8 weeks, by using spectrophotometric analysis.

**Results:** Serum Ca levels in test group were significantly lower than in the control group at 4, 6 and 8 weeks ( $P < 0.05$ ). However, there were no statistically significant differences between the groups in terms of the serum ALP levels ( $P > 0.05$ ).

**Conclusion:** Within the limits of this study, we suggest that CycA may have an effect to decrease the initial alveolar bone destruction in rats.

This study was supported partly by Novartis Pharma AG and Selcuk University Scientific Research Fund.

49

#### Stem cells in human periodontal ligament cultures

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**Objectives:** Recently stem cells have been observed in human dental tissues including PDL. Our objectives were to identify and characterize postnatal stem cells in primary PDL cultures.

**Methods:** Cells were derived from PDL of human extracted third molars. PDL tissue was separated from root surfaces using sterile scalpels. PDL tissue was digested in a collagenase type I and dispase containing solution. PDL cells were cultured using  $\alpha$ -MEM supplemented with FCS and incubated at 37 °C in 5% CO<sub>2</sub>. Cell viability of the PDL cultures was estimated by MTT-assay. To measure colony-forming efficiency, 14 day old cultures were fixed with 4% formalin, and then stained with 2% Giemsa. Mesenchymal stem cell marker STRO-1 immunohistochemistry and FACS analysis was performed on PDL cell cultures.

**Results:** Cell cultures were successfully established from PDL, then cultivated for up to 25 passages. Cell cultures showed typical

fibroblast-like morphology, demonstrating clonogenic activity. The colony-forming efficiency level of approximately  $10^5$  cells was 20–30 colonies. In the PDL cultures 8–10% of the cells showed STRO-1 immunoreactivity.

**Conclusions:** We established primary cell cultures from human PDL. The cultures contained cells capable of colony-formation. STRO-1 immunoreactivity indicates the steady level of stem cells in the primary cultures for up to 25 passages. These findings open a new avenue in our research aiming the application of PDL originated stem cells in periodontal disease.

50

### Moxifloxacin as an alternative antibiotic in treatment of *Porphyromonas gingivalis*-associated periodontitis - conclusions from *in vitro* studies

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**Objectives:** The aim was to evaluate experiments about the *in vitro* activity of moxifloxacin against *Porphyromonas gingivalis* (P.g.).  
**Methods:** Minimal Inhibitory Concentration (MIC) of moxifloxacin against 16 strains of P.g. was determined by Etest. The spontaneous mutation rate and the induction of resistant strains by the 0.25fold MIC of the antibiotic were determined. To find the target of resistance the *gyrA*- and *gyrB*-genes were sequenced. Finally the efficacy of moxifloxacin to P. g. ATCC 33277 within biofilms or within epithelial cells (KB cells) was evaluated.

**Results:** Moxifloxacin had very low MIC values (0.006–0.032 mg/L), but subinhibitory concentrations induced very fast mutations. The spontaneous mutation rate was up to  $5 \times 10^{-8}$  after the two-fold MIC and  $1.2 \times 10^{-8}$  after the eight-fold MIC. The mutants exhibited a resistance up to  $\geq 32$  mg/L. All mutants bore Ser-83→Phe substitution in *gyrA*. The 5-fold MIC eliminated P.g. ATCC 33277 within biofilms after 24 h, and the 100-fold MIC was able to kill all intracellular P.g.

**Conclusions:** Moxifloxacin showed a very good activity against planktonic P. g. and P.g. within biofilms. In high concentrations it was also efficient to intracellular bacteria. A rapid development of resistance was observed under *in vitro* conditions. Moxifloxacin might be an alternative in the antibiotic treatment of P. gingivalis-associated periodontitis, but clinical studies should focus not only on improvement of clinical parameters but also on occurrence of resistant strains.

51

### The effects on enamel matrix derivatives (EMD) on connective tissue growth factor (CTGF) expression via TGF- $\beta$ signaling pathway in osteoblastic cells

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Enamel matrix proteins (EMD) stimulate the production & release of growth factors such as TGF- $\beta$ . CTGF is a downstream mediator of TGF- $\beta$  & mediates cell growth stimulatory actions of TGF- $\beta$ . Interactions between EMD and TGF- $\beta$  on osteoblastic cell growth & bone formation have been described but whether EMD is affecting CTGF expression is still unknown. Our study investigates the effects of EMD on CTGF expression in osteoblasts & whether EMD-induced CTGF expression is modulated by TGF- $\beta$  signaling pathway. We also examine if CTGF plays a role in EMD-induced cell proliferation in osteoblasts. Human osteoblasts (SaOS-2) were treated with 25–100  $\mu$ g/mL EMD, with & without TGF- $\beta$  inhibitors. TGF- $\beta$ -treated cells served as positive control. CTGF steady-state mRNA expression was detected by RT-PCR. CTGF protein levels were assayed by Western Blot & quantitated

densitometrically. Cell proliferation was determined by BrdU assay, with & without CTGF inhibitors. Western blot & RT-PCR analysis demonstrated a dose- and time- dependent increase of CTGF expression by EMD. In the presence of TGF- $\beta$  inhibitors, CTGF expression of EMD-stimulated cells was significantly reduced, indicating a modulation of CTGF via TGF- $\beta$  pathway. The BrdU assay revealed significant increase of the cell proliferation exceeding 2.5-fold ( $P < 0.01$ ) in EMD & TGF- $\beta$  stimulated cells but no significant effect when CTGF inhibitors were added. We can conclude that EMD increases CTGF expression via TGF- $\beta$  signaling pathway in osteoblasts.

52

### Effect of experimental hemolytic anemia on peri-implant bone tissue

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Anemia is a disease resulting from a decrease in the normal amount of circulating hemoglobin (Hb). In previous studies we observed that hemolytic anemia causes alterations in bone healing and remodeling. The aim of the present study was to perform a histological and histomorphometric evaluation of peri-implant bone tissue under experimental hemolytic anemia. Male Wistar rats (60  $\pm$  7 g) were anesthetized and a laminar Ti implant was placed in the tibiae. Animals were assigned to one of three groups: GI (Control) ( $n$ :10); GII (Mild to Moderate Anemia, Hb10–12 g/dL) ( $n$ :15); GIII (Severe Anemia, Hb < 10 g/dL) ( $n$ :15). On day 15, the anemia groups were injected with phenylhydrazine every 48 h; control animals were injected with saline. Guidelines of the NIH were observed. In all animals, body weight (b.w.), hematocrit (Hct), and Hb were recorded every 48 h. Animals were killed 30 day after the onset of the experiment. Tibiae were resected, fixed, radiographed, and embedded in methylmethacrylate. Ground sections were stained with toluidine blue. Animals of GII and GIII exhibited a reduction in b.w. (20% and 39%), Hct (20% and 43%), Hb (31% and 51%) respectively as compared to control. Histomorphometric evaluation revealed a statistically significant reduction in peri-implant bone volume (71% and 82%) and percentage of bone to implant contact (66% and 87%) in GII and GIII respectively. Hemolytic anemia and/or associated factors cause qualitative and quantitative alterations in the peri-implant bone tissue. CONICET PIP 6042 UBA O020.

53

### Experimental bony defect models in rats: which are critical-size defects? a histologic study

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A variety of biomaterials have been placed in bony defects in order to facilitate and/or promote bone regeneration. A critical-size defect (CSD) is the ideal model with which to evaluate their regenerative potential. However, studies have reported conflicting results regarding the size of CSD's in different experimental models in rats. The purpose of this study was to histologically analyse bone healing in surgically created defects in rat calvaria, tibia and mandibular angles. 48 rats were divided into three groups: Group 1 - a 5 mm diameter calvarial defect was made; Group 2 - a 4 mm diameter tibial defect was made; Group 3 - a 5 mm diameter mandibular angle defect was made. Each group was subdivided ( $n = 8$ ) for euthanasia at either 30 or 90 days post-operative. Histometric and histologic analyses were performed. Newly formed bone area (NFBA) was calculated as percentage of total area of original defect. Data were statistically analysed (ANOVA, Tukey,  $P < 0.05$ ). In Groups 1 and 3, no defect completely regenerated

with bone at either 30 or 90 days post-op. In Group 2, complete bone regeneration was seen in five animals ( $n = 8$ ) at 30 days and four animals ( $n = 5$ ) at 90 days post-op. three animals were lost due to tibial fractures. Group 2 had significantly more bone formation than Groups 1 and 3 at both periods of analysis. It can be concluded that a 5 mm diameter calvarial or mandibular angle defect is a CSD, while a 4 mm diameter tibial defect is not.

54

### Three-dimensional type I collagen cell culture system for the study of periodontal ligament cell differentiation

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Three-dimensional (3-D) type I collagen cell culture systems composed of reconstituted collagen fibers are able to support short- and long- growth of various cell types, including endothelial cells, osteoblasts and fibroblasts and to sustain or even enhance cell differentiation, *in vitro*. The aim of this study was to investigate phenotypic differences arising when periodontal ligament (PDL) cells populations were cultured in both mono-layer and 3-D type I collagen cell culture systems. PDL cells were isolated from the mid-root of three maxillary premolars extracted for orthodontic reasons. Freshly isolated collagen fibers depleted of telopeptides and proteoglycans containing endogenous and exogenous collagen cross-links were used to form collagen gels. PDL cells were allowed to grow in collagen gels and in mono-layer cultures for 4 to 7 days. Immunohistochemical analysis of the expression profile of Vimentin (indicator of mesenchymal origin) and  $\alpha$ -Smooth Muscle Actin ( $\alpha$ -SMA, indicator of fibroblastic differentiation) was used to assess phenotypic variations in the different culture media. Mono-layer cells cultures were found to express  $\alpha$ -SMA and vimentin while cells cultured in the 3-D collagen system expressed only vimentin. In the 3-D type I collagen system, PDL cells differentiate in a different manner than in mono-layer cell cultures. Such a culture system may act as an important experimental tool to elucidate mechanisms of growth and differentiation of PDL tissues.

55

### Resistance of the implant/abutment morphology to lateral force

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**Aim:** The aim of *in vitro* study was to evaluate the influence of different implant - abutment connection (I/A) morphology resistance to lateral force. Three Types of I/A connection were evaluated. Type A: external hexagon in implant; Type B: internal conical surface; Type C: internal hexagon.

**Method:** Ten I/A pcs. of each Type were evaluated. The implants were firmly screwed in to testing device. The tip of the machine was pressed on the abutment from lateral side at the level of I/A connection. The force was increasing from 0 N, through the irreversible plastic deformation, till breaking the abutment screw.

**Result:** The average breaking force for the Type A was 650.6 N (SD = 85.7), for the Type B 814.7 N (SD = 72.7), for the Type C 658.7 N (SD = 49.2). The mean plastic deformation of the screw before breaking for Type A was 2.09 mm (SD = 0.44), for Type B 2.16 mm (SD = 0.26), for Type C 1.19 mm (SD = 0.09). The Type B I/A show significant ( $P = 0.01$ ) higher breaking force for the abutment screw than the Type A and Type C. The irreversible deformation of the abutment screw of Type A and Type B I/A morphology show significant higher rate ( $P = 0.01$ ) as the Type C.

**Conclusion:** The Type B I/A connection morphology show improved resistance to lateral force compared to the type A and C.

56

### Evaluation of dental implants used as orthodontic anchors: a comparison of histomorphometric and finite element analysis results

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**Aim:** The aim of this study was evaluation of dental implant used as orthodontic anchors by means of comparison of histomorphometric and finite element analysis results.

**Materials and methods:** Eight SLA implants with a 4.1 mm diameter and length of 10 mm were placed in the maxilla and mandible 8 dogs after 12-week healing period after extraction. Superelastic nickel-titanium coil springs were activated between implants and the canines, producing a force of 200 g (2 N). Two unloaded implants served as controls. A two-dimensional finite element analyses of the implant-abutment complex are carried out using FEA software.

**Results:** Histologic analysis showed a corticalization of bone trabeculae, thicker at the loaded than at the unloaded implants. New bone formation at the level of the crest was slightly superior in the test implants. A difference between the tension and compression areas could not be observed in the test implants. The mean bone-implant contact values of the test implants for the maxilla and mandible were 40.23% and 49.33%. In the control implants, the bone-implant contact value was 67.91% for the maxilla and 49.23% for the mandible. The finite element analysis results indicated that the maximum stresses located around the neck of the implant, in cortical bone.

**Discussion/Conclusion:** According to histomorphometric and finite element analysis results stress areas should be preserved clinically in order to maintain bone-implant interface structurally and functionally.

57

### Periodontal wound healing following regenerative procedures in furcation degree III defects: histomorphometric outcomes

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**Objectives:** To describe the periodontal healing following different regenerative modalities in degree III furcation defects.

**Materials and Methods:** Degree III furcation defects were surgically created at teeth 36, 37, 46 and 47 in three monkeys. Spontaneous healing of the defects was prevented by placing impression material into the defects. After 6 weeks, four treatment modalities were performed: (i) Emdogain (EMD), (ii) GTR (iii) EMD and GTR (iv) coronally repositioned flap (control). In all groups root surfaces were conditioned with 24% EDTA gel. After 5 months of healing, 3 central sections 100  $\mu$ m apart, were used for histomorphometric analysis. Measurements were performed at six zones on each tooth. Zones 1–4 were within the furcation area. (1): At the level apically to the notch (2): At the level of the notch (3): At the most coronal level of regenerated bone between the notch and the fornix (4): At the level of the fornix. Zones 5–6 were on the pristine external surface of the root. (5): on the level of the fornix. (6): on the level of the notch.

**Results:** In all specimens, new cementum 30–60  $\mu$ m wide, with inserting collagen fibres was formed. Its linear extension within the defects varies according to the treatment modality. Regeneration

extending in the furcation fornix was observed only in the GTR or EMD and GTR treated specimens.

**Conclusion:** The predictability of regeneration in furcation degree III defects depends mainly on the presence or absence of barrier membranes (GTR).

58

### Effect of chlorhexidine/thymol and fluoride varnishes on dental biofilm formation *in vitro*

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The aim of the present study was to evaluate the effect of two varnishes containing either chlorhexidine/thymol (CHX/T) or fluoride (F) on a biofilm model *in vitro*. Hydroxyapatite discs were coated with varnish and exposed for various times up to 20 h to a bacterial suspension containing *A. naeslundii*, *F. nucleatum*, *S. oralis*, and *V. dispar*. Uncoated discs served as controls. In some experiments discs were immersed in saline for 0, 3, 7 or 14 days before incubation with bacteria. Bacterial growth and biofilm development were monitored by culture and by scanning electron microscopy (SEM). Bacterial vitality was examined by live/dead fluorescence staining. In the CHX/T-treated group, the total number of bacteria was significantly lower than in controls at each observation time. SEM observations showed that bacterial colonization was delayed compared to control discs. In the F-treated group, the total number of bacteria did not differ from control discs although the number of *S. oralis* was lower compared to controls. Bacterial vitality in the CHX/T (86.8 %) and the F (78.6%) group did not differ from that in controls (74.9%). Total numbers of bacteria on CHX/T-treated discs immersed into saline for 3, 5 and 14 days were significantly higher than non-immersed discs. Bacterial attachment and subsequent biofilm development were inhibited on CHX/T varnish-coated discs but not F-treated discs. The effect of CHX/T varnish decreased following immersion of varnish-coated discs in saline.

59

### *In vitro* effect of positive and negative pressure on the permeability of a self-etch adhesive used to treat dentinal sensitivity

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Based on the hydrodynamic theory, adhesive systems have been used to treat dentinal sensitivity (CDS). *In vitro* studies show successful results while clinical studies not. The aim of study is to compare the effect of positive pressure on the permeability of a self-etch adhesive (AdheSE) proposed for treatment of CDS.

**Materials and methods:** Sixty dentin discs obtained from molars were divided into two groups: G1 (positive pressure) and G2 (negative pressure). Discs were mounted in a perfusion device based on the Pashley's system. Our device allows control pressure of perfusion fluid during the whole process. I disk of each perfusion was studied under scanning electron microscopy to check acid etch opened dentine tubules. In G1 AdheSE was applied keeping a positive pressure of perfusion fluid during the whole process. In G2 no pressure of the perfusion fluid was allowed until it was applied. Permeability was measured after 1 and 2 hours. Chi Square Test and *t*-test statically analysed the results.

**Results:** G2 showed significant lower dentinal perfusion rates and a greater amount of tubular occlusion than G1 in all cases.

**Discussion:** Recently studies have used a tubular positive pressure and found the presence of 'Water Tree' that can justify the postoperative sensitivity. These results indicate that positive

pressure should be incorporated in the new '*in vitro*' studies of CDS to simulate more accurately *in vivo* conditions.

**Conclusion:** Positive pressure significantly affects the rate of dentinal perfusion.

60

### Comparison of naturally occurring and ligature induced peri-implantitis bone defects in humans and dogs

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The aim of the present study was to compare naturally occurring and ligature induced peri-implantitis bone defects in humans and dogs. Twenty-four patients undergoing bone augmentation procedures due to advanced peri-implantitis were included in this study ( $n = 40$  implants). Furthermore, peri-implantitis was induced by ligature placement and plaque accumulation in five beagle dogs ( $n = 15$  implants). The ligatures were removed when about 30% of the initial bone was lost. During open flap surgery, configuration and defect characteristics of the peri-implant bone loss were recorded in both humans and dogs. Open flap surgery generally revealed two different classes of peri-implant bone defects. While Class I defects featured well-defined intrabony components, Class II defects were characterized by consistent horizontal bone loss. The allocation of intrabony components of Class I defects regarding the implant body allowed a subdivision of five different configurations (Classes Ia-e). In particular, human defects were most frequently Class Ie (55.3%), followed by Ib (15.8%), Ic (13.3%), Id (10.2%), and Ia (5.4%). Similarly, bone defects in dogs were also most frequently Class Ie (86.6%), while merely two out of 15 defects were Classes Ia and Ic (6.7%, respectively). Within the limits of the present study, it might be concluded that configurations and sizes of ligature-induced peri-implantitis bone defects in dogs seemed to resemble naturally occurring lesions in humans.

61

### Effects of bisphosphonate and doxycycline on alveolar bone resorption in rats with experimental periodontitis

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Disodium chlodronate, a bisphosphonate that is a potent inhibitor of osteoclast-mediated bone resorption is clinically used in the treatment of metabolic bone diseases associated with bone resorption. Doxycycline has been widely used in periodontal treatment for its antimicrobial and anti-enzymatic effects. The aim of this study was to compare the effects of doxycycline and bisphosphonates to bone loss from prophylactic and curative aspects in an experimental periodontitis. Thirty-six Wistar rats were used. Maxillary left molars ligated with silk suture to induce periodontitis. The contralateral side served as a control group. The rats were divided into six groups: (1) prophylactic chlodronate, (2) prophylactic doxycycline, (3) prophylactic control, (4) curative chlodronate, (5) curative doxycycline, (6) curative control. In prophylactic groups, drugs were injected for 7 days, starting at day 0 and until day 7, while in curative groups the drugs were injected from day 5 until day 11. At designated times, histological evaluations were accomplished. The results showed that bone loss in the doxycycline and chlodronate groups were statistically less than control group. Prophylactic groups were most effective in reducing bone loss. No detectable statistically significant differences between the doxycycline and chlodronate groups. This study suggests that local application of doxycycline and chlodronate can be used as an adjunct in periodontal therapy for reducing bone resorption.

62

### Characterization of ofloxacin encapsulated chitosan microspheres for periodontal applications

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The objectives of this study were to prepare ofloxacin (OFL) encapsulated chitosan microspheres and to investigate the effect of the formulation parameters on the properties of the prepared microspheres. *In vitro* and *in vivo* release profiles from periodontal pockets in aggressive periodontitis patients were further compared. OFL encapsulated chitosan microspheres were prepared by precipitation method. The effect of chitosan, drug and cross-linking agent concentrations on particle size, encapsulation capacity and release properties of the microspheres were the investigated parameters. *In vitro* OFL release from microspheres was performed in PBS (pH 7.4) at  $37 \pm 0.1^\circ\text{C}$  and the released amount of OFL was analysed at 1, 3, 5 hours and 1, 3 and 5 days spectrophotometrically. Microspheres were applied into 4 periodontal pockets ( $\geq 5$  mm and + bleeding) in each of six patients. Gingival crevicular fluid samples were taken at 1, 3 and 5 hours and at 1, 3 and 5 days and the samples were evaluated with HPLC. The encapsulation efficacy of the microspheres, with the size range of 1.05 and 2.08  $\mu\text{m}$ , changed between 48.8 and 89.2 %. All formulation parameters affected the particle size, encapsulation capacity and release profiles of the microspheres. The cumulative *in vitro* release of OFL from the particles was found similar with OFL levels in the GCF samples. It can be concluded that OFL encapsulated microspheres might be a potential delivery system for periodontal applications.

63

### The effect of Nd: YAG laser irradiation on human cultured cells derived from the periodontal tissue and MC3T3-E1

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**Background and objective:** Nd: YAG lasers (laser) have been widely used for various dental treatment procedures. However, the biological effects of laser irradiation on cultured cells derived from periodontal tissue have not been determined. The growth rate, and the morphological and biological responses of cultured cells derived from periodontal tissue and subjected to laser irradiation were investigated in this study.

**Materials and methods:** PDL cell and HGF were isolated from human periodontal ligament and gingiva attached to permanent first premolars extracted during orthodontic therapy performed in subjects with a clinically healthy periodontium. MC3T3-E1 used in place of Osteoblast derived from human periodontal tissue. A total of 1500 cells were cultured in each 96-well plate, and laser irradiation was performed. The variable parameters were pulse energy (100~400 mJ), pulse rate (20~100 Hz). The cell proliferation activity after irradiation was assessed using the modified MTT method, and cytomorphology was observed under a phase-contrast microscope. TGF- $\beta$ 1 expression after irradiation was measured using an ELISA.

**Results and conclusion:** The proliferation and size of PDL cell, HGF and MC3T3-E1 increased laser irradiation (PDL cell: 100~250 mJ, HGF: 100~400 mJ, MC3T3-E1: 100~200 mJ). Furthermore, the production of TGF- $\beta$ 1 also increased in each cell type. Thus, laser irradiation may be useful for regenerating periodontal tissue *in vitro*.

64

### Implications of oxidant/antioxidant interactions of nicotine, coenzyme Q<sub>10</sub>, pycnogenol and phytoestrogens on healing in fibroblasts and osteoblasts

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**Background:** There is a growing awareness that oxidative stress may play a role in periodontal disease. The aim of this investigation was to investigate potential oxidant/antioxidant interactions of nicotine with antioxidants (Coenzyme Q10 (CoQ), Pycnogenol) and phytoestrogens in a cell culture model.

**Methods:** Duplicate incubations of human periosteal fibroblasts and osteoblasts were performed with <sup>14</sup>C-testosterone as substrate, in the presence or absence of CoQ (20  $\mu\text{g}/\text{ml}$ ), Pycnogenol (150  $\mu\text{g}/\text{ml}$ ) and phytoestrogens (10 and 40  $\mu\text{g}/\text{ml}$ ), alone and in combination with nicotine (250  $\mu\text{g}/\text{ml}$ ). At the end of a 24-hour incubation period, the medium was solvent extracted with ethyl acetate, and testosterone metabolites were separated by thin-layer chromatography and quantified using a radioisotope scanner.

**Results:** The incubations of fibroblasts and osteoblasts with CoQ, Pycnogenol or phytoestrogens stimulated the synthesis of the physiologically active androgen DHT, while the yields of DHT were significantly reduced in response to nicotine compared to control values ( $P < 0.001$  for phytoestrogens). The combination of nicotine with CoQ, Pycnogenol or phytoestrogens increased the yields of DHT compared with incubation with nicotine alone in both cell types ( $P < 0.005$  for the association of nicotine with phytoestrogens).

**Conclusion:** This investigation suggests that the catabolic effects of nicotine could be reversed by the addition of antioxidants such as CoQ or Pycnogenol and phytoestrogens.

65

### Anorganic bovine-derived hydroxyapatite matrix coupled with a synthetic cell-binding peptide (PepGen P-15) for bone regeneration

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The aim of the study was to evaluate the effect of PepGen P-15 implantation on the healing of craniectomy defects. Sixteen, 4-month old rats were used in the study. A 5.0 mm round, full thickness defect was created in each parietal bone of the animals. In each animal, one defect was loosely filled out with the graft (test), while the defect in the other side was left empty (control). Groups of 8 animals were sacrificed after 2 and 4 months and undecalcified sections approx. 10  $\mu\text{m}$  thick were produced. The most central section from each defect was selected for histometric analysis. Histological examination showed limited bone formation in both tests and controls. Complete bone bridging of the defect was never observed. In test sites, the defect was basically occupied by graft particles embedded in loose fibrous connective tissue and new bone had formed only at the margins of the defect. In the controls, minimal new bone formation was observed again only at the periphery of the defect, while a rather narrow band of fibrous connective tissue bridged its margins. After two months of healing, the residual defect was 94.6% and 90.6% ( $P = 0.06$ ) of the original defect size in control and test sites, respectively. After four months, the corresponding values were 89.9% and 85.0% ( $P = 0.33$ ). Although somewhat more new bone formation was observed in the grafted defects, PepGen P-15 does not seem to enhance bone formation since the vast portion of the grafted defects did not heal with bone.

66

### Systemic administration of TTC delays degradation of 3 different collagen membranes in the rat

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**Background:** Degradation of bioabsorbable collagen membranes (BCM) can be altered by enhancing their structural integrity or by delaying the degradation process using matrix metalloproteinase (MMP) inhibitors. We have previously shown that immersing BCMs in Tetracycline (TTC) solution before implantation delays their degradation *in-vitro* and *in-vivo*. This study evaluated the effect of systemic TTC administration on the degradation of three different BCMs in the rat calvaria.

**Methods:** Three different BCM disks (Biomend, Biogide, Ossix), 5 mm in diameter, were labeled with biotin and implanted in similarly sized, surgically created intra-bony defects in rat calvaria. IP injections of either Deoxytetracycline (60 mg/kg) or saline (control group) were administered every three days. Specimens were harvested on day 14 or 28. After histological processing and staining with HRP-Streptavidin complex, BCM degradation was measured by image-analysis and analysed using ANOVA and paired *t*-test.

**Results:** In the control group, Ossix membrane degraded by 5% after 14 days and by 10% after 28 days. Degradation of Biogide membrane was more pronounced - 10% and 40% respectively, and that of Biomend was the greatest - 30% and 60% respectively. Systemic TTC administration significantly reduced significantly the degradation of all three membranes at 14 days and that of Ossix and Biomend also at 28 days.

**Conclusion:** Systemic TTC administration is effective in reducing collagen membrane degradation *in-vivo*.

67

### Vertical ridge augmentation with guided bone regeneration in association with dental implants in the canine model

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**Aim:** To evaluate the effect of guided bone regeneration with a titanium reinforced e-PTFE membrane in alveolar bone defects with titanium implants.

**Materials and methods:** Following extraction of three mandibular premolars and a molar in both sides of the jaw in three dogs, alveolar bone defects (depth: 5–7 mm) were produced. At 4 months three implants were inserted per defect to a depth of 5 mm, so that the coronal portion protruded about 5 mm. Four sides in the dogs were assigned to a test group and two sides to a control group. 12 test implants were covered with a reinforced e-PTFE membrane. The space under the membrane was filled with peripheral venous blood from the animal and flaps were sutured over the membrane. Six control implants received no membrane. Animals were sacrificed at 6 months.

**Results:** Histologic and histomorphometric analyses revealed a significantly ( $P = 0.08$ ) larger bone fill in the test group (52.77%, 62.07%) than in the controls (13.78%, 9.51%), and clinical evaluation of one test site showed the implants were completely covered with tissue resembling bone. In most of the specimens, bone had grown in height close to, or in direct contact with the membrane. However, the new bone generally was not in direct contact with the implants. A zone of dense connective tissue was interposed between the implants and the newly formed bone.

**Conclusion:** Formation of even considerable amounts of bone following vertical ridge augmentation with GBR and implants

was not accompanied by predictable osseointegration of the implants.

68

### The effect of platelet-rich plasma on the proliferation of human periodontal ligament cells, alone or in combination with a bone allograft

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**Objectives:** A novel therapeutic technique for periodontal regeneration is the application of platelet-rich plasma (PRP). PRP is a blood component and is considered to be a rich source of growth factors. The purpose of this study was to investigate the mitogenic effect of homologous PRP on human PDL cell lines *in vitro*.

**Material and methods:** Human PDL cultures were derived from the mid-root of three maxillary premolars extracted for orthodontic reasons. PRP obtained from three donors was prepared from plasma by centrifugation. Each PRP preparation (5% v/v) was added in all cell lines, in the absence or presence of 10 mg/mL of a demineralized freeze-dried allograft (DFBA). Cells were also treated with 25 ng/mL bFGF (positive control). Consequently, cells were incubated for 24 hours at 37 °C, in humidified atmosphere of 5% CO<sub>2</sub>, 95% air. After the 24-hour treatment, cell proliferation was determined using Trypan Blue Exclusion Assay.

**Results:** The results of this study suggested that all PRPs as well as bFGF significantly ( $P < 0.001$ ) induced cell proliferation in all cell lines, in contrast to DFBA. When the allograft was combined with all PRPs, cell proliferation was significantly induced. Furthermore, PRP preparations alone exhibited an advantage over bFGF.

**Conclusion:** PRP alone or in combination with DFBA exhibits an important mitogenic effect on periodontal ligament cells, providing a significant biological approach in the periodontal regeneration *in vivo*.

69

### How valid is the experimental gingivitis model?

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Experimental gingivitis (EG) is often used as a model to study the effects of plaque accumulation on clinical and immunological parameters. However, it is not clear whether effects seen after up to 28 days of EG mirror effects of more prolonged periods of plaque accumulation and gingivitis. In the present randomised controlled trial we thus compared EG with chronic gingivitis (CG). 24 students without periodontitis volunteered for participation. When included in the study they all suffered from gingivitis at least five of six papillae of the posterior teeth of the maxilla. They were randomly assigned either to the EG condition or to the CG condition. After professional tooth cleaning and a period of excellent and professionally controlled oral hygiene to achieve perfect gingival health EG subjects refrained from any oral hygiene for a period of 28 days. CG subjects received no professional tooth cleaning and were instructed just to continue their regular oral hygiene procedures. Plaque and bleeding on probing were assessed twice a week, while gingival crevicular fluid samples were taken weekly from the six papillae and analysed for Interleukin-1 $\beta$  and Interleukin-8. After 28 days groups did not differ with respect to clinical parameters. They did, however, differ significantly with respect to interleukins. These data indicate that at least from an immunological point of view experimental gingivitis might not be a valid model for more chronic stages of gingivitis.



70

### Comparative study of the antibacterial effect of the second ( $\lambda=532$ nm) and third ( $\lambda=355$ nm) harmonics of a Nd: YAG laser on a dental biofilm

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**Background/aims:** To establish an *in vitro* biofilm model which allows the standardized formation of dental plaque and to evaluate and compare the antibacterial effect of two different wavelengths of irradiation on the accumulated biofilm.

**Materials and methods:** An acetate splint was designed to maintain four sterile disks of hydroxyapatite in contact with the teeth of the maxillary arch of a patient. The disks were used to accumulate dental plaque during 12 h. Immediately after the acetate splint was removed, the disks were irradiated with a Q-switched Nd: YAG laser (Spectron SL282G) operating at the second ( $\lambda = 532$  nm) and third harmonic ( $\lambda = 355$  nm). Next, the microbiological analysis of the disks and the bacterial count by means of CFU/mL were carried out by culture. A double blind study was carried out with 22 cases and 28 controls.

**Results:** The design employed allowed the accumulation of dental plaque without any type of manipulation and its immediate examination. Both irradiation wavelengths successfully reduced the biofilm from the disks. ANOVA and KRUSKAL-WALLIS statistical analysis confirmed a significant reduction of aerobic and anaerobic microorganisms with the different wavelengths employed ( $P < 0.05$ ). Biofilm absorptivity at the different wavelengths appears to be the most important parameter for explaining the differences in the bactericidal effect between irradiation at 355 nm and 532 nm.

**Conclusions:** The UV radiation at 355 nm showed a greater bactericidal potential.

71

### Histological and immunohistochemical analysis of early periimplant tissue reactions to chemically modified SLA® titanium implants in dogs

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The aim of the present pilot study was to investigate early tissue reactions to modified (mod) and conventional sandblasted/acid-etched (SLA) titanium implants. Implantation of modSLA and SLA implants was performed bilaterally in both the mandible and maxilla of dogs. The animals were sacrificed after healing periods of 1, 4, 7, and 14 days. Non-decalcified tissue sections were evaluated using conventional histology (H) and immunohistochemistry (IH) using monoclonal antibodies to transglutaminase II (TG) (angiogenesis - AG) and osteocalcin (OC). Bone density and bone to implant contact (BIC) were assessed morphometrically. IH analysis at day 1 revealed an early TG antigen reactivity in the provisional fibrin matrix adjacent to both implant surfaces. Day 4 was characterized by the formation of a collagen-rich connective tissue, which revealed first signs of OC synthesis adjacent to modSLA surfaces. IH staining for TG revealed a direct correlation between AG and new bone formation (BF). BF was clearly identifiable after 7 days by means of H, as well as a positive OC and TG staining. After 14 days, mod SLA surfaces seemed to be surrounded by a firmly attached mature, parallel-fibered woven bone. Within the limits of the present study, it might be concluded that the combination of immunohistochemical and conventional histological stainings at non-decalcified tissue sections is a valuable technique to evaluate early stages of wound healing around endosseous titanium implants.

72

### Bone healing in critical-size-defects treated with platelet rich plasma. A histologic and histometric study in rat calvaria

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Platelet Rich Plasma (PRP) has been used either alone or in combination with bone grafts in order to enhance bone healing. There are only a few histologic studies evaluating the healing of bone defects treated with PRP alone. The purpose of this study was to histologically analyse the influence of PRP on bone healing in surgically created critical-size-defects (CSD) in rat calvaria. 32 rats were divided into 2 groups: C (control) and PRP (Platelet Rich Plasma). An 8 mm diameter CSD was created in the calvarium of each animal. In Group C the defect was filled by blood clot only. In Group PRP it was filled with Platelet Rich Plasma. Both groups were divided into subgroups ( $n = 8$ ) and euthanized at either 4 or 12 weeks post-operative. Histometric, using image analysis software, and histologic analyses were performed. Amount of new bone was calculated as percentage of total area of original defect. Percentage data were transformed into arcsine for statistical analysis (ANOVA, Tukey,  $P < 0.05$ ). No defect completely regenerated with bone. Group PRP had a statistically greater amount of bone formation than Group C at both 4 (17.68% and 7.20%, respectively) and 12 weeks (24.69% and 11.65%, respectively) post-operative. Within the limits of this study, it can be concluded that PRP significantly enhanced bone healing in critical-size-defects in rat calvaria.

73

### Inhibitory effects of green tea polyphenol (-)-epigallocatechin gallate (EGCG) on the osteoclast survival *in vitro*

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**Background:** Alveolar bone resorption involves removal of mineral and organic constituents of bone matrix. Osteoclasts are principally responsible for this process. Recently, (-)-epigallocatechin gallate (EGCG) has been shown to induce apoptotic cell death of osteoclasts and modulate caspase activation. In the present study, we investigated inhibitory effect of EGCG on osteoclast survival and examined if EGCG mediates osteoclast apoptosis via caspase pathway.

**Materials and methods:** Effect of EGCG on osteoclast survival was examined by TRAP staining in osteoclasts differentiated from RAW264.7 cells. In addition, we evaluated apoptosis of osteoclasts by EGCG using DNA fragmentation analysis. Involvement of caspase in EGCG-mediated osteoclast apoptosis was evaluated by examining effect of EGCG on activity of caspase-3, which was assessed by western blotting and colorimetric activity assay.

**Results:** EGCG significantly inhibited survival of osteoclasts differentiated from RAW264.7 cells. It was confirmed by DNA fragmentation pattern that this inhibitory effect was mediated by apoptosis. Moreover, EGCG stimulated activity of caspase-3.

**Conclusions:** From these findings, we could suggest that EGCG might prevent alveolar bone resorption by inhibiting osteoclast survival through the caspase-mediated apoptosis. This study was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea (03-PJ1-PG1-CH08-0001).

74

# **Evaluation of ablation of naturally grown biofilm by laserfluorescence**

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**Aim:** With laser devices it may be possible to remove selectively calculus. It is unknown if a low power laser irradiation removes completely biofilm without ablating root cementum. The purpose of the present study was to evaluate the ablation of biofilm and the removal of root cementum with a KEY-laser-III with different power settings.

**Methods:** Fifty extracted teeth were treated with a KEYlaser-III and water-spray in the former pocket region. Biofilm was stained with toluidine-blue. Each tooth and a handpiece were fixed at a computer-assisted-bench, which allowed a standardized treatment field ( $3 \times 1.65$  mm/min). The glass tip was applied perpendicular to the root. On each root surface a control field was irradiated with 160 mJ. The treatment was performed either with 120 mJ, 100 mJ or 80 mJ. Afterwards digital images were taken to evaluate the biofilm ablation. To assess the amount of tooth substance removal the roots were cut into 200  $\mu$ m thick slices (3 slices per tooth). The Wilcoxon-test was used to compare 160 mJ with 120 mJ, 100 mJ or 80 mJ. Mann-Whitney U-test was used for comparisons among 120 mJ, 100 mJ or 80 mJ.

**Results:** Root substance removal was  $243.7 \mu\text{m} \pm 159 \mu\text{m}$  (median  $\pm$  IQR) with 160 mJ,  $59.3 \mu\text{m} \pm 116.7 \mu\text{m}$  at 120 mJ,  $55.9 \mu\text{m} \pm 121.3 \mu\text{m}$ , at 100 mJ and constant 0  $\mu\text{m}$  with 80 mJ. ( $P < 0.001$  for all comparisons) Biofilm was ablated to 100% at 100–160 mJ and at 80 mJ to 63%.

**Conclusion:** It has to be evaluated which setting between 80–100 mJ will remove biofilm completely without ablating tooth substance.

75

# **The effect of deer antler growth factor on the viability and proliferation of human osteoblast cells *in vitro***

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Deer Antler Growth Factor (DAGF) is a product which is extracted from deer antler. In Traditional Chinese Medicine DAGF is used for the treatment of osteoporosis and fracture healing. This study examined the influence of different DAGF concentrations on the viability and proliferation of osteoblast cells.

**Material and methods:** Primary human osteoblast cells were used in the experiments. The cells were cultured in Dulbeccó's modified Eagle's medium (DMEM), supplemented with 10% calf serum, 1%

streptomycin at a humidified air atmosphere of 5% CO<sub>2</sub>. The cells were exposed to various concentrations of DAGF (0  $\mu$ g/mL, 12.5  $\mu$ g/mL, 25  $\mu$ g/mL, 50  $\mu$ g/mL, 100  $\mu$ g/mL, 250  $\mu$ g/mL). After an exposure period of 48 hours, cellular viability and proliferation were evaluated with a cell counting kit and a MTT cell proliferation assay.

**Results:** The cell number and the cell viability of human osteoblast cells increased by concentration of DAGF, the results are significant ( $\alpha < 0.05$ ).

**Conclusions:** The results demonstrate that DAGF increases the proliferation of human osteoblasts *in vitro*. Further research is indicated to understand the interaction of DAGF with osteoblasts and to assess the potential of DAGF for bone regeneration and improved fracture healing.

76

# **Implant stability in relation to osseointegration. An experimental study in the Labrador dog**

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**Background:** Resonance frequency analysis (RFA) is frequently used to determine implant stability and the time interval at which loading can be initiated. The correlation between RFA values and degree of osseointegration is unclear.

**Aim:** The objective of the present experiment was to study RFA in relation to early phases of osseointegration.

**Material and methods:** All mandibular premolars in 20 Labrador dogs were extracted. 3 months later, four implants were placed in each premolar region. The implants were designed with either a turned (TS) or a SLA surface (SLA). The animals were divided into four groups and each group represented two different healing times. Hence, the healing following implant installation could be examined after 2 hours, 4 days, 1 week, 2 weeks, 4 weeks, 6 weeks, 8 weeks and 12 weeks. RFA assessments were performed at the time of implant placement and at each biopsy interval. Block biopsies from each implant site were processed for histological analysis.

**Results:** The histological analysis revealed that the degree of osseointegration (BIC%) increased from 1 to 4 weeks, while changes from 4 weeks to 12 weeks were small. The BIC% was significantly higher at SLA than at TS implants at all healing periods examined. The changes of the RFA values during the course of healing were small and no differences were observed between the 2 types of implants.

**Conclusion:** The RF analysis did not correlate with BIC% changes assessed in specimens representing the various implant sites.

## Posters: Biomaterials and surfaces

77

# **Load capability of root canal treated teeth restored with adhesively cemented fibre reinforced posts depends on bone support**

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**Aim:** It was aim of the present investigation to study the influence of a reduced bone support on fracture resistance of endodontically treated teeth (ETT) restored by glass fibre-reinforced posts (FRC).

**Methods:** Forty caries-free maxillary central incisors were divided into four groups ( $n = 10$ ). Endodontic treatment was performed. Teeth were flattened 2 mm above the cemento-enamel junction. Group I (control) simulated a clinical situation without horizontal bone loss. In group II a horizontal bone loss of 25%, in group III of 50% and in group IV of 75% was simulated. All specimens received FRC and composite core restorations. All-ceramic crowns were adhesively cemented. Specimens were exposed to chewing simulation (TCML) and finally statically loaded until failure. For statistical analysis, the

72

Kruskal-Wallis test was applied followed by the Mann-Whitney test as post hoc testing.

**Results:** The median fracture load values (min. / max.) were: group I = 501 (326 / 561), group II = 422 (323 / 495); group III = 352 (266 / 406). Two specimens each of group II and III and all specimens of group IV failed during TCML. Statistical analysis revealed statistically significant differences between all test groups regarding maximum fracture load.

**Conclusions:** The fracture resistance of ETT restored adhesively with a FRC, composite core, and all-ceramic crown restoration dependent on the level of surrounding supporting periodontal bone. The level of fracture decreases as the level of bone moved apical.

78

### Bone microstructure and mineral density in two different interimplant distances. A dog study

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**Background:** The peri-implant bone level will affect the position of gingival soft tissues, and have a significant impact on the aesthetic outcome of the implant therapy.

**Aim:** The aim of this study was to evaluate the influence of two different interimplant distances on crestal bone microstructure and mineral density level.

**Materials and methods:** Forty-eight implants (DENTSPLY-FRIADENT, Mannheim, Germany) were placed in the mandibular bone of six dogs. Each animal received eight implants. The implants were divided into two groups: in group A the interimplant distance was 2 mm, while in group B the distance was 3 mm. The implants received after 12 weeks, temporary prostheses than, 4 weeks later, metallic crowns. The animals were killed after four additional weeks.

**Results:** For groups A and B, respectively, the interimplant area was  $29.813 \pm 6.421$  pixels and  $23.574 \pm 4.238$  pixels. The area covered by bone was  $15.988 \pm 4.322$  pixels and  $11.843 \pm 4.528$  pixels. In group A the marrow spaces represented 99%, while in the group B they represented 86.4%. In group A, 44.5% of the bone area was highly mineralized while 35.7% showed a low mineral density. In group B, 41.2% of the bone area appeared highly mineralized while 22.4% showed a low mineral density. No statistically significant differences were obtained.

**Conclusions:** The distances of 2 and 3 mm between implants did not result in statistically significant differences in the bone microstructure characteristics and mineral density.

79

### Finite element analysis of dental implants placed at varying mesio-distal angulations and connected by 3-unit bridges

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**Objectives:** Proximity of the maxillary sinus to the alveolar ridge sometimes prohibits implant therapy in the posterior maxilla. Placement of one or more implants at an angle to avoid the sinus has been proposed, but so far clinical evidence is very limited. The aim of this study is to test for stresses generated by different implant mesio-distal angulations, at and around implants supporting 3-unit bridges.

**Method:** Two-dimensional models were created of 3 unit bridges supported by implants (13 mm length, 4 mm wide) placed at 0, 10 or 20 degrees to the vertical axis, embedded in bone blocks. Material properties were taken from the literature. Finite element models were constructed and forces applied on the angulated

implants via the bridges. The stress distribution on the bridges, implants and alveolar bone was evaluated for every model and then cross-compared.

**Results:** The numerical results showed little or no difference in stress distribution between the models.

**Conclusions:** Within the limitations of finite element analysis, it was demonstrated that the constructed models were not compromised by the implant angulation.

80

### Surface microstructure and fibrin clot extension on titanium laser sintered specimens

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**Background:** Fibrin seems to be an important factor in the early healing processes at the interface with dental implants. The aim of the present study was a quantitative evaluation of the *in vitro* fibrin clot extension on a new implant surface obtained throughout laser powder sintering.

**Materials and Methods:** Ten disk-shaped of Ti6Al4V sintered titanium alloy were used in the present study. The specimens were covered by 0.2 mL of human whole blood drawn during implant placement. The blood was immediately dropped onto the surface of each specimen. Contact time was 5 minutes at room temperature. After processing, all the specimens were observed under SEM and Confocal Laser Scanning Microscopy (CLSM).

**Results:** Quantitative analysis performed under CLSM on a mean of  $12.543 \text{ mm}^2$ , showed that a 33% ( $4.144 \text{ mm}^2$ ) of the surface was 40  $\mu\text{m}$  deep, 36.6% ( $4.597 \text{ mm}^2$ ) was 20  $\mu\text{m}$  deep and 17.8% ( $2.237 \text{ mm}^2$ ) was 5  $\mu\text{m}$  deep. The analysis of the fibrin clot extension on a total of  $786432 \pm 42.386$  of the investigated surface showed a  $78807 \pm 41.027$  pixels of metal surface free from the fibrin network. The 90% of the surface resulted covered by fibrin clot while only a 10% of the metal surface remained uncovered. There was a statistically significant correlation between the quantity of fibrin and surface roughness ( $P < 0.05$ ).

**Conclusions:** The results of this *in vitro* study indicated that there was a correlation between implant surface roughness and fibrin clot extension.

81

### Proliferation and differentiation of osteoblast like cells cultured on different bone graft materials: an *in vitro* investigation

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Aim of this study was to investigate the biological activity of eight bone graft materials analysing their inductive potential of osteoblast proliferation and differentiation. The materials were classified as: Synthetic materials: Sintbone, Fisiograft and Ostim Animal derived materials: BioGen, Bio-Oss, and Osteograft Mixed materials: Biostite and Pep Gen P-15 The bone graft materials have been tested by the MTT Test using osteoblasts SaOS-2, coming from human osteosarcoma. Afterwards the ALP enzymatic assay was performed. Both tests were repeated three times for each specimen at three, four and five days. After the cellular growth evaluation by MTT test we quantified the absorbance value for each sample using a spectrophotometer at 405 nm and we calculated the ALP/MTT ratio. Data were statistically analysed (ANOVA one way and post-hoc Scheffè test) The mean values of MTT (proliferation test) and ALP enzymatic activity (differentiation test) at three, four and five days were different between tested materials. Within the limits of the *in vitro* studies it can be concluded that: Tested bone graft materials induced different amounts of osteoblast proliferation Biostite

(mixed material) shown the lowest proliferation and differentiation values at three, four and at five days (this bone graft material has a cytotoxic effect) Others tested bone graft materials induced a good osteoblast differentiation At five days Pep-Gen, Bio-Oss and Osteograft showed the highest ALP activity values.

82

# **Histological evaluation of using bio-gide membrane with or without deproteinized bovine bone mineral (DBBM) in healing of bony defects in rabbit**

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Guided Bone Regeneration (GBR) doesn't always produce consistent results and bone filling within the space provided by membrane can be incomplete. The aim of this animal study was to evaluate the effect of using a collagen membrane (Bio-Gide) in combination with or without deproteinized bovine bone mineral (DBBM) on healing of calvarial defects. 12 New Zealand white rabbits were used in this randomized blind prospective study. In all animals, four equal defects were created on the calvarium. Four defects per rabbit were randomly assigned to one of the following treatments: 1-no treatment (control) 2-Bio-Gide 3- DBBM 4-Bio-Gide + DBBM. The results showed no significant differences between control group and the group with Bio-Gide. There was a significant difference between the group with DBBM and the group with DBBM + Bio-Gide in comparison with the control group. The difference between the group with Bio-Gide and the group with DBBM + Bio-Gide was insignificant at 1 and 2 months intervals. Within the limits of this study no additional benefits were seen with regard to use of collagen membrane.

83

# **Clinical evaluation of treatment of intrabony periodontal lesions using autogenous bone graft and ostim**

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The ultimate goal of periodontal treatments is restoring the lost periodontium. In spite of the autogenous bone graft being the gold standard material for reconstructive methods, in some cases with not enough source, using a bone substitute material can be helpful. This study evaluates the treatment of intrabony periodontal defects using the autogenous bone graft and a bone grafting material (Ostim). In this clinical trial study, 12 patients aged 23–40, who had bilateral intrabony defects, were treated with Ostim and ABG. In each patient, one side was treated with Ostim and the other side with ABG randomly. Clinical attachment levels (CAL) and probing pocket depths (PPD) were recorded before the operation and 6-months post-operation. Radiovisography was also carried out at the same intervals. The results of this study showed no significant differences between Ostim and ABG. The mean reduction of PPD after 6-months was 3.92 mm for ABG and 3.02 mm for Ostim and the mean attachment gain was 3.79 mm for Ostim and 4.18 mm for ABG. The mean bone fill was 2.29 mm for Ostim and 2.77 mm for ABG. Due to the above results, Ostim can be considered as a safe and appropriate bio-material for treatment of intrabony periodontal defects, especially in cases with not a suitable source of ABG. Histological evaluation of the regenerative potential of this bio-material is also recommended.

84

# **Effects of gamma radiation sterilization on morphology, mechanical properties, and biocompatibility of ostrich eggshell membranes**

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Eggshell membrane has been introduced as resorbable biological membranes for guided tissue regeneration technique but the results have demonstrated that chicken egg collagen membrane failed to promote bone healing probably due to its lack of rigidity and the structural characteristics of collagen. This study aimed to evaluate the morphology, mechanical properties, and the biocompatibility of a more rigid ostrich eggshell membrane (OEM). Specimens were performed for microscopic analyses and for tensile test. *In vitro* cell proliferation and adhesion tests was performed using fibroblasts obtained of healthy gingival explants and MTT spectrophotometrical detection, after cultivate by 10 and 20 minutes in a 98 wells culture plates blocked with BSA, with gamma radiation OEM sterilized and a control membrane. The differences were analysed by unpaired *t*-test with  $P < 0.05$ . The gamma radiation process did not affect morphology and tensile strength. The cells cultivated in the presence of OEM showed greater optical density values. The mean of OD increased progressively with the time of incubation in both control and OEM culture, but the cellular adhesion was significantly greater in OEM ( $P = 0.000$ ). The ostrich eggshell membrane sterilized by gamma radiation, has good mechanical properties and is biocompatible with gingival human fibroblasts, and it shown characteristics that allow a full cellular adhesion mediated by integrins and the conservation of cellular vitality.

85

# **Efficacy of tetracycline adsorbed xenogenic bone substitute for periodontal therapy**

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**Objectives:** (1) To investigate the *in vitro* release of tetracycline hydrochloride (TCH) onto deproteinised bovine bone minerals (Bio-Oss) (DBBM) (2) to evaluate its antimicrobial efficacy.

**Material and methods:** DBBM granules were incubated in TCH solutions of different concentrations (0.1, 0.5, 1 mg/mL) and adsorption of TCH onto DBBM was followed for 216 hours (h). The TCH release from the DBBM particles was carried in phosphate buffered saline for 96 h. The adsorption and release of different concentrations of TCH from DBBM were determined using UV spectroscopy. The effect of TCH adsorbed onto DBBM was tested on *Actinobacillus actinomycetemcomitans* (A.a), *Porphyromonas gingivalis* (P.g), and *Staphylococcus aureus* (S. aureus). The bacterial growth was determined by the total viable colony count (TVC) of the bacteria in agar cultured plates and was followed for 96 h in the presence of DBBM with or without TCH.

**Results:** Most of TCH adsorption onto DBBM took place during the first 73 h. The highest concentration of TCH resulted in increased adsorption onto DBBM granules. The TCH release profile was fast during the first 10 h, and was reduced gradually over time. The TCH adsorbed DBBM showed reduction in growth of A.a, P.g, and S. aureus for all TCH concentrations. High concentration of TCH adsorbed onto DBBM resulted in increased reduction in the growth of all bacteria.

**Conclusions:** DBBM granules are able to adsorb and release TCH. This effect might provide clinical utilisation.

86

### The bone regenerative effects of tetracycline blended chitosan membranes on the calvarial critical size defect in sprague-dawley rats

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The aim of this study is to evaluate the osteogenesis of tetracycline blended chitosan membranes on the calvarial critical size defect in Sprague Dawley rats. An 8 mm surgical defect was produced with a trephine bur in the area of the midsagittal suture. The rats were divided into five groups: Untreated control group versus four experimental groups. Four types of membranes were made and comparative study was been done. Two types of non-woven membranes were made by immersing non-woven chitosan into either the tetracycline solution or chitosan-tetracycline solution. Other two types of sponge membranes were fabricated by immersing chitosan sponge into the tetracycline solution, and subsequent freeze-drying. The animals were sacrificed at 2 and 8 weeks after surgical procedure. The specimens were examined by histologic analyses. The results are as follows: (1) Clinically the use of tetracycline blended chitosan membrane showed great healing capacity. (2) The new bone formations of all the experimental group, non-woven and sponge type membranes were greater than those of control group. But, there was no significant difference between the experimental groups. (3) Resorption of chitosan membranes were not shown in any groups at 2 weeks and 8 weeks. These results suggest that the use of tetracycline blended chitosan membrane on the calvarial defects in rats has significant effect on the regeneration of bone tissue in itself.

87

### Histopathological evaluation of the effects of alveolar bone and bone marrow originated cell on healing around various root surfaces

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Periodontium contains various mesenchymal cell populations with specific differentiation potential capacity of those cells for tissue formation still remains unclear. Sementoblast precursors within periodontium reported to found on endosteal surfaces of alveolar bone. The aim of this study is evaluation of the effects of alveolar bone and bone marrow originated cell on healing around various root surfaces. According to this purpose, cortical layer of the alveolar bone was removed by  $3 \times 3 \times 1$  mm (width, length, height) dimensions at the level of maxillar and mandibular molars level in five pigs for preparations of bone defects. Furthermore, those defects were covered with  $3 \times 3$  mm root particle caps with cement, dentin and fibronectin coated dentin surfaces and Atrisorb membranes were plated over those root particle caps. After the operations, pigs were sacrificed at 15<sup>th</sup> and 45<sup>th</sup> days and histopathological examination of the samples were performed by light microscope. Results of those observation showed that the cells originated from endosteal gaps of the alveolar bone has regenerative potential not towards sementoid formation. In conclusion, the results showed that periodontal ligament cells has regenerative potential.

88

### Microvessel density (MVD) and vascular endothelial growth factor (VEGF) expression in sinus lifting procedures using anorganic bovine bone (Bio-Oss®)

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**Aim:** The aim of this study was to conduct a comparative immunohistochemical evaluation of microvessel density (MVD) and Vascular Endothelial Growth Factor (VEGF) expression in sinus augmentation procedures using anorganic bovine bone (Bio-Oss®) after 3 and 6 months.

**Material and methods:** Twenty patients participated in this study. The sinuses were filled with 100% Bio-Oss®. Implants were inserted after 3 months in Group A, and 6 months in Group B. As control, the pre-existing subantral bone was used.

**Results:** The mean MVD in control bone was  $23.6 \pm 1.8$ , while in the sites augmented with Bio-Oss®, at 3 months, the MVD was  $23.3 \pm 2.1$ , and at 6 months the MVD was  $29.5 \pm 2.4$ . The difference in MVD between control bone and Group A was not statistically significant. The difference between the control bone and Group B was statistically significant ( $P$ -value  $< 0.05$ ). Moreover the difference in MVD between Group A and Group B was statistically significant ( $P$ -value  $< 0.05$ ).

**Discussion and conclusions:** Bio-Oss® seemed to induce an increase in the MVD that reached a higher value after 6 months. VEGF decreased from Group A (85% of vessels stained) to Group B (65% of vessels stained). Our data could point to a close spatial relationship between angiogenesis and osteogenesis due to an higher percentage of vessel and stromal cells positive to VEGF and higher MVD values in the front of osteoconduction compared to the center of bone maturation.

89

### Attachment and spreading of human fibroblasts on suprather® - membranes

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Human fibroblasts adhere to surfaces with integrins and show a typical morphology. Attachment and spreading of cells can be classified into different stages described by Rajaraman et al. (1974). The aim of this study was to examine cell spreading of human fibroblasts on two material surfaces at different incubation times. For the experiments human periodontal ligament cells (PDL) and gingival fibroblasts (HGF) were used. The materials, cover slip and resorbable Suprather® membrane, were tested for cell compatibility. Cells were seeded in duplicates for 3, 6, 18 and 24 hours on cover slips and membranes. PDL and HGF were analysed by light and scanning electron microscope (SEM). SEM photographs were used for cell counting and defining stages of spreading. Mann-Whitney U-Test ( $P < 0.05$ ) was used to analyse the differences between the stages on the various materials. Early cell spreading events could be observed after 3 hours on both materials. Significant differences between the stages 1, 2 and 3 were shown for cover slip and Suprather® after 3 and 6 hours ( $P < 0.05$ ). After 18 hours incubation there were significant differences between stages 1, 2 and 4 ( $P < 0.05$ ) for both materials. After 24 hours only a low number of cells was completely attached to Suprather®. For the first 24 hours the results showed a well attachment and spreading of HGF and PDL on Suprather® compared with cover slip. Incubation times over 24 hours indicate lower attachment because of membrane degeneration.

90

# **Microvessel density (MVD) in sinus augmentation procedures using anorganic bovine bone (Bio-Oss®) and autologous bone: 3 months results**

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Bio-Oss® is a xenogeneic bone graft material of bovine origin that has been demonstrated to be biocompatible and osteoconductive. Angiogenesis plays a key role in bone formation and maintenance. Aim of the present study was an immunohistochemical evaluation of microvessel density (MVD) in sinus augmentation with autologous bone and Bio-Oss®. Twenty-four patients participated in this study. Twelve sinus augmentation procedures were performed with 100% autologous bone, with in the other twelve patients 100% Bio-Oss® was used. Endosseous implants were inserted after a mean period of 3 months. As control, the portions of pre-existing subantral bone were used. The mean value of the MVD in control bone was  $23.4 \pm 1.3$ . The mean value of the MVD in the sinuses augmented with autologous bone was  $29.0 \pm 2.4$ . The mean value of the MVD in the sinuses augmented with Bio-Oss was  $23.8 \pm 2.2$ . The statistical analysis showed that the differences of the MVD between control bone and sinuses augmented with Bio-Oss® were not statistically significant ( $P = 0.52$ ), while the difference of the MVD between sinuses augmented with autologous bone and Bio-Oss was statistically significant ( $P = 0.0008$ ). This fact can, probably, be explained with the hypothesis that autologous bone may act not only as a passive filling material in bone defects but also through the release of osteogenic growth factors and that the particles of autologous bone seem to contain vital osteoprogenitor cells.

91

# **Peri-implant bone evaluation combining confocal laser scanning microscopy and scanning electron microscopy backscattered electrons imaging**

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**Background:** Aim of this report was to present a new investigative approach of the peri-implant tissues based on the correlation of the qualitative and quantitative details by overlapping different images obtained on the same specimen through different investigative techniques.

**Materials and methods:** Unloaded titanium dental implants (3 XiVE plus and 2 Frialit2, DENTSPLY-Friadent, Mannheim, Germany), retrieved from the mandibles of four patients after a mean period of 6 months period, were used in the present study. Samples of resin-embedded peri-implant tissues were evaluated under SEM using a Backscattered Electrons (BSE) signal, Confocal Laser Scanning Microscopy (CLSM), Circularly Polarized Light (CPL) and Light Microscopy (LM).

**Results:** The SEM BSE images were used to identify the mineral density around the implants. The CLSM images was used to provide information about cells and bone marrow spaces. The CPL images gave the collagen fibers orientation in the peri-implant bone, while; LM images were used as controls. To overlap the images we used a program based on a linear transformation matrix; the overlapping was made by calculating three corresponding points in the BSE and CLSM figures.

**Conclusions:** The combination by overlapping of two images of the same section of the same specimen made possible to have, in the same image, a complete evaluation of both the peri-implant bone tissue and of the cellular elements, the not yet mineralized osteoid matrix, the newly-formed bone.

92

# **Experimental evaluation in rabbits of the effects of thread concavities in bone formation with different titanium implant surfaces**

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Aim of the present study was an evaluation of the osseous response in machined implants (M), in implants blasted with apatitic calcium phosphate (TCP/HA blend) particles (Resorbable Blast Texturing, RBT), and in implants coated with HA (HA). Threaded M, RBT and HA coating commercially pure titanium screw shaped implants (BioHorizons<sup>R</sup>, Birmingham, AL, USA) were used in this study. Twelve New Zealand white mature male rabbits were used for this study. The implants were inserted into the tibia. Each rabbit received four implants, two in the left tibia and two in the right tibia. A total of 48 implants (16 M, 16 RBT and 16 HA) were inserted. Three animals were killed, with an overdose of intravenous pentobarbital, respectively after 1, 2, 4, and 8 weeks. A total of 48 implants were retrieved. The specimens were processed to obtain thin ground sections with the Precise 1 Automated System (Assing, Rome, Italy). Higher and highly statistically significant differences were found in the percentages of bone that had formed in the concavities rather than the convexities of the implants in the specimens retrieved after 1 ( $P = 0.000$ ), 2 ( $P = 0.000$ ), and 4 weeks ( $P = 0.004$ ). No statistically significant differences were found in the percentages of bone that had formed in the concavities rather than the convexities of the implants in the specimens retrieved after 8 weeks ( $P = 0.703$ ).

93

# **Evaluation of abm/p-15 hydrogel in a rabbit cranial bone model**

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**Background:** ABM/P-15 is a combination of anorganic bovine-derived HA bone matrix (ABM) with a synthetic cell-binding peptide (P-15). An evolution of this biomaterial was achieved through the combination with a hydrogel carrier to optimize its clinical handling and efficacy. The aim of this study is to compare the standard ABM/P-15 particulate with the ABM/P-15 hydrogel in a preliminary bone defect animal model.

**Materials and methods:** Twelve male adult rabbits were used. Cranial bilateral full thickness circular 8 mm defects were created. Defects randomly received either the ABM/P-15 particulate or the ABM/P-15 suspended in a hydrogel composed by glycerol and carboxymethylcellulose, or remained empty as controls. At time periods of 2 and 4 weeks post surgery, six animals from each group were sacrificed. Skulls were harvested *en bloc* and prepared for histological processing of undecalcified specimens for histomorphometric evaluation.

**Results:** Control defects healed with connective fibrous tissue. None of the graft materials showed adverse tissue reactions. ABM/P-15 particulate promoted significantly the formation of new bone when compared to control defects. The hydrogel produced an unexpected increased migration of particles, determining a poor efficacy on bone regeneration.

**Conclusions:** The ABM/P-15 particulate enhances new bone formation. Nevertheless, the hydrogel vehicle does not seem suitable to be applied in non-contained bone defects as a carrier for bone graft substitutes.

94

### Evaluation of p-15 in a new hydrogel bone graft in a rabbit cancellous bone model

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**Background:** Coating anorganic bone mineral (ABM) with the cell-binding small peptide P-15 (ABM/P-15) simulates the ligand role of native collagen, which can have the potential to enhance osseous regeneration. The purpose of this study is to evaluate the efficacy of the synthetic peptide by comparing the ABM matrix with and without the P-15 in an inert hydrogel carrier in a cancellous bone defect model.

**Materials and methods:** Twelve male adult rabbits were used. Rabbits randomly received either the ABM or the ABM/P-15 suspended in a hyaluronic acid hydrogel, and as controls, only the hydrogel and empty defects. Four cylindrical defects (4 mm diameter by 10 mm depth) were created per rabbit in the distal femur and medial tibia. At time periods of 1, 2, 4 and 8 weeks post surgery, five animals from each group were sacrificed. Bones were harvested *en bloc* and individual specimens fixed for histomorphometric evaluation.

**Results:** All the rabbits recovered with no negative response to the surgical procedure or graft material. Sections receiving no graft material or hydrogel carrier showed minimal bone ingrowths. At 1 week, bone formation was comparable but, with increasing time, histological sections at 2, 4 and 8 weeks showed greater bone formation with ABM/P-15 hydrogel.

**Conclusions:** The significant results of this study were attributed to the P-15, which provides a biomaterial with an ability to stimulate cells interaction with their extracellular matrix, mimicking the role of collagen.

95

### Morphological, structural and chemical characterization of bone graft substitutes

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**Aims:** The purpose of this study is to characterize bone graft substitutes with different origins using techniques currently available.

**Materials and methods:** Five materials (DFDBA, Bio-Oss<sup>®</sup>, PepGen P-15<sup>TM</sup>, Osteogenos<sup>®</sup> and Biocoral<sup>®</sup>) were characterized with respect to their physical and chemical properties. The samples were observed by light and scanning electron microscopy to qualitatively evaluate morphological aspects. Size was characterized by laser diffraction spectrometry and image analysis. Sample porosity was quantified by mercury intrusion porosimetry. Density was determined by gas pycnometry. Physical characterization was complemented by X ray diffraction, for crystalline phase identification, and by FTIR, to qualitatively assess samples functional groups. Ash content was determined by incineration.

**Results:** It was detected organic material in the allograft and animal xenografts. The materials kept the microscopic organization typical of bone tissue, however pores size distribution curves revealed that the majority of the particle pores have submicron sizes not suitable to cellular colonization.

**Conclusions:** These results suggest that, first, some of the manufacturing techniques are not able to completely eliminate organic components and, second, interparticle spaces play an important role in the final performance of these materials. Therefore attention should be paid not only to the particles properties but also to the processing methods and packing characteristics.

96

### BGP-15, a new type insulin sensitizer is protective in rat experimental periodontitis

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Previously our group identified a hydroxylamine derivative, BGP-15 with insulin sensitizer activity. BGP-15 inhibits poly (ADP-ribose) polymerase, stimulates the expression/function of heat shock proteins and constitutive nitric oxide synthases (NOS), and through these mechanisms it improves mitochondrial biogenesis/function. Considering that diabetes is a major risk factor of periodontitis, furthermore some of the observed biochemical activities of BGP-15 might be beneficial in periodontitis even in the lack of diabetes, the potential therapeutic efficacy of BGP-15 was tested in periodontitis model. Periodontitis was produced by a silk ligature placed around the cervix of the lower left 1st molar in the rat. Animals were gavaged with 20 or 50 mg/kg BGP-15 or vehicle once a day for 7 days. On Day 8, the gingivomucosal tissues encircling the mandibular 1st molars were harvested and the level of inflammation in the tissue was evaluated by Evans blue extravasation technique. The heat shock, NOS, and COX-IV protein contents were determined by Western blot, and the alveolar bone loss by videomicroscopy. Ligation-induced periodontitis resulted in marked inflammation in the gingivomucosal tissue and led to alveolar bone destruction. Both of the applied doses of BGP-15 significantly reduced gingival inflammation. The insulin sensitizer and cytoprotective BGP-15 is active in experimental periodontitis and it represents a new drug candidate in the treatment of periodontal disease.

97

### Ultrastructural features of regenerated bone in maxillary sinus augmentation using a porous porcine-derived allograft

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Natural anorganic porcine-derived hydroxyapatite matrix in form of 0.12–1 mm granules (Apatos, Tecnos, Turin, Italy) has been used in this study. Aim of the present study was to report the light microscopy and transmission electron microscopy results in 12 specimens retrieved 5 months after a sinus augmentation procedure using this biomaterial. Each specimen was cut in half and one part was fixed in formalin and treated to be observed under light microscopy; the other part was fixed in glutaraldehyde and paraformaldehyde, decalcified and treated to be observed under a transmission electron microscope. Light microscopy showed that most of the particles were surrounded by newly formed bone. In some areas osteoid matrix was present. In transmission electron microscopy, all phases of bone formation (osteoid matrix, woven bone, lamellar bone) were observed around the biomaterial particles. Some reactive connective tissue was present and a few inflammatory cells were identified. Bone-biomaterial interface showed a close contact between the partially demineralized porcine bone particles and the bone tissue that had mainly features of mature bone with numerous osteocytes. This is, according to our knowledge, the first study presenting data on transmission electron microscopy of porcine-derived hydroxyapatite used in a sinus augmentation procedure in man. Our data, in conclusion, show, at an ultrastructural level, the high biocompatibility and effectiveness of this biomaterial.

98

# **Proliferation and differentiation of human gingival fibroblasts on titanium implant surfaces**

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Surface characteristics of dental implants seem to modulate the cellular phenotypic expression and metabolic activity thus affecting perimplant tissues healing. In order to investigate *in vitro* the effects of surface topography on the biological behaviour of fibroblasts, human gingival fibroblast cells from primary cultures were cultured in DMEM with 10%FCS at 37°C in 5%CO<sub>2</sub> on c.p. titanium disks with two different surface topographies: machined, smooth(S) and dual acid-etched, Osseotite(O)(3i-Biomax,Italy). Cell adhesion and DNA synthesis were tested by means of <sup>3</sup>H-thymidine incorporation while production of extracellular matrix(ECM) proteins was assessed by indirect enzyme-linked immunoassay *in situ* and immunoblotting using specific monoclonal antibodies. The adhesion of gingival fibroblasts measured after 3h of culture and cell proliferation up to 14 days of culture did not show any statistically significant difference between the surfaces. The ECM proteins collagenI(CoI), fibronectin(FN) and tenascin(TN) were progressively produced between 1 and 14 days of culture on both surfaces with a greater expression of CoI and FN on the surface O. The preliminary results indicate that *in vitro* both the smooth and the Osseotite surfaces show a favourable interaction with human gingival fibroblasts supporting cell proliferation and differentiation and suggest that the microtopography of a dual acid-etched surface may not modify *in vivo* the biological behaviour of human gingival fibroblasts.

99

# **Emdogain improves the proliferation and differentiation of osteoblasts on the surface of titanium implants**

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**Background:** Enamel matrix derivative (EMD) supports periodontal tissue regeneration of cementum, periodontal ligament (PDL) and alveolar bone. The purpose of this study was to study the effect of EMD on osteogenesis and behaviour of osteoblasts on the surface of titanium implants.

**Material and methods:** Human osteoblast-like MG-63 cells were incubated on the surface of sand-blasted and acid-etched (SLA) titanium discs (Straumann Co) in culture medium containing EMD (50 and 100 µg/mL). After 48 hours the supernatants were collected and osteoprotegerin (OPG) and TGF-β were determined by ELISA Kits, alkaline phosphatase (AP) activity with an enzyme assay. Cells were stained with cell tracker orange and monitored under time-lapse microscopy for 6 days.

**Results:** Treatment with EMD resulted in higher levels of OPG and TGF-β that were produced from cells growing either on titanium surface or on the culture surface, compared to control groups in the absence of EMD ( $P < 0.01$ ). AP activity was enhanced by EMD ( $P < 0.01$ ). Cytofluorescence staining showed increased density of cells on the titanium surface in the presence of EMD in 3-day-old cultures and mineralized nodule formation after 5 days.

**Conclusion:** Our results demonstrated that EMD stimulates osteoblast-like cells on titanium surfaces to induce OPG, TGF-β and AP activity. This study gives evidence that EMD may positively participate in osseointegration and bone formation on the surface of titanium implants.

100

# **Bone healing around resorbable ceramic/autologous platelet lysate constructs: the role of anatomical site and of thrombin**

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The effects of autologous platelet lysates (APL) on the resorption of calcium carbonate ceramic particles (CP) and on new bone formation were investigated in an animal (rabbit) model at two critical-size defects: calvaria and femur. PL for autologous use was prepared according to published methods for human PL. Defects were filled with CP, CP plus APL, and CP plus APL with or without thrombin (THR). Bone formation and CP resorption were determined histomorphometrically after 6 weeks of implantation. Resorption of CP occurred under all conditions tested. Compared to respective CP alone, addition of APL resulted in significantly ( $P < 0.01$ ) higher CP resorption at both defect sites. The presence of THR prevented reduction of both CP diameter and number in the femoral sites. Addition of APL to the CP resulted in a significant ( $P < 0.03$ ) two-fold decrease in new bone area at the calvarial, but not at the femoral sites; moreover, when THR was added to the CP plus APL constructs, bone formation in was significantly ( $P < 0.05$ ) reduced. The results of this study provided evidence that APL affects bone healing when used with a resorbable ceramic material; the differences observed in CP resorption and bone formation between the two sites tested may be due to the differences in the respective cellular environment and mechanical loading. These results provide new information on the conditions needed to ensure the success of platelet concentrates used with biomaterials for bone reconstruction.

101

# **Different titanium surface treatment influences initial adhesion and proliferation of human osteoblast-like cells (MG-63). an *in vitro* study.**

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Surface characteristics play a prominent role in determining bone tissue response to dental implants and entirely to clinical outcome. The major objective of the present study was to determine the effect of four commercially available titanium surfaces: smooth-machined, osseotite, sand-blasted/acid-etched (SLA) and hydroxyapatite coated (HA) to initial cell attachment and proliferation. Six implants of each surface morphology were obtained from commercially available sources. Implants were incubated in semiconfluent cultures of human osteoblast-like cells (MG-63) for 1, 3, 7, 12 days. At the end of each period, implants were removed from culture media. Viable MG-63 cells attached to implant surface were calculated using the Trypan Blue Exclusion Assay. A Poisson regression analysis statistical model was employed to determine significant variations in attachment and proliferation rates for different surfaces and time intervals ( $P < 0.001$ ). Initial attachment for MG-63 cells (day 1) was significantly higher in HA surfaces ( $P < 0.001$ ). MG-63 cells proliferate on each different surface in a time dependent manner ( $P < 0.001$ ). For days 3, 7, 12 MG-63 cells proliferate in a significant higher rate on the SLA surface when compared to other surfaces ( $P < 0.001$ ). Although cells grown on all surfaces exhibited good adhesion and proliferation potential, a clear early adhesion profile was exhibited by the HA surface. Moreover, SLA surface revealed a unique long-term proliferation capability.



102

### Randomized comparison of periodontal indices for HA- or TPF-coated IMZ implants in 313 partially edentulous mandibles followed up for 5 years

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**Background:** The clinical effectiveness of bioactive coating of implants remains controversial, evidence from randomized controlled trial to compare the surfaces of implants is required.

**Materials and methods:** Hydroxyapatite (HA) and titanium-plasma-flame (TPF) coated intramobile cylinder (IMZ) implants have been compared in 313 partially edentulous mandibles in a randomized controlled trial. The patients had been followed up for at least 3 (average 5) years after placement of prostheses. Plaque-free, inflammation-free, and mobility-free intervals are compared between the implants and the abutment teeth as well. Kaplan-Meier survival curve and log-rank test are used for the analyses.

**Results:** Two year cumulative survival rates for HA and TPF implants are 29% (95% CI: 19–38%) and 46% (95% CI: 36–56%), respectively, for plaque-free interval, 49% (95% CI: 38–59%) and 50% (95% CI: 40–61%), respectively, for inflammation-free interval, and 99% (95% CI: 96–100%) and 97% (95% CI: 93–100%), respectively, for mobility-free interval. HA had a statistically significantly shorter plaque-free interval than TPF ( $P = 0.04$ ). No other statistically significant difference in terms of periodontal indices has been suggested between two coatings.

**Conclusion:** In restoration of partially edentulous mandibles, HA coated implants seem to attract plaque more easily than TPF-coated ones, while there are no differences with respect to the survival time of mobility and inflammation.

103

### Development of an injectable composite for periodontal regeneration

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**Aim:** Various biomaterials have been used to facilitate periodontal regeneration. Because several tissues constitute the periodontium it is challenging to find a single biomaterial that may fulfil basic requirements including biocompatibility, porosity, mechanical properties, and resorption rate to in concert support regeneration of alveolar bone, cementum, and the periodontal ligament. Ideally, the biomaterial is combined with suitable growth factor(s) that will orchestrate and accelerate regeneration of all relevant tissues. The present study describes the development of a novel injectable, *in-situ* setting composite combined with rhGDF-5 for regeneration of periodontal defects.

**Materials and methods:** Different composites consisting of bioresorbable poly (lactic-co-glycolic acids) (PLGA) and inorg. fillers were characterized in terms of porosity, mech. stability, degradation profile, and injectability. The stability of rhGDF-5 within the formulation and its release kinetics from the carrier were determined using RP-HPLC, SEC, and ELISA.

**Results:** Injectable, *in-situ* setting composites based on PLGA and inorg. fillers can be combined with rhGDF-5 to achieve a ready-to-use formulation that shows high porosity after application and degrades in an appropriate time to allow a maximum regeneration rate.

**Conclusion:** The results suggest that the formulation may have ideal properties to allow regeneration of all relevant tissues in periodontal defects.

104

### Osteoblast functions on functionalized PMMA-based polymers exhibiting *S. aureus* adhesion inhibition

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The effect of ionic composition on functionalized poly (methyl methacrylate) PMMA-based terpolymers on both *S. aureus* adhesion and osteoblast select functions were investigated, in order to identify biomaterial surface modifications which inhibit bacteria adhesion but do not interfere with osteoblast functions pertinent to new bone formation. Terpolymers bearing randomly distributed carboxylate and sulfonate groups were synthesized by radical polymerization, characterized by nuclear resonance spectroscopy and classified by the ratio  $R = [\text{COO}^-/\text{COO}^- + \text{SO}_3^-]$  in the range 0.5–0.8. *S. aureus* adhesion and select functions of rat calvaria osteoblasts were examined using established methods. Compared with results obtained on PMMA, adhesion of *S. aureus* on the functionalized terpolymers was highly (more than 90%) inhibited. In contrast, and compared to results obtained on PMMA, 4-hour adhesion of osteoblasts onto these terpolymers was decreased only by 20%. However, only osteoblast cultured on the PMMA-based terpolymers with  $R$  equal to 0.7 and 0.8 exhibited proliferation, alkaline phosphatase activity and calcium accumulation similar to that observed onto non-functionalized PMMA. The results provide evidence that since functionalized PMMA-based terpolymers with a ratio  $R$  of 0.7 and 0.8 simultaneously inhibit bacteria adhesion and support osteoblast functions, they could be used as coating of dental implants to render them both bone compatible and less vulnerable to bacterial infection.

105

### Bioactive implants: first clinical experience with a fluoridized surface

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The successful integration of enossal implants depends on several factors like, e.g., material, design and surface characteristics of the implant. An Astra Tech implant system with a fluoridized surface has been available since 2004. By means of this modified surface an improved osseous integration is rapidly achieved even in structurally weak osseous beds. This type of implant was applied in 17 patients at the Heidelberg University department of oral and maxillofacial surgery in 2003 within the scope of an international multicenter survey and before launch onto the market. Of a total of 53 implants, 12 were inserted into the mandible and 41 into the maxilla. All implants healed in transgingivally. Six weeks later the abutments were mounted, according to the manufacturer's instructions, with a force of 25 Ncm and provided with cemented superconstructions within two weeks. One implant, inserted into the mandible, with a length of 8 mm, was lost during the healing phase. All other implants remain in function. After one year in function they show the same clinical and radiological results as the Astra Tech implants with the traditional healing protocol, applied at the department of oral and maxillofacial surgery since 1992. The fluoridized implant surface allows an improved osseous integration in best time and even in structurally weak osseous areas.

# Posters: Diagnosis and risk factors

106

## Amlodipine and nifedipine used with cyclosporin a induce different effects on gingival overgrowth

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Gingival overgrowth (GO) is a side effect associated with administration of Cyclosporin A (CsA) and calcium channel blockers (CCB). This study aimed to determine and compare GO prevalence and severity in three groups of renal transplant recipients (RTR) who had been medicated with CsA, CsA-nifedipine and CsA-amlodipine.

**Methods:** A cohort of 93 RTR was selected. Thirty-one RTR had been medicated with CsA, 31 with CsA-nifedipine and 31 with CsA-amlodipine. Drug, demographic data and presence and severity of GO for each subject were recorded and photographs of the anterior buccal gingiva were taken.

**Results:** There were no significant differences for age, gender distribution, and CsA dose among the three groups. There were significant differences for GO prevalence and GO severity among the three groups. A higher proportion (90.3%) of the CsA-nifedipine group were categorized as having GO compared with CsA-amlodipine group (58.1%) and CsA group (51.6%). A greater percentage of subjects in the CsA-nifedipine group expressed severe GO (22.6%) when compared to the CsA group (0%) and CsA-amlodipine group (16.1%).

**Conclusion:** Prevalence and severity of GO in RTR maintained on CsA-nifedipine is significantly greater than those treated with CsA-amlodipine and CsA. The GO prevalence and severity is greater in CsA-amlodipine group than CsA group. This fact should lead the clinicians to avoid the use of these drugs together with CsA or to choose amlodipine instead of nifedipine.

107

## Hepatocyte growth factor in saliva is a novel marker of advanced periodontal disease

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**Background:** Hepatocyte growth factor (HGF) – a pluripotential reparative cytokine is a new factor in the pathogenesis and progression of periodontal disease (PD), due to the excessive stimulation of gingival epithelial cell growth and impaired regeneration of deep collagenous structures.

**Aims:** To compare for the first time: (i) HGF levels in unstimulated whole mixed saliva from PD patients vs. that from healthy subjects, and (ii) to test relationships between the salivary HGF levels and clinical indices in PD patients.

**Methods and results:** Immunoreactive HGF levels were determined in 26 patients with PD, and in 20 healthy subjects. Salivary HGF was detected in all PD patients and was 0.06–5.38 ng/mL (a mean of  $1.87 \pm 1.32$  ng/mL). In healthy subjects, HGF level was 0.68 ng/mL (full range: 0–7.33 ng/mL), and almost 3-fold lower as compared with that in PD patients (Mann-Whitney  $P < 0.0001$ ). Periodontal parameters in the patients were as follows: gingival index (GI) 2.0 (0–2.8), papillary bleeding index (PBI) 2.2 (0–3.2), plaque index (PI) 2.0 (0–3.0), and the loss of clinical attachment level (CAL) 4.7 (1.1–10.6) mm. Direct and logarithmic Spearman correlations between salivary HGF and GI ( $P = 0.004$ ), PBI ( $P = 0.046$ ) and PI ( $P = 0.001$ ), but not between HGF and the loss of CAL ( $P = 0.172$ ), teeth number ( $P = 0.279$ ) and patients' age ( $P = 0.362$ ) were found.

**Conclusion:** Salivary HGF concentration may be a novel, promising and the cause and effect-oriented marker of advanced periodontal disease.

108

## Evaluation of sampling methods for subgingival bacteria by real-time PCR

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**Objective:** To compare curette and paper point sampling of subgingival bacteria by quantitative Real-Time PCR.

**Materials and methods:** Twenty patients with chronic periodontitis ( $\geq 1$  maxillary incisor with PPD  $\geq 6$  mm) were randomised into groups A & B. Before scaling and root planning (SRP) and after 10 weeks samples in group A were taken first with a paper point then with a curette at the same site. In group B samples were taken in opposite sequence. Samples were analysed for *A. actinomycetemcomitans* (A.a.), *F. nucleatum ssp.* (F.n.), *P. gingivalis*, *P. intermedia*, *T. denticola*, *T. forsythia* (T.f.) and total bacterial count (TBC) by quantitative Real Time - PCR (meridol Perio Diagnostics®, GABA Int., CH).

**Results:** Curette samples yielded higher TBCs than paper points. The proportions of T.f. (group A: 3.5–6.5%; group B: 3.1–4.4%), and F.n. (group A: 1.8–3.8%; group B: 2.4–3.7%) were comparable with both methods, with only limited effects of SRP. A.a. was much higher before (4.6–9.9%; except PP group A) than after SRP (0.0–0.7%).

**Conclusion:** Within the limitations of the experimental design, we found a relatively good agreement between two sampling methods of subgingival bacteria as evaluated by the novel method of quantitative Real Time-PCR.

109

## Completely unerupted wisdom teeth increase the risk for periodontitis

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This study investigated the relation between the clinical and microbiological findings on the distal surfaces of 2nd molars (zone of contact to completely unerupted 3rd molars), and 1st molars and lower incisors (reference teeth). 202 subjects aged 13 to 65 years were examined clinically and microbiologically. The test group consisted of 144 subjects with a mean PD of  $> 3.5$  mm, whereas the control group was comprised of 56 subjects with a mean PD of  $< 3.5$  mm at the reference teeth. The subgingival samples were taken from the distal sites of 2nd molars and the mesiobuccal sites of 1st molars. Two samples were taken from the lower incisors. PCR was employed to detect F.n., A.a., P.g., T.f., T.d., and P.i. Compared to the control group, the subjects of the test group had a significantly greater number of periodontopathogenic species in both regions (test group 1.12 vs. controls 0.18). The risk for harbouring *P. gingivalis* in the reference sites is higher by a factor of 14.2 if these species occur on the distal site of the second molar (CI 95% 5.2–38.7).

Furthermore the risk for a periodontitis at the reference teeth is six times higher if at least one of the species is found on the distal site of a 2nd molar (CI 95% 2.5–14.4). The results of this study suggest that completely unerupted wisdom teeth could be associated with increased numbers of periodontopathogenic species and increased pocket depths at first molars and lower incisors.

110

**Evidence based diagnosis of dysfunction in periodontal patients**

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**Aim:** The aim of the study was to investigate the possible correlation between dysfunctional syndrome of stomatognathic system and periodontal diseases.

**Materials and methods:** The research was realized on 87 individuals, with ages between 18–72 years old, diagnosed with periodontal disease and one or more sign and symptoms of stomatognathic dysfunction. The diagnosis of oral dysfunction and periodontal disease is based on correlation between clinic and complementary exams (EMG, KMG, gnathosonic and radiological exam).

**Results:** The study showed a significant statistical relevance between periodontal disease and different degrees of malfunction of muscles, TMJ and occlusion.

**Conclusion:** Periodontal disease can be the consequence of TMD, but in the same time can be the etiologic factor for dysfunctional syndrome of stomatognathic system.

111

**Dental health and periodontal disease in pregnancy**

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**Background:** It is very important to know the oral status of pregnant women in order to recommend effective preventive measures.

**Aim:** To determine the prevalence of dental caries, gingivitis, periodontal disease and tooth loss from mothers who recently delivered live-born infants.

**Material and methods:** In the period from May to July 2005, a cross-sectional study was carried out at the Hospital Rancagua (Chile) among 610 pregnant women who recently delivered live-born infants. Following variables were recorded: age, number of children, the Decayed, Missing and Filled Teeth Index, the Gingivitis, Plaque, periodontal disease, and dental treatment during pregnancy. Examination and questions were designed to obtain information from mothers who recently delivered live-born infants.

**Results:** Prevalence of gingivitis was (66.0%), periodontal diseases was 93.2%. The number of tooth loss was upper jaw (833) and lower jaw (809). D.M.F. index was 10.39. Dental care use during pregnancy was 33.9%. Among mothers reporting a dental problem, 42 were treated for extractions and 142 for restorative treatment. Mother's age was 29.5% (15 to 20 years old); 26 % (21 to 25 years old); 21.8 % (26 to 30 years old); 12 % (31 to 35 years old); 10.7 % (36 to 40 years old).

**Conclusions:** Pregnancy was shown to be associated with a higher prevalence of gingivitis, and periodontal disease. Poor oral healths represent strong reasons for activation of dental health care in this period.

112

**Assay of the total antioxidant capacity (TAC) of crevicular fluid in healthy subjects and in periodontopathic patients**

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Oxidative stress is involved in the pathogenesis of numerous diseases, many of which of an inflammatory type, such as periodontal disease. Several studies have highlighted the influence

of free radicals and reactive oxygen species (ROS) on the pathogenesis of tissue damage in the course of inflammation. The aim of this study was to determine the total antioxidant capacity of gingival crevicular fluid (GCF) in a group of healthy subjects and to compare the findings with those from a group of periodontopathic patients. We enrolled 18 patients 6 m 12 f with clinically healthy gums and 15 patients suffering from moderate periodontitis, 7 m 8 f. GCF samples were collected using Periocol strips inserted in the sulcus and then placed in an Eppendorf test tube containing 10 µL of a solution of PBS Tween 20 at pH 7.4 diluted at 0.05%. The test tubes were vortexed and stored at -80 °C until analysis, which was carried out by a spectrophotometric method. GCF did not show high TAC as measured by the LAG phase, either in healthy or periodontopathic subjects. Some technical problems are likely related to difficulties in assaying the GCF protein content, to which results should be referred. This could be due to the binding affinity between paper and proteins, and by the fact that the strips are designed to measure the GCF volume of the sample and they may not completely release the absorbed material.

113

**The relationship between cotinine and periodontal disease in smokers and non-smokers**

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**Background:** Tobacco smoking is an established risk factor for periodontitis but self-reported smoking status may be inaccurate. Cotinine (Cot), a major metabolite of nicotine is a stable biochemical marker of smoking status. The relationship between Cot and periodontitis is unclear.

**Aim:** To clarify the relationship between Cot and mean probing pocket depth (PPD), loss of attachment (CAL), and recession.

**Methods:** We studied 135 adults (age  $55.4 \pm 12$  years) with moderate-advanced periodontitis. Plasma Cot concentration was measured using ELISA. Cumulative exposure to smoking was represented by cotinine years (CotY) where  $CotY = Cot \times \text{number of years smoked}$ . Associations were assessed using Pearson correlations.

**Results:** Smokers, when compared with non-smokers, were younger (mean age  $49.7 \pm 10.8$  vs.  $60.2 \pm 11.4$  years,  $P < 0.0001$ ) and had higher levels of plasma Cot ( $249.6 \pm 149$  vs.  $2.7 \pm 7$  ng/mL,  $P < 0.0001$ ). CotY was significantly related to mean recession ( $r = 0.31$ ,  $P = 0.02$ ) and a trend toward greater CAL ( $r = 0.24$ ,  $P = 0.06$ ) but not associated with PPD. No significant relationship was found between Cot concentration and the periodontal markers.

**Conclusion:** In this population, cumulative exposure (cotinine years) was associated with recession and a trend towards greater CAL although no significant relationship was seen with plasma Cot. Smokers had comparable periodontal disease at a younger age than non-smokers.

114

**Untreated periodontitis in diabetes adult patients in Lithuania- a pilot study**

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**Aim:** Natural course of periodontitis disease not interfered by complex periodontal treatment was studied in diabetes patients in Lithuania.

**Materials and methods:** Periodontitis was evaluated in a sample of 50 diabetes patients from Vilnius area. One calibrated examiner performed clinical recordings with a Florida Probe. Clinical parameters were oral hygiene index, by individual numbers of:

sites with bleeding on probing, pockets 5.5 mm or more, teeth with furcation involvement and number of mobile teeth. Non-clinical variables were smoking and satisfaction with oral health. Bivariate associations were assessed by means of Spearman's correlation.

**Results:** The number of deep pockets was statistically significantly correlated to oral hygiene index (0.509), number of bleeding on probing sites (0.587), number of teeth with furcation involvement (0.605) and to the number of mobile teeth (0.725). Smoking was significantly related only to oral hygiene index (0.280). The satisfaction with oral health was correlated to the individual number of teeth with furcation involvement. The mean number of pockets 5 mm or more was higher, although non-significant in a current smoker's group compared to a group of ex-smokers and never smoked group.

**Conclusion:** A few clinical indicators related to periodontal disease experience were frequently observed in Lithuanian diabetes patients. The high level of untreated periodontitis in diabetes patients indicates that this group of patients is a risk group.

115

### Oral bacteraemia caused by chewing in patients with periodontal disease

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**Background:** Transient bacteraemia caused by chewing is considered a risk factor for infective endocarditis (IE) in at-risk patients. Previous chewing studies have shown conflicting results in bacteraemia incidence (0%–55%) and have not classified the periodontal status of subjects. Most failed to obtain pre-chewing blood samples.

**Aim:** To determine if chewing in patients with untreated chronic periodontitis (CP) or plaque-induced gingivitis (GV) causes bacteraemia.

**Method:** 21 CP (32–75 year old) and 19 GV (26–54 year old) patients chewed a standard wax medium for 4 min. Blood samples were drawn before, during and 5 min post-chewing. Aerobic and anaerobic BACTEC system culturing was performed for 21 days and positive bottles subcultured and isolates identified to genus level. Following sampling, full periodontal analysis was performed (PPD, CAL, BOP, PI, GI, mobility). Radiographs were assessed for alveolar bone loss.

**Results:** No bacteraemia of oral origin was detected in any patients. Skin contaminants (*Staphylococcus epidermis*, *Propionibacterium spp*) were detected in three patients (2 CP, 1 GV). Mean CAL for CP was 4.4 mm  $\pm$  0.9 mm and GV was 2.5 mm  $\pm$  0.3 mm; BOP (CP 46%  $\pm$  14%, GV 19%  $\pm$  11%); PI (CP 1.9  $\pm$  0.4, GV 1.2  $\pm$  0.5); GI (1.9  $\pm$  0.2, GV 1.1  $\pm$  0.4). Total number of mobile teeth > Grade I was GV = 0, CP = 134.

**Conclusion:** Chewing did not cause bacteraemia in CP or GV patients and may not be a risk factor for IE, even in severe CP. (Grants from AustPerioResFndt, AustDentResFndt).

116

### Analysis of pain experienced during periodontal probing in smokers

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**Background:** The aim of this study was to investigate the factors that can affect the pain responses to periodontal probing and to establish whether smoking will cause different periodontal pain levels and discomfort.

**Methods:** The study population consisted of 40 patients 20–65 years old, with a negative medical history and with absence of current analgesic therapy. The following data were collected: the

smoking exposure, clinical periodontal and endodontic evaluation. The subject intensity and quality of the pain on periodontal probing was assessed using a Visual Analogue Scale (VAS).

**Results:** At site level statistical analysis revealed in both groups that pain responses to periodontal probing were statistically significant higher in older group (> 50 years), in females and in teeth with higher probing pocket depth, with higher percentage of sites with bleeding upon probing ( $P < 0.001$ ) and in vital teeth ( $P < 0.05$ ). Results indicated that approximal sulci of all teeth were the most pain sensitive ( $P < 0.05$ ).

**Conclusions:** These data suggest that pain on periodontal probing depends on several variables as age, gender, tooth type, site type, probing pocket depth, bleeding on probing, tooth vitality.

117

### *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* in periodontitis patients of Greek origin

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**Background:** Different distribution for *Porphyromonas gingivalis* (*Pg*) and *Actinobacillus actinomycetemcomitans* (*Aa*) might exist in various geographic locations.

**Aim:** To evaluate the levels of these pathogens in chronic and aggressive periodontitis patients of Greek origin.

**Materials and methods:** Data from 78 patients with chronic periodontitis (mean age 53.6  $\pm$  7.4) and 44 patients with aggressive periodontitis (mean age 35.23  $\pm$  6.81) never treated previously were included in the present study. *Pg* and *Aa* were quantified in 485 subgingival samples from chronic periodontitis patients and 394 subgingival samples from aggressive periodontitis patients previously analysed using the 'checkerboard' DNA-DNA hybridization. Quantification was performed using image analysis software (Total Lab 100, Nonlinear Dynamics).

**Results:** The sites from chronic periodontitis patients had mean probing depth 4.78  $\pm$  0.83 mm and the sites from aggressive periodontitis patients had mean probing depth 5.39  $\pm$  1.17 mm. Chronic periodontitis patients displayed mean and SEM of 2.72  $\times 10^5 \pm 4.1 \times 10^4$  bacterial cells of *Pg* and 1.19  $\times 10^5 \pm 2.1 \times 10^4$  of *Aa*, while the respective values for aggressive periodontitis patients were 2.7  $\times 10^5 \pm 0.27 \times 10^4$  and 0.87  $\times 10^5 \pm 0.2 \times 10^4$ . Counts for the two species did not differ between the two groups (Mann-Whitney test  $P > 0.05$ ).

**Discussion/Conclusions:** Subgingival samples from chronic and aggressive periodontitis patients of Greek origin displayed high counts of *Pg* and *Aa*.

118

### Subgingival calculus detection employing IR-laser fluorescence and a led-based optical probe *in vitro*

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Objective subgingival calculus detection techniques are desirable for systematical periodontal treatment procedures. Therefore, the aim of the present study was to compare two systems for detecting subgingival calculus evaluating both sensitivity and specificity. 20 freshly extracted human teeth with calculus on the root surface were fixed in an artificial pocket model. Measurements on the root surfaces were performed with the Detectar<sup>TM</sup>-system (DE) and the Diagnodent<sup>TM</sup>-system (DI) using a novel periodontal tip. Each tooth was scanned in increments of 1 mm with an angulation of 0°–10° between the tip of the optical fiber and the root surface. The values of the blindly examined root surfaces were compared to the clinical findings. Clinically apparent calculus on the root surface

was detected by both systems ( $P < 0.05$ ). Regarding the exact position of mineralized deposits, sensitivity for calculus detection of 30% (DE) and 46% (DI) and a specificity of 99% (DE) and 95% (DI) were observed. However, irrespective of the exact localisation of calculus, sensitivity was 61% (DE) and 78% (DI), values for specificity was 95% and 80% respectively. The present study indicates that the systems do not appear to allow a reliable assessment of the exact localisation of mineralized deposits. However, both systems offer the possibility of subgingival calculus detection and may, therefore, be suited to determine the endpoint of root surface instrumentation during non-surgical periodontal therapy.

119

### Relationship between unstimulated and stimulated whole saliva, DMFT and CPI in young adults

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**Aim:** Saliva is considered to be an important factor in oral health. Few studies have investigated the correlation between salivary function, periodontal health and caries. There is no conclusive data on gender differences in salivary flow, and the correlation to periodontal disease and caries.

**Material and methods:** It was the goal of this study to evaluate possible differences between genders in unstimulated and stimulated salivary flow rate, periodontal status (by means of Community Periodontal Index, CPI), and caries (by means of Diseased, Missing and Filled Teeth index, DMFT), in a young population (mean age  $23.8 \pm 1.1$ ).

**Results:** Males show more increase in salivary flow rate after stimulation, but there is inconclusive data concerning salivary flow rate and CPI and DMFT values. It seems that subjects with healthy periodontium have lower DMFT values.

**Conclusion:** Saliva represents an important mechanism of periodontal and dental health. Although there were no statistically significant differences between genders, it is believed that males are not so prone to regular dental check-ups, but there is a drive to encourage gum chewing, as the values of sugarless gum chewing stimulated salivary flow rate is 50% greater than ascorbate stimulated salivary flow rates.

120

### Association between labial frenum insertion and mucogingival problems

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**Introduction:** The labial frenum is a fibrous band of connective tissue, muscular or both, which joins the lip with the alveolar mucosa or gingiva. It can be related with periodontal, prosthetic, orthodontic or phonetic problems. Placek presented a classification of the labial frenum, depending on whether its insertion was on mucosa, attached gingiva, interdental papilla or papilla-penetrating.

**Aim:** The purpose of this study is to analyse if there are significant differences related to clinical attachment level, height of attached gingiva, or gingival width, of the adjacent teeth, depending on the type of labial frenum insertion.

**Material and methods:** An examination of subjects from the Universitat Internacional de Catalunya was made and prevalence of the labial frenum attachment was determined. The following measurements were examined in each subject: type of the frenum attachment as mentioned above, probing depth, attachment level, height and width of attached and queratinized gingiva.

**Results:** Means were obtained for each type of labial frenum and repeated measures analysis of variance (ANOVA) was used to determine if the different periodontal parameters were correlated with labial frenum classification.

**Discussion:** An extent periodontal exploration should consider the type of the frenum attachment and its relation with the periodontal tissues. This simple consideration should help us to detect future mucogingival problems.

121

### Comparison of the qualitative PCR technique results from two independent laboratories

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**Introduction:** An accurate diagnosis is of utmost importance in order to give patients the best treatment in each case. Then, the development of molecular techniques to detect the main specific microorganisms that are found in periodontal diseases has meant a great advance as a possible diagnostic tool.

**Aim:** The aim of this study is to set the validity and usefulness of the qualitative PCR technique as a good and reliable diagnostic tool.

**Material and methods:** Thirty patients from the school of dentistry were evaluated. Samples from the same localization and taken at the same time were sent to two independent laboratories in order to detect specific periodontal pathogens using a qualitative PCR technique.

**Results:** The provisional results show only few cases where the results provided from the two laboratories were similar.

**Discussion and conclusion:** With the limitations of the study, we question severely the usefulness and validity of the qualitative PCR technique as a good and reliable diagnostic tool.

122

### The analysis of the relationship between smoking and periodontal disease according to serum cotinine levels

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The aim of the study was to evaluate the relationship between chronic periodontal disease and smoking in regard to serum cotinine levels (SCL) and to analyse the effect of co-existence of these two situations on the biochemical parameters that reflect systemic health. In the clinical part of the study, plaque index (PI), gingival index (GI), probing pocket depth (PPD), bleeding on probing (BOP), clinic attachment level (CAL), gingival recession (GR), calculus index (CI), have been examined in order to determine the periodontal situation of patients with chronic periodontitis. In the laboratory part of the study, SCL, taken from subjects have been analysed. Triglyceride, HDL-C, LDL-C, VLDL-C values have been analysed to determine cholesterol level and haematological analyses have been investigated. Results derived indicate that SCL of the patients was significantly associated with daily tobacco consumption (DTC). Although no association of smoking with PI and CI observed, its inverse association with GI and BOP levels, and significant positive association with PPD, CAL and GR have been found. SCL was also significantly associated with CAL and PPD, and this result has displayed the relationship between smoking and periodontal destruction on a biochemical basis. SCL was significantly inversely related to systemic HDL-C, and significantly positively related to WBC and eosinophil amount. DTC was also significantly associated with haemoglobin, hematocrite, MCH and leucocyte number.

123

### Prevalence of gingival stippling in children

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**Aim:** In this cross-sectional study prevalence and characteristics (morphometric data, distribution on gingiva, relation to oral hygiene and gingival inflammation) of gingival stippling in children were determined.

**Material and method:** Two hundred and seventy four children (♂ 148, ♀ 126) aged 0 to 10 years (mean age 4.98) were examined. API, PBI, dmft/DMFT and race were obtained. Stippling, documented by photo camera or replica (Affinis light), was scanned and analysed regarding density, height and width of the stipples with the software analysis 3.0. For statistical analysis  $\chi^2$ -Test ( $P < 0.05$ ) was used (SPSS 12.0).

**Result:** Stippling was found in 68.2% of the children and was evident from an age of 1 year. There was no relation between stippling and sex, race, caries, oral hygiene or gingival inflammation. It was more frequently in mixed dentition and in anterior region as well as in upper jaw. It was mainly localized to the attached gingiva (interradicular > radicular > subpapillar area). Morphometric data showed a density of 2.54 stipples/mm<sup>2</sup>, a height of 0.10 mm and a width of 0.09 mm. Older children had a higher density. Height and width were not influenced by sex or by age.

**Conclusion:** Stippling is a normal characteristic of healthy keratinized gingiva. In our study it appeared at an age of 1 year and increased with age. But it cannot be used as a major criterion in clinical assessment of gingival or periodontal inflammation, because missing stippling does not always coincide with presence of inflammation.

124

### Microbiological testing: changing the target. Exploring antibiotic resistances in a sample of periodontal patients in Spain

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**Initial background:** The adjunctive use of certain antibiotics (Amoxicillin + Metronidazole) in the treatment of periodontitis, especially in the most aggressive forms, has shown to be effective especially when certain putative periodontal pathogens (*P. Gingivalis*) are present. But some patients do not benefit from this treatment, maybe because of the absence of these specific periodontal pathogens. One of the virulence factors of the bacteria is the quantity. Based on this, we consider our target the most common bacteria in the sub gingival plaque of our patients and we wanted to know its sensitivity to the commonly used antibiotics.

**Materials and method:** We took sub gingival plaque samples of 234 consecutive periodontal patients. We search for the most common bacteria of the sample. Antibiotic sensitivity test was carried out in each of the cases. Clinical parameters were also recorded at the time of the first visit (baseline) and at the end of the periodontal treatment.

**Results:** The result showed 44 different bacteria, almost equally distributed along the sample, and a very high incidence of resistance for the most commonly used antibiotics, especially Metronidazole (89.3%).

**Discussion/conclusion:** Due to the high incidence of resistance to certain antibiotics in our sample we consider microbiological testing and antibiogram desirable, especially if we want to use antibiotics as an adjunctive treatment in our periodontal patients.

125

### Periodontal status of patients receiving hemodialysis at Rancagua hospital, chile

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**Background:** The number of patients on dialysis is growing. Good dental hygiene reduces the risk of oral infections. Healthy dentition becomes increasingly important when a patient is a candidate for renal transplantation. The incidence of periodontal disease seems greater among dialysis patients.

**Aim:** The aim of this study was to examine the levels of periodontal status of patients on hemodialysis at Rancagua hospital.

**Method:** Completion of a questionnaire and a non-invasive oral examination was obtained from all hemodialysis patients at Rancagua hospital, between March 1 and June 30 of 2005. A total of 36 patients, were examined for gingivitis, gingival index (GI), the periodontal index (CPITN), Oral Hygiene and DMFT index. Information gathered from the medical chart included the patient's age, sex, duration of dialysis, dialysis modality and so on.

**Results:** The mean age of dentate patients was 49.9 and 64 year in edentulous. Mean dialysis duration (months) was 28.5. Most hemodialysis patients have complex medical conditions, including hypertension and diabetes and polycystic kidney disease. The GI revealed 2.29 scored, CPI of 2.34, and almost all (90%) had gingivitis. Dental hygiene and frequency of dental control found was regular to bad. 72.2% of dental visit was for pain and only 27.8% or prevention. The group DMFT index mean was 16.8.

**Conclusion:** Gingival and periodontal diseases are prevalent in this patients studied. They need more dental prevention.

126

### The potential impact of the sampling site on the resting volume of gingival crevice fluid

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**Background & aim:** Volumetric features of gingival crevice fluid are influenced by a vast majority of factors, including various sampling variables. As attempts to standardize such factors may enable a more precise methodology, the possible impact of the clinical periodontal status and the distinct location of sampling site on fluid volume was analysed.

**Materials & methods:** Clinical parameters were recorded and fluid samples were obtained from 931 maxillary sites. The potential site-specific volumetric differences among healthy, gingivitis and periodontitis sites and between multi-rooted or single-rooted teeth and mesio-buccal or disto-buccal sampling sites, and the correlations between volume and clinical measures were statistically analysed.

**Results:** Although volume increased in a disease-related pattern (healthy < gingivitis < periodontitis) ( $P < 0.05$ ), the distribution range of volume was wide with prominent overlaps between different clinical periodontal conditions. Multi-rooted teeth presented more fluid volume, and even mesio-buccal or disto-buccal sites exhibited volumetric differences ( $P < 0.05$ ). Constant correlations between fluid volume and clinical parameters could only be observed at gingivitis sites ( $P < 0.05$ ).

**Discussion/conclusion:** These findings support the disease-related spectrum values of fluid volume and the clear impact of the unique dimensional features of different teeth groups and the distinct sampling site on the volumetric features of this biologic fluid.

127

### Correlation of five gene polymorphisms with periodontal conditions in a Greek population

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**Background:** Various studies have examined possible correlations between a number of cytokine gene polymorphisms and periodontal disease in populations of different origins. This study sought the correlation between four Single Nucleotide Polymorphisms (IL-1A + 3954, IL-1B + 4845, TNF-A -308, COL1A1 Sp1) and a Variable Number of Tandem Repeats Polymorphism (IL-1RN intron 2) and periodontal conditions in subjects of Greek origin.

**Methods:** One hundred and ninety two healthy subjects representing four periodontally distinct conditions (healthy, gingivitis, chronic and aggressive periodontitis) participated in the present study. Genotyping was performed by PCR based techniques using primers and conditions described in the literature. The frequencies of genotypes and alleles between study groups were compared using Genepop genetic software. Nei's genetic distances were calculated using GeneAlex genetic software. Data were also analysed with subjects stratified as smokers or non-smokers.

**Results:** No differences were observed among the four groups concerning the distributions of alleles, genotypes and haplotypes under investigation, regardless of smoking status.

**Conclusions:** Carriage rates of the polymorphisms under investigation in healthy Greek subjects are within the range reported for Caucasians but these polymorphisms cannot discriminate between four distinct periodontal conditions. Our findings do not support the incorporation of testing for these polymorphisms in clinical practice.

128

### Screening for pre-diabetic and diabetic patients using a capillary blood glucose meter in a specialist periodontal practice

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Diabetics who are undiagnosed or uncontrolled can suffer severe periodontal disease. This study aimed to detect type II pre-diabetic or diabetic patients using an Accu-chek® Advantage blood glucose meter in 280 consecutive new patients referred to a specialist periodontal practice. Patients were asked if they were high risk for type II diabetes based on the following criteria: >55 years old; >45 years with high blood pressure; >45 years, overweight and/or elevated blood fats; >45 years and an immediate family member with diabetes; has/had heart disease or a heart attack; has/had high blood sugar during pregnancy; has/had borderline blood glucose levels; has polycystic ovary syndrome and overweight; >35 years old and Aboriginal, a Torres Strait Islander, from the Pacific Islands, Indian subcontinent or of Chinese origin. From 159 patients who admitted to being high risk 116 (73%) agreed to be screened. The maximum probing depth was recorded for each patient (mean = 7.3 mm). Diabetes had already been diagnosed in 14 patients (4 = type I; 10 = type II). Blood glucose levels  $\geq 5.7$  mmol/L were detected in 54 patients (46%) signifying possible impaired fasting glucose, impaired glucose tolerance or diabetes (>11 mmol/L). Thirteen patients (11%) had readings between 7 and 18.9 mmol/L. Patients with >5.7 mmol/L were referred to their medical practitioner for further testing. Capillary blood glucose screening helped identify potential and unstable diabetic patients in a specialist practice.

129

### Risk indicators of aggressive periodontitis in Syrian adolescents

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**Background:** Little is known about the etiological factors and risk indicators of aggressive periodontitis (AgP) in developing countries. We assessed the occurrence and characteristics of this disease among a representative group of youths in Syria.

**Material and methods:** 1,795 subjects 11–18 years of age in 27 public schools were randomly selected from four districts in Syria, and were clinically examined to assess the attachment loss at six sites on all permanent teeth. Subgingival dental plaque at six deepest pockets was collected using sterile paper points from cases and controls, and the presence of the putative pathogens *Actinobacillus actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi), *Tanarella forsythensis* (Tf), and *Treponema denticola* (Td) was assessed. Epithelial cells were collected by buccal swab, and the occurrence of IL-1 $\beta$  + 3953, IL-1 $\beta$  + 511, and IL-1 $\alpha$ -889 polymorphisms were investigated.

**Results:** Forty-nine (2.7%) subjects had clinical features of aggressive periodontitis. The disease was more prevalent among females (3.4%) than males (2%) ( $P = 0.07$ ), and in subjects with a large family size ( $P = 0.01$ ) and poor education ( $P = 0.02$ ). Only Td showed significant association with AgP. A significantly higher frequency of IL-1 $\beta$  + 511 allele 2 polymorphism was found in cases than controls ( $P < 0.05$ ).

**Conclusion:** The findings suggest significant association of AgP with socio-economic factors and polymorphism of IL-1 $\beta$  + 511 allele 2.

130

### *Actinobacillus actinomycetemcomitans* as indicator for aggressive periodontitis

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**Objective:** Comparison of the subgingival microflora in aggressive and generalized severe chronic periodontitis.

**Methods:** In 41 patients (22 female) suffering from untreated aggressive (AgP: 17) or generalized severe chronic (ChP: 24) periodontitis subgingival plaque samples were obtained from the 4 pockets with the deepest probing depths. Two sterile paper points were inserted simultaneously in the periodontal pockets. One paper point from each pocket was put in a separate transport-vial, the second was pooled with the three other paper points of a respective patient in a transport-vial. The content of each vial was analysed separately for *Actinobacillus actinomycetemcomitans* (AA), *T. forsythensis*, *P. gingivalis*, and *T. denticola*.

**Results:** Using the separate analysis the detection rate of AA was statistically significant higher in AgP than in ChP ( $P = 0.012$ ). Further, log-transformed numbers of AA were lower for ChP than AgP ( $P = 0.067$ ). Detection of AA as a diagnostic test for AgP had a sensitivity and positive predictive value of 65%. For all other tested pathogens with neither sampling strategy the analysis failed to detect statistically significant differences between AgP and ChP regarding log-transformed bacteria numbers and detection frequency.

**Conclusion:** The AA was detected in higher numbers and frequency in AgP than in ChP. Detection of AA may confirm the clinical diagnosis and influence choice of therapy. To be used as a diagnostic test alone its sensitivity was too low.

131

### CARD15 gene variants in aggressive periodontitis patients

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**Background:** The *CARD15* protein acts as an intracellular receptor of bacterial products. *CARD15* gene variants have been shown to be associated with Crohn's disease (CD), an inflammatory bowel disease.

**Aim:** The study aimed to investigate the association of the three most common mutations of the *CARD15* gene (R702W, G908R, and L1007fsX1008) with aggressive periodontitis (AgP).

**Methods:** Eighty six patients with AgP as well as 67 periodontal healthy controls were genotyped for the three main mutations in the *CARD15* gene. The carrier and allele frequencies were compared between the study groups.

**Results:** The carrier and allele frequencies of the three variants in the groups were not statistically significant different. All study subjects were further classified into carriers and non-carriers of at least one of the three mutations. No significant difference was found between the groups. Additionally, a logistic regression analysis adjusted for gender and smoking showed no association between carriers of any of the studied variants and periodontal status.

**Conclusion:** Since similar pathogenic mechanisms may be involved in both CD and periodontitis, a common genetic background may be inferred. However, our results reject the hypothesis that the major *CARD15* gene mutations are involved in generalized AgP susceptibility. Our data find support on two recent studies that could not show an association between *CARD15* gene mutations and chronic periodontitis (Folwaczny et al. 2004, Laine et al. 2004).

132

### Association between genotype and microbacterial profile of patients with periodontitis – an introductory report

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Polymorphism of IL-1 seems to have an influence on the progression of periodontal disease. Oral mouth microflora can also have an effect on the disease process. The aim of the work was to evaluate the association of microbacterial pathogens in the periodontal pockets of patients with positive and negative genotype with their clinical status. Study group comprised of 16 patients, aged 25–50 years. Only patients with severe generalized form of chronic periodontitis were included into the study. After clinical examination patients were subjected to the IL-1 genotype evaluation (Genotype PST, Hain Lifescience GmbH, Germany) and PCR examination of selected bacteria pathological for periodontium (Perio-Analyse, Pierre Fabre Medicament, France). 7 out of 16 individuals were diagnosed as genotype positive. No association is observed between clinical parameters and the genotype of the patients. Total count of bacteria from so called 'red complex' (*P. gingivalis*, *T. forsythensis*, *T. denticola*), and 'orange complex' (*F. nucleatum*, *P. micros*, *P. intermedia*, *C. rectus*) were respectively 2, 4-fold and 1, 3-fold higher in group with positive genotype, despite the fact that group was smaller (7 vs. 9 persons with negative genotype). Number of bacteria seems to correlate with plaque and bleeding indices and percentage of pockets deeper than 4 mm. Observed association may have an influence on increased severity of periodontal disease in patients with positive genotype.

133

### Lip piercing: periodontal complications and etiologic factors

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**Background:** There are only limited studies assessing the prevalence of periodontal complications in association with lower-lip studs. This clinical controlled trial investigated the prevalence of periodontal complications of mandibular incisors and their possible etiologic factors in association with lower-lip studs.

**Methods:** The study involved 88 females and 12 males (mean age  $21.76 \pm 2.66$ ). The test group ( $n = 50$ ) included subjects with a lower-lip stud, controls ( $n = 50$ ) were matched according to sex, age and smoking status. Patients with a prior diagnosis of periodontitis, need of antibiotic prophylaxis, pregnant or lactating women were excluded. Clinical examination included plaque indices, bleeding on probing, probing depth, recession, attachment level, width of keratinized gingiva, gingival biotype, insertion of the frenulum, evaluation of the hard tissues, trauma of occlusion, characteristics of the stud, radiographs and photographs.

**Results:** Recessions of the test group were noted in 68% as compared to 4% in controls ( $P = 0.001$ ). Localized periodontal disease limited to the mandibular incisors was noted in 4% of the test group ( $n = 2$ ). Stud-closures positioned on the CEJ, and time of wear showed a significant association to gingival recessions.

**Conclusion:** The prevalence and severity of gingival recessions is strongly associated with labial piercing. The position of the intraoral disc and time of wear might be a significant predictor for the development of recessions.

134

### Factors associated with oral hygiene and gingival health in flemish pre-school children

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**Objectives:** The study aimed to identify factors associated with dental plaque accumulation and gingival health in young children.

**Methods:** Cross-sectional data from 1250 3-year old and 1283 5-year old children from four geographical areas in Flanders (Belgium) were analysed. Children were examined at school by eight trained dentist-examiners, using standard criteria and calibrated examination methodology. Buccal plaque accumulation and gingival health were assessed on six index teeth. Data on oral hygiene and dietary habits, oral health behaviour and socio-demographic variables were obtained through structured questionnaires, completed by the parents.

**Results:** Roughly, 31% of 3-year-olds and 37% of 5-year-olds presented with visible plaque accumulation. In both age groups, only 3 to 4% of children presented with signs of gingival inflammation. Multivariable logistic regression models revealed that brushing frequency had a significant impact on plaque accumulation in both age groups; in 3-year-olds, other significant covariates were age, educational level of the mother and frequent consumption of drinks. With gingival health as dependent variable, multivariable logistic regression analysis revealed that plaque accumulation as well as the age at start brushing had a significant effect in 5-year-old children.

**Conclusion:** Parents should be motivated to start brushing the dentition at an early age and brush frequently in order to maintain good oral health in their off-spring.



135

### Proximal caries in localized aggressive periodontitis Moroccan patients

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A number of studies have made an effort to demonstrate an association between caries and periodontal disease. Some studies have found no association while others have found a positive association between the two diseases. In order to determine if an inverse relationship (Fine and coll, 1984) exists between periodontal disease and proximal caries in a group of Localized aggressive periodontitis (LAP) patients as compared to a group of control patients (CP), 55 subjects (14–26 years old) were examined in the Dental Health Centre of the university of Casablanca. 33 were LAP affected and 25 were periodontally healthy. In these patients the plaque Index (PI) (silness and loe, 1964), the gingival index (GI) (loe and silness, 1963) and the number of decayed, missing and filled teeth were assessed. Carious lesions were assessed using radiographic evaluation (bite wing) (Marthaler and German, 1970). Concerning the mean value of PI, it appears that there is no difference between the LAP and control. Concerning the periodontal status, the GI revealed no significant difference between LAP and control. Concerning the dental status, no statistically significant difference occurred when the total number of decayed missing and filled teeth was compared in the two groups. In the opposite, results indicated that LAP patients had significantly less proximal decay than the control group.

136

### Effects of zinc-deficiency on oral tissues and periodontal diseases in rats

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The aim of this study was to investigate the alterations of the oral tissues in zinc-deficient rats and to compare these results with those of control rats. The study was carried out on 14 Sprague-Dawley rats, cessation of lactation on 24th day of birth. Rats were randomly divided into two groups. Group I rats were fed with a Zn-deficient diet, and Group II rats with a Zn-containing diet. At the end of the fourth week on experimental diets, alterations of the oral tissues in both groups were recorded and oral tissue samples were investigated by light microscopy. Then, the blood samples were taken and serum Zn levels were measured by atomic absorption spectrophotometry. The zinc-deficient findings were observed at the 10–16 days in rats. Although body weight, body length and tail length were retarded in zinc-deficient rats, they were advanced in rats fed with a Zn-containing diet. Although, the mean PI and GI for Group II was lower than that for Group I ( $P < 0.001$ ), there was not a significant difference as regards the PPD between two groups of rats. According to histological findings, there was no any difference related with the epithelial keratinization of the hard palate between the two groups. However, hyperkeratosis was found on the dorsal surface of the tongue in zinc-deficient rats. The findings indicated that oral health was better in Group II rats than that in Group I rats. We suggest that Zinc-deficiency can be a potential risk factor for oral and periodontal diseases.

137

### Identification of four periodontopathogens in adults with advanced chronic periodontitis

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**Objectives:** To identify *Porphyromonas gingivalis* (Pg), *Tannarella forsythensis* (Tf), *Actinobacillus actinomycetemcomitans* (Aa) and

*Prevotella intermedia* (Pi) in subgingival plaque of three ethnic groups of Malaysia (Malays, Chinese, Indians) with Advanced Chronic Periodontitis and to correlate these findings with their periodontal status.

**Methods:** Thirty diseased adults were age, sex and ethnically matched with 30 healthy individuals. Clinical parameters were assessed for all. Subgingival plaque samples were collected from each subject for identification of Pg and Tf (on only 44 subjects) and Aa and Pi using PCR (Polymerase chain reaction).

**Results:** Prevalence for Pi was (83.3%); Pg (59%); Tf (54.5%) and Aa (6.7%) in the total subject population. Pg, Tf, Pi and Aa were more prevalent in diseased (87.5%, 81.9%, 86.7%, 10% respectively) than in healthy (30.5%, 26.4%, 80%, 3.33% respectively) subjects. There was a significant association between Pg (OR = 21.5) and Tf (OR = 11.1) with diseased status. Among the ethnic groups, only presence of Pg was significantly associated with Indian subjects. When tested by General linear model analysis, Pg had a significant causal effect on PPD ( $P < 0.01$ , Observed power = 0.99) and CAL ( $P < 0.01$ , Observed power = 0.99) while Aa had a moderately significant causal effect on CAL ( $P < 0.05$ , Observed power = 0.68).

**Conclusion:** *Porphyromonas gingivalis* was significantly associated with the Indian subjects. Pg had a significant causal effect on PPD and CAL while Aa had a moderately significant causal effect on CAL.

138

### Microbiological pattern and periodontal pathogens resistance to metronidazole in patients needing prosthetic treatment

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**Aim:** Evaluating the microbiological pattern, antibiotic sensitivity and resistance with culture methods in a group of 14 adult patients affected by aggressive periodontitis requesting prosthetic treatment. Supragingival debridement was performed with curettes and sonic devices, sterile paper points were inserted in two pockets, removed after 40 sec, inserted in test tubes, in a vial of anaerobic media and brought to a microbiological laboratory. Culturing assessed the main periodontal pathogens. Susceptibility to the following antibiotics was tested: Penicillin G, Ampicillin, Amoxicillin, Amoxicillin/Clavulanate, Metronidazole, Clindamycin, Cloramfenicole, Imipenem, Cefotetan, Cefoxitine, Piperacilline. Among the bacteria identified 12 were Tf, 14 were Pg, 2 Aa, 12 Td, and 2 Pi. 11 out 14 patients showed the 'red complex' (Tf, Pg and Td), in two cases presenting also Pi. All the bacteria showed normal susceptibility to all the drugs except for metronidazole. Regarding the 'red complex' in 10 out 11 cases Tf showed resistance to metronidazole, in another one Pg was resistant. In one patient with the only Pg this bacteria was also resistant and in one patient with Aa and Td Aa was resistant to metronidazole. There was a dramatic and clinically relevant antimicrobial resistance to metronidazole in most of patients that did not allow to use the combination amoxocilline and metronidazole together

139

### Characterization of progressive periodontal lesions in chronic periodontitis patients

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**Aim:** The aim of this work was to determine the quimiokines, cytokines, MMP-13, periodontal pathogens and inflammatory cells

levels in periodontal sites characterized by active periodontal connective tissue destruction.

**Materials and methods:** Periodontitis was characterized by at least six sites with probing depth 35 mm, clinical attachment level 33 mm, and radiographic bone loss. Selected patients had not previous periodontal therapy, antibiotic treatment during last 6 month, any systemic disease, and pregnant. Periodontitis progression was determined by tolerance method. Samples of gingival crevicular fluid, subgingival plaque and a gingival biopsy according to surgical requirements were obtained from active sites. RANKL, MPC-1, TNF $\alpha$ , IL-1 $\beta$ , MMP-13, *A. a.*, *P. g.*, and inflammatory cells levels were determined. Data were expressed as mean  $\pm$  SD and *t*-test and chi-square test were used.

**Results:** Twenty one active sites were detected. Higher quimiokines, cytokines and MMP-13 levels were observed in active sites vs. inactive sites ( $P < 0.05$ ). Although statically non-significant, not only *Actinomyces comitans* and *P. gingivalis* but also lymphocytes and macrophages levels were higher in active than inactive sites.

**Conclusion:** In an active periodontal site, which is characterized by attachment loss 32 mm after 2 month clinic following, there are cellular and molecular markers that are characteristics and different to inactive sites.

This work was financed by Fondecyt 1050518 project.

140

### New perspective on gingival overgrowth

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Gingival Overgrowth (GO), mainly medicamentously induced, is still problem of nowadays. GO occurs during immunosuppressive (Cyclosporin A, Azathioprin, Mycophenolate), antihypertensive (Calcium Channel Blockers, e.g. nifedipine) and anticonvulsive therapy (Phenytoin). It is very complicated status, where many factors play important role, e.g. sex, duration of treatment, parallel medication, hormones, genetic.

**Aim1:** We decided to establish new classification of GO. Our scale has four stages: 0-without any lesion, 1-lobulation localized on interdental papilla, 2-GO to 1/3 of clinical crown, 3-GO to 2/3 of clinical crown, 4-GO to incisal (occlusal) line.

**Results1:** We evaluated CPITN, PBI, GO and X-ray index in 40 patients. There were some correlations in the data (sex, time after transplantation).

**Aim2:** Not only proliferation of the cells has effect on GO. On the other side there should be the key for controlling proliferation/apoptosis. The other our view was focusing on apoptosis (Caspase molecules).

**Results:** We found some differences in the groups of patients (antihypertensive, immunosuppressive and anticonvulsive therapy).

**Conclusion:** It can be guidance for the treatment induct with focusing on interdisciplinary coordination between doctors and periodontists.

141

### Reduced antioxidant defense in serum and gingival crevicular fluid in menopause and chronic periodontitis

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Recent evidence suggests menopause-oxidative stress, menopause-periodontal destruction links and increased reactive oxygen species generation and oxidative stress in periodontitis. Serum and gingival crevicular fluid (GCF) total antioxidant capacity (TAOC) and superoxide dismutase (SOD) concentrations were compared between pre- and postmenopausal women with chronic periodon-

titis. 32 postmenopausal chronic periodontitis (PMCP), 31 premenopausal chronic periodontitis (CP) patients, 25 periodontally healthy postmenopausal controls (PMPH), 26 premenopausal controls (PH) were studied. After clinical measurements and samplings, TAOC and SOD were measured by automated and spectrophotometric tests respectively. Clinical parameters were higher in periodontitis groups. There was no significant difference between PMCP and CP groups. TAOC and SOD were the lowest in PMCP and the highest in PH groups ( $P < 0.05$ ). The values were lower in postmenopausal women than in periodontally matched premenopausal women, in periodontitis than in controls ( $P < 0.05$ ). Moderate correlations were found between clinical parameters and AO values ( $P < 0.05$ ). TAOC and SOD were significantly reduced in both menopause and periodontitis. Menopause seemed more related with AO reduction in serum while periodontitis seemed more effective in GCF. The findings showed a clear reduction in systemic and local AO defense in menopause and periodontitis. The lowest values in PMCP group suggest menopause a risk factor for periodontitis.

142

### The correlation of gingival crevicular fluid matrix metalloproteinase-8 and prostaglandin E<sub>2</sub> levels with each other and clinical parameters

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**Background:** Chronic periodontitis arises from the interaction between bacterial factors and the host response. Perpetuation of this immunoinflammatory response in susceptible host disrupts homeostatic mechanisms. In return inflammatory mediators such as matrix metalloproteinase-8 (MMP-8) and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) are released. Since levels of both mediators are shown to be higher in active and lower in inactive periodontal sites, they can be implicated as useful parameters in the assessment of disease activity. The purpose of this study is to further assess the relationship between GCF MMP-8 and PGE<sub>2</sub> levels and clinical parameters.

**Methods:** GCF samples were obtained from 640 interproximal sites with at least 4 mm pocket depth in 40 chronic periodontitis patients. Plaque index, sulcus bleeding index and probing pocket depth were recorded. Half of the samples were used for MMP-8 and the remaining were analysed for PGE<sub>2</sub> detection with enzyme immunoassay method. The correlations of MMP-8 and PGE<sub>2</sub> levels with each other and with the corresponding clinical recordings were evaluated using Linear Regression Analysis.

**Results:** GCF MMP-8 and PGE<sub>2</sub> levels exhibited positive correlations with clinical parameters ( $R_{sq} = 0.79$  and  $R_{sq} = 0.76$  respectively) ( $P < 0.05$ ) and with each other ( $R_{sq} = 0.86$ ) ( $P < 0.01$ ).

**Conclusion:** The assessment of GCF MMP-8 and PGE<sub>2</sub> levels may reflect disease severity. Periodontal sites with elevated levels of MMP-8 may also be expected to have higher levels of PGE<sub>2</sub>.

143

### Correlation between inflammatory mediators in GCF and clinical signs of periodontal disease in a Greek population

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**Objectives:** To compare the levels of IL-1 $\beta$ , IL-8, and MMP-8 in GCF samples from patients with periodontitis and healthy controls and correlate their levels to clinical signs of periodontal disease.

**Methods:** Nineteen periodontitis patients and 19 periodontally healthy subjects were studied. After the clinical exam, 30-second

GCF samples were collected from the mesial aspect of each tooth using periopaper (882 samples). Samples were stored at  $-70^{\circ}\text{C}$  and after elution, analysed for their content of IL-1b, IL-8, and MMP-8 using checkerboard immunoblotting. The Mann-Whitney test was used to determine differences between the two clinical groups. Correlations between the three biomarkers and clinical parameters were sought using the Spearman correlation coefficient.

**Results:** There were significant differences between the two groups for PD, AL, % of sites with BOP and GCF volume ( $P < 0.001$ ). The mean levels of IL-1b ( $P < 0.001$ ), IL-8 ( $P < 0.001$ ) and MMP-8 ( $P < 0.05$ ), were higher in the periodontitis vs. control group. There were significant correlations between all three pairs of biomarkers and significant correlations between all three mediators and PD, AL and % of sites with BOP. When only healthy sites from both groups were compared; there were still significant differences between the groups for all biomarkers.

**Conclusions:** There is an association between levels of IL-1b, IL-8, and MMP-8 and the traditional clinical measures of periodontal disease.

Supported by NIDCR grants DE14242 and DE12108.

144

### Effects of self-ligating and conventional bracket systems on gingival crevicular interleukin-6 levels

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Fixed orthodontic appliances induce accumulation and retention of bacterial plaque, which constitute a risk of white spot lesions and periodontal disease development during orthodontic treatment. Interleukin-6 (IL-6) plays an important role in the local regulation of bone remodelling and in the acute inflammation found at the onset of orthodontic tooth movement. The aim of this study was to investigate and compare the effects of two bracket systems, with different ligation type, on gingival crevicular fluid (GCF) IL-6 levels. 30 patients between the ages of 12–18 with Angle Class I, II and III anomalies were selected to participate the study. Subjects were divided in two groups: group I included patients treated with roth brackets and group II; subjects treated with in-ovation brackets. Quigley-Hein plaque index (QPI), gingival index and probing depths were recorded. GCF was collected before orthodontic treatment and after 3 months of force application from the upper six anterior teeth. The results were compared with T and Wilcoxon Signed Rank Tests. At baseline and 3rd month no significant differences were found for GCF IL-6 levels between groups. At 3rd month, group I demonstrated higher QPI and GI than group II ( $P < 0.001$ ,  $P < 0.05$ ). Although no significant differences could be detected during the 1st month of the study, the group with self-ligating brackets improved better oral hygiene at 3rd month correlating with the decrease of GCF IL-6 levels.

145

### Periodontal damage of impacted third molar: prevalence of lower third molar impaction in orthodontic patients treated nonextraction and with extraction

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The periodontal damage in the second molar caused by an impacted lower third molar or by its surgery is poorly documented in the literature. With this in mind, three orthodontic cases groups have been studied in order to analyze lower third molar impaction: lower first molar extraction cases, lower first bicuspid and non extraction cases. Eruption space for the wisdom lower molar has

been established, through different values: distance to the anterior border of the ramus and inclination of the third molar crown. Results of the study show a better eruption pattern in the lower first molar extraction cases, and a more favourable uprighting of the wisdom molar in the bicuspid extraction cases. Both values results show a bad eruption pattern in the non extraction cases. Therefore, we will have a lower potential periodontal damage of the lower third molar working with extractions groups than non extraction group.

146

### The importance of psychologic factors on periodontal disease

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**Background/Aim:** Clinical observations and epidemiological studies suggest that psychosocial stress and adverse psychological conditions may represent risk factors for periodontal disease. The purpose of the present study was to verify the influence of some psychosocial factors on periodontal disease.

**Materials and methods:** One hundred and two subjects were examined: 51 cases and 51 controls. Plaque index, gingival index, Decayed Missing Filled Surfaces index (DMFS) and Periodontal Screening and Recording index (PSR) were registered for all patients. Life Stress Events by Holmes and Rahe, the Coping Humor Scale and 10 couples of semantic differentials to be associated once to one's own teeth and once to one's own gums were used to investigate the psychosocial aspects.

**Results:** A statistically significant correlation ( $P < 0.01$ ) between stress and gravity of periodontal disease was observed. Coping Humor Scale was not correlated to stress and periodontal disease. In the semantic differentials, a statistically significant difference ( $P < 0.05$ ) between perception of one's own teeth and one's own gums between the group of cases and the one of controls was detected.

**Conclusions:** Stress did not act modifying aetiological factors of periodontal disease, but it can be assumed, through complicated mechanisms like biohumoral factors which act on target organs. As to the semantic differential, the distinction of teeth and gums in the cases and in the controls bolsters the theory of target organs.

147

### Comparison of the periodontal screening index (PSI) and radiographic (OPG) findings – a double blind study

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The PSI indicating reliable assertion of the periodontal situation is an integral part of the dental check-up. The x-ray completes the initial diagnostic finding by showing the bone level approximately. The aim of this study was to examine if both diagnostic tools generate similar results relating to the periodontal situation. The clinical examination was performed by one investigator and includes the following parameters: PSI, DMF-T and QHI. The category 'no periodontitis' was defined up to PSI score 2; PSI score 3 and 4 indicated 'periodontitis'. Separated by location and time, two other investigators evaluated the radiographs (OPG) in agreement. The classification followed according to the two categories above. 112 males aged 18–58 years ( $\bar{X}$ :  $37.7 \pm 8$  years) without any previous periodontal treatment participated. The mean QHI was  $2.4 \pm 1.3$ , the mean DMF-T  $14.6 \pm 4.9$ . With regard to the PSI 17 patients had 'no periodontitis' and 95 patients had 'periodontitis'; according to the radiographic evaluation 42 patients revealed 'no periodontitis' and 70 patients 'periodontitis'. Correspondingly, the result of both diagnostic methods was 'no

periodontitis' in 17 patients and 'periodontitis' in 42 patients. However, in 53 cases the result was different. Statistics showed significant differences between both methods ( $P = 0.00$ ; kappa = 0.194). The results show that both diagnostic methods have different outcomes. In addition, there is a difference in the informative value of both findings.

148

### Interleukin-1 gene polymorphism and periodontal status in a Spanish population

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**Background:** Find a possible association between presence of polymorphisms in IL-1A (+4845) and/or IL-1B (+3954) genotypes and the initiation and progression of chronic periodontal disease (CPD). On the other hand, correlate the [IL-1 $\beta$ ] in the gingival crevicular fluid (GCF) with the disease activity and with the presence of genetic polymorphisms.

**Materials and methods:** The study extended to 50 patients, 25 healthy and other 25 patients with active (CPD). Two samples of GCF were taken from every patient and [IL-1 $\beta$ ] was determined using (ELISA). Besides that, 10 ml of blood were taken in order to determine their ADN and to detect possible polymorphisms in IL-1A (+4845) and IL-1B (+3954) genotypes.

**Results:** The [IL-1 $\beta$ ] in GCF of patients with active (CPD) was higher [4.161 + 2.845 (pg/ $\mu$ L)] than control group [2.304 + 1.459 (pg/ $\mu$ L)]. ( $P = 0.016$ ). No statistically significant differences were found (SE) between the [IL-1 $\beta$ ] in GCF and the presence of polymorphisms in any genotype. With respect to polymorphisms prevalence, no differences (SE) were found between both groups.

**Conclusions:** Our results show that [IL-1 $\beta$ ] is higher in patients with (CPD), almost double than in healthy patients. On the other hand, the presence of polymorphisms in IL-1A (+4845) and/or IL-1B (+3954) genotypes, does not implicate an overproduction of [IL-1 $\beta$ ] in GCF. And finally, the presence of polymorphisms in those genotypes, do not represent a risk factor to suffer chronic periodontal disease.

149

### Comparison of two different microbiological assays to characterize the subgingival microflora

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**Aims:** Sensitivity of clinical assays for detection of microorganisms can show significant differences. The goal of this study was to find out if a new assay for quantitative detection of *Actinobacillus actinomycetemcomitans* (A.a.) in samples of subgingival plaque (Meridol real time PCR) exhibits a different sensitivity when compared with an established assay (IAI Pado Test).

**Methods:** Fourteen patients that suffered either from aggressive Periodontitis (AP) or severe chronic Periodontitis (sCP) participated in the study. Inclusion criterium was a negative test result for A.a. with the IAI Pado test. Subgingival plaque samples were drawn according to the manufacturer's instruction of the IAI Pado and the Meridol real time assay.

**Results:** With the IAI Pado test, none of the 14 patients was tested positive for A.a. In contrast, the meridol real time PCR could detect A.a. in subgingival plaque samples of 5 out of 14 patients (35.7%).

**Conclusion:** If A.a. concentrations in samples of subgingival plaque are close to the lower detection threshold, selection of a certain microbiological assay can decide if a patient is A.a. positive or not. Since the detection of A.a. can have therapeutic

consequences in patients with sCP and AP, it should be recommended to choose an assay with a maximum sensitivity.

150

### Composition of supra- and subgingival biofilm of subjects with health and diseased implants

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Forty four subjects (mean age  $48.9 \pm 13.51$  years) with at least one implant restored and in function for at least 1 year were split in two groups: peri-implantitis ( $n = 22$ ) – subjects presenting peri-implant sites with radiographical defects  $\geq 3$  mm, bleeding on probing and/or suppurating; and control ( $n = 22$ ) – subjects with healthy implants. The clinical parameters evaluated were gingival redness, plaque index, bleeding on probing, suppuration, probing depth and clinical attachment level. Supra- and subgingival biofilm samples were taken from the deepest sites of each implant and analysed for the presence of 38 microorganisms by Checkerboard DNA-DNA Hybridization. All clinical parameters were statistically higher in the diseased implants, except for plaque index. The species evaluated were found supragingivally and subgingivally in the two groups. The total counts of red complex species (*Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*) were higher in the peri-implantitis group for both supra- and subgingival plaque ( $P < 0.001$ ). The presence or absence of *P. gingivalis* and *P. intermedia* in supragingival biofilm showed high correlation with the presence or absence of the same species in the subgingival plaque. The main differences between supra- and subgingival biofilm, as well as between healthy and diseased implants were in the proportions and counts of orange and red complex species.

151

### Oral condition in patients undergoing dialysis due to chronic renal failure

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**Aim:** Clinical assessment of oral condition and cytology of the oral buccal mucosa in patients on hemodialysis due to chronic renal failure compared to patients on peritoneal dialysis and healthy controls.

**Materials and methods:** One hundred and twenty patients have been examined in three groups: I – 40 patients on hemodialysis. II – 40 patients on peritoneal dialysis. III – 40 healthy controls. Dental conditions were assessed using the DMF, CPITN and OHI indexes. Cytological assessment of the oral mucosa specimen included the following indicators: cell maturation, acidophilia and karyopycnosis. Statistical analysis was carried out by means of the Statistical package ( $P < 0.05$ ).

**Results:** The results showed hard tissues of the teeth of the patients on dialysis to be more susceptible to caries. The patients' oral hygiene was poor and changes in periodontal tissues were found to be more frequent. High levels of urea in the blood of the patients on dialysis caused pathological changes in oral mucosa. The cytology of the mucosa was also found to deviate from the norm. The above disorders were most often observed in patients on hemodialysis due to chronic renal failure.

**Conclusions:** The collaboration between nephrologists and dentists is indispensable to prepare the patients properly and to avoid complications due to the primary and secondary sources of infection inside the mouth.

152

### A decision support system for determining prognosis and survival time in periodontics (an innovation)

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**Background:** In traditional methods prognosis is determined based on number of current clinical parameters without considering risk ratios of each parameter and especially on self implications of clinicians which often lead to false conclusions.

**Aim:** The aim of this project is construction and evaluation of computer software (Decision Support System) for determining primary prognosis to decrease cited problems.

**Materials and methods:** First, project algorithms based on risk ratios of most important clinical factors with mathematical and statistical calculation designed. Next, among 30 periodontal patients, 61 teeth were selected as case and information form for each of cases was given to three experienced periodontists and they determined prognosis (clinical prognosis). Afterward, algorithm prognosis was determined for each case. Finally, a computer software (DSS) was written based on designed algorithms and accuracy calculated.

**Results:** The DSS result was same as gold standard (algorithmic prognosis) and to conclude Accuracy was 100%. Moreover, there was a significant difference between three clinical prognosis and also between clinical prognosis and algorithmic prognosis (DSS) which was the main rationale to doing this study.

**Conclusion:** With regard to including most important clinical parameters with risk ratios of each parameter which is not performed by mankind accurately, we can conclude that this DSS is reliable and useful.

153

### Real-time polymerase chain reaction quantification of the periodontal pathogens variations after occlusal therapy

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**Background:** The relationship between occlusal forces and the initiation or progression of periodontal disease has been controversial for over a century.

**Aim:** The present study was performed to assess the influence of occlusal therapy on periodontopathic flora.

**Method:** One hundred and seventy two patients with advanced destructive periodontitis were recruited. The participants met the following inclusion criteria: (i) probing pocket depth of  $\geq 5$  mm at  $\geq 3$  teeth in the upper anterior group and dental mobility (value 1 or 2 on Miller's scale) at the same teeth; (ii) non-smokers. Permanent splinting of teeth was performed in 110 patients. In the remaining 62 patients, a prosthetic solution was adopted that involved applying a provisional resin bridge. Occlusal therapy was not preceded or followed by topical or systemic drug treatment or by surgical or non-surgical treatment of the affected periodontal tissues. Clinical parameters (probing depth, bleeding on probing, presence of pus) were measured and the subgingival flora was detected by real-time PCR before treatment, after 20 days and lastly after 60 days for each patient.

**Results:** At each follow up visit and for each patient a statistically significant improvement of clinical parameters towards baseline was recorded. After 60 days of treatment, a statistically significant reduction of periodontal pathogens was recorded in all patients.

**Conclusions:** Removal of the occlusal trauma brings about a reduction in pathogenic subgingival microflora levels.

154

### Assessment of preoperative bone loss with different X-ray techniques

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**Aims:** The aim of this study was to find out if the amount of bone loss that can be diagnosed in patients suffering from aggressive Periodontitis (AgP) or severe chronic Periodontitis (CP) depends on the applied X-ray-techniques.

**Method:** A total of 65 patients (33 female, 32 male) with AgP ( $n = 37$ ) or severe CP ( $n = 28$ ) were included. Panoramic X-rays (OPTG) and intraoral radiographs (IO) were analysed for each patient: (i) computer assisted (software: Friacom®) (ii) manually with the Schei-straightedge. Distances Cemento-enamel-junction (CEJ)- Alveolar Crest (AC), CEJ- deepest point of Bony defect (BD) and CEJ-Apex were measured.

**Results:** Comparative analysis showed a higher percentage of bone loss using Friacom® vs. the Schei-straightedge. The amount of difference depended on I) the examined location (maxillary molars: 18.6% difference between Friacom® and Schei-straightedge, mandibular incisors 2.6%) as well as on chosen reference points (BD distal: 13.8% difference between Friacom and Schei-straightedge, LA mesial -0.9%). Not measurable sites were mostly found in the maxillary premolar region. Intraoral radiographs showed better contrast between AC and BD than OPTG.

**Conclusion:** With panoramic radiographs, a pre-orientation concerning the expected bone loss is possible. The computer-assisted analysis of single intraoral films provides larger diagnostic potential concerning the estimation of the defect localization compared to the conventional Schei-straightedge.

155

### Influence of biopsy localization on diagnosis of gingivitis desquamativa

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Direct immunofluorescence (DIF) has increasingly been used to determine the disease causing desquamative gingivitis. However, in some cases there are still diagnostic difficulties concerning the localization of biopsy where immunoreactive deposits can be observed. Therefore, the aim of the present study was to evaluate and compare the influence of biopsy localization on the reliability of DIF diagnosis of gingivitis desquamativa. A total of 14 patients (mean age  $61 \pm 11$  years) suffering from non specified gingivitis desquamativa (GD) were included. Three incisional biopsies were taken from each patient including (i) affected (2x lesional), and (ii) non-affected (1x non-lesional) oral sites and prepared for histopathological (HP) (lesional) and DIF (lesional and non-lesional) examinations. Additionally, patients sera were taken and analysed for indirect immunofluorescence (IIF). In nine patients, DIF of lesional biopsies exhibited autoimmune reactions to basement membrane proteins (i.e. IgG: eight patients; IgG and IgA: one patient), while respective non-lesional biopsies were negative. In five patients, both lesional and non-lesional biopsies were negative for DIF. However, IIF was positive in all patients. The combination of lesional DIF, IIF and HP examinations classified GD as mucous membrane pemphigoid in all patients. Within the limits of the present study, it might be suggested that a combination of lesional DIF, IIF and HP diagnosis seems to be appropriate in order to specify GD.

156

# **Rapid detection of periodontopathogenic species from subgingival paper points samples with a commercial PCR based method**

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A poor treatment response in adults with periodontitis has been related to the persistence of periodontopathogenic species. Periodontitis patients may benefit from molecular testing to identify the presence of periodontopathogenics after periodontal treatment in order to consider further therapy.

**Objective:** To assess a commercial PCR method (MicroDent) for control testing of adult periodontitis after active treatment. Subgingival plaque was sampled by inserting paper points in the deepest periodontal pockets from several teeth (4–5) from 22 adult patients with periodontitis following actual periodontal treatment. DNA extraction was carried out with DNeasy Spin Columns kit (Qiagen) within 48 h after sampling. The amplification was realized by multiplex-PCR of 16S rDNA (MicroDent). The detection of periodontopathogenics was realized by hybridization with species-specific probes. The mean age of patients 37.4 years, being 82% female, 18% male and 36% were active smokers. A positive hybridization result for periodontopathogenics yielded in 86% patients. DNA from *T. forsythia* (95%) was mostly detected as well as *T. denticola* (79%), and *P. intermedia* (63%). In 11 cases *P. gingivalis* was still found and only in two patients *A. actinomycetemcomitans* remained. The molecular MicroDent assay has been shown a rapid and reliable procedure in clinical practice to evaluate incomplete removal of subgingival pathogens, without the problems during sample collection and transport.

157

# **The influence of oral hygiene practices on the inflammatory periodontal diseases in the adult population of Riyadh, Saudi Arabia**

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**Aim:** This study investigated the influence of oral hygiene practices on the prevalence and severity of inflammatory periodontal diseases in the adult population of Riyadh.

**Methods:** Using a two stage stratified random sampling method a sample size of 1053 subjects was selected for the study. The subjects, aged 16–69 years, composed of 538 females and 515 males. The methods of oral hygiene employed were one or a mixture of the following: tooth cleaning by 'miswak' (a fibrous substance made from the roots of Arak Tree), tooth brushing and interdental cleaning. The study was carried out in two parts: clinical examination and questionnaire interview.

**Results:** The effect of each of the oral hygiene methods on inflammatory periodontal disease and gingivitis was assessed by the measurement of CPITN and Bleeding Indices. Chi-square test was used to test the correlation between oral hygiene practices and inflammatory periodontal disease status. Logistic regression was used to determine the effect of each method on the prevalence of both gingivitis and periodontal inflammatory disease when the influence of age and gender was controlled. A 5% level of significance was employed.

**Conclusions:** The results indicate that all the methods of oral hygiene significantly reduced the prevalence and severity of gingivitis and inflammatory periodontal disease. Toothbrushing was shown to have the greatest impact on inflammatory periodontal diseases as measured by the indices used.

158

# **Association of the IL10 gene polymorphism at position -1082 with generalized aggressive periodontitis in an Iranian Khorasanian population**

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**Aims:** Polymorphisms in cytokine genes have recently been described in susceptibility to periodontitis. IL-10 is a multi-functional cytokine thought to play a role in periodontitis by down-regulating the production of pro-inflammatory cytokines. The aim of this study was to investigate the association of the –1082(G/A) polymorphism with generalized aggressive periodontitis.

**Materials and methods:** This study included 37 Iranian Khorasanian subjects suffering from generalized aggressive periodontitis (GAP) referred to periodontology department of Mashhad dental school. They were compared to 43 healthy controls of the same race. The DNA was isolated from peripheral blood cells and genotyping was performed by means of ARMS-PCR method. Data was analysed using chi-square test.

**Results:** There was a marked difference in allele frequencies between controls and GAP patients. The G allele carriage was significantly more prevalent among the GAP subjects, while the A allele was significantly associated with healthy periodontal status ( $P = 0.040$ ). Moreover, the proportion of subjects, which showed the AA genotype in comparison to subjects exhibiting a genotype other than AA, was significantly larger in the control group ( $P = 0.040$ ).

**Conclusion:** We concluded that the polymorphic nucleotide A at position –1082 of the IL-10 gene protects against GAP. This may help us to investigate the role of genetic variations among individuals in susceptibility to periodontal disease.

159

# **Microbiological profile of subgingival plaque in patients with aggressive and severe chronic periodontitis**

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**Aims:** The goal of this study was to find out the prevalence of *Actinobacillus actinomycetemcomitans* (*A. a.*), *Tannerella forsythensis* (*T. f.*), *Porphyromonas gingivalis* (*P. g.*) and *Treponema denticola* (*T. d.*) in samples of subgingival plaque obtained from patients with aggressive (AgP) and severe chronic periodontitis (CP).

**Methods:** Eighty eight patients with either AgP ( $n = 26$ ) or sCP ( $n = 62$ ) participated in the study (inclusion criteria). After removal of the supragingival plaque, papertips were applied into the deepest periodontal pockets of four different teeth to obtain subgingival plaque. Microbiological analysis of pooled samples was performed with a 16s-RNS-gene probe test (IAI-Pado-Test).

**Results:** There were no differences between AgP and sCP at the beginning of the study, regarding oral hygiene (PCR), inflammation (GBI, BOP), mean pocket probing depths and clinical attachment levels. There were only small differences in the percentages of patients tested positive for *A. a.* (57.7 vs. 40.3%), *T. f.* (88.0 vs. 96.8%), *P. g.* (69.2 vs. 83.9%) and *T. d.* (88.5 vs. 95.2%) when comparing AgP and sCP Patients. Medians for different bacteria species in the AgP and sCP group were  $3.0 \times 10^5$  and  $0.1 \times 10^4$  (*A. a.*),  $9.6 \times 10^6$  and  $3.7 \times 10^6$  (*T. f.*),  $15.5 \times 10^6$  and  $4.4 \times 10^6$  (*P. g.*),  $4.7 \times 10^6$  and  $1.8 \times 10^6$  (*T. d.*).

**Conclusions:** In patients with the clinical diagnoses AgP and sCP there are no significant differences concerning the microbiological profile of the analysed periodontal pathogens.

160

## Two subgingival plaque sampling strategies used with RNA-probes

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In 158 patients suffering from aggressive or generalized severe chronic periodontitis clinical examinations were obtained before ( $n = 82$ ) or after ( $n = 76$ ) combined mechanical and antibiotic anti-infectious periodontal therapy. Subgingival plaque samples were obtained from the three pockets with the deepest probing depths. Two sterile paper points were inserted simultaneously in the periodontal pockets. One paper point from each pocket was put in a separate transport-vial, the second was pooled (MT3) with the two other paper points of a respective patient in a transport-vial. The content of each vial was analysed separately for *A. a.*, *T. f.*, *P. g.* and *T. d.* with a commercially available RNA-probe test. The log-transformed number of bacteria was higher in pooled samples than the mean value of the results of the separate samples for all tested pathogens ( $P < 0.001$ ). However, for all four pathogens analysis failed to detect statistically significant differences between the single samples and the MT3 regarding the detection frequency. These findings were observed over all samples as well as after evaluation of samples before and after therapy separately. Pooling of subgingival plaque samples increased the bacterial counts per analysis compared to separate samples and thus may increase the probability to detect existing pathogens. This observation had no statistically significant effect on the detection frequency of the tested pathogens.

161

## Periodontal alterations in users of marijuana and cocaine

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A patient is characterized as special when it presents some shunts of the normality standards, which need differentiated cares, then, the inclusion of narcotics users is justified in this group. The illicit psychoactive drugs have right effect on the structures of the mouth (mucous, tooth, tongue), and the caries and the periodontal illness happen more frequently with drugged, particularly, of marijuana and cocaine. This study evaluated the periodontal alterations in forty patients of mental illness and manning upheaval, all male, attended in the Dental Sector of the Juliano Moreira Hospital. The sample was divided in twenty using patients of marijuana and cocaine and twenty patients that are not using. The gotten data had been compared and submitted to statistics analysis. The periodontal diagnosis was made by only one examiner duly calibrated, in intention to evaluate the depth the level of clinical insertion, for this, it was used the periodontal probe Williams by Hy-Friedy. The epidemiologist index of Silness Loe and Ainano Iday was applied to evaluate the indices of biofilm and gingival bleeding. The data were registered in clinical handbook especially created for this study. A high prevalence of the inflammatory periodontal illness in the drugged patients was observed, what it confirms the hypothesis of this study.

162

## Comparative evaluation of periodontal statuses in Khorasan twins

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**Background:** Study on the twins has helped us to know about the roles of genetic and environmental factors in periodontal diseases. The purpose of this study was the evaluation of periodontal condition in twins.

**Materials and methods:** This study was carried out on 30 pairs of twins between 12–35 years old including 12 pairs of monozygotic and 18 pairs of dizygotic twins in Mashhad. Periodontal parameters studied, consisted of: Probing Pocket Depth (PPD), Clinical Attachment Level (CAL), and Bleeding on Probing (BOP). Moreover factors such as age, sex, education, dental hygiene and inheritance index was studied.

**Results:** (i) PPD and CAL were significantly different between MZ and DZ, while no significant differences in BOP was observed between two groups. (ii) CAL and PPD were significantly different according to sex in dz group. This difference was not significant in MZ. (iii) PPD, CAL and BOP were significantly different according to age in DZ. This difference was not significant in MZ. (iv) with improvement in dental hygiene, BOP decreased significantly in both groups, but PPD and CAL were not related to dental hygiene. (v) study of inheritance index showed that BOP could be affected by environmental factors (0.41) while CAL and PPD affected by genetic factors (–0.70, –0.61) respectively.

**Conclusion:** In twins, PPD and CAL are affected by genetic factors, while BOP is affected by environmental factors.

163

## Single nucleotide polymorphisms of osteoclastogenesis-related genes in aggressive periodontitis

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**Background:** Aggressive periodontitis (AgP) is characterized by rapid attachment loss and bone destruction. While attempting to identify genetic polymorphisms associated with AgP, we focused more on single nucleotide polymorphisms (SNPs) in the key mediators of osteoclast differentiation and activation, which involve receptor activator of nuclear factor- $\kappa$ B (RANK), RANK ligand (RANKL) and osteoprotegerin (OPG) in the Japanese population. The aim of this study was to evaluate the association of RANK/RANKL/OPG gene polymorphisms with AgP in the Japanese population.

**Materials and methods:** We examined 99 Japanese patients with AgP and 89 controls to explore the possibility of RANK/RANKL/OPG loci as candidate regions associated with the disease. These three candidate genes were amplified by PCR, followed by direct sequencing, and polymorphisms were identified by comparing the sequences obtained from 48 subjects.

**Results:** We identified 27 SNPs in RANK including 10 novel SNPs, and 7 SNPs each in both RANKL and OPG. A pairwise linkage disequilibrium analysis using the  $r^2$  statistic showed that some SNP pairs from the three loci are in tight linkage disequilibrium. An association analysis with allelotypes showed that SNPs identified in the RANK/RANKL/OPG genes have no significant association with AgP in the Japanese population. The study will be continued by further functional analysis of some SNPs that might have important roles in the genetic molecular mechanism of aggressive periodontitis.

164

## The association of tissue plasminogen activator and plasminogen activator inhibitor-1 gene polymorphisms in patients with chronic periodontitis

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**Aim:** Tissue plasminogen activator (t-PA) and plasminogen activator inhibitor type 1 (PAI-1) may be involved in the pathogenesis of periodontitis by controlling proteolytic events in the extracellular matrix. This study was designed to investigate the

association of t-PA and PAI-1 genes with chronic periodontitis (CP).

**Methods:** A total of 163 subjects were included. Genomic DNA was obtained from peripheral blood of 66 CP and 83 periodontally healthy subjects. We used polymerase chain reaction and endonuclease digestion to genotype for 4G/5G polymorphism in the promoter region of the PAI-1 gene and the Alu-repeat insertion/deletion (I/D) polymorphism in intron 8 of t-PA gene.

**Results:** The genotype distributions and allele frequencies of t-PA gene did not differ between CP and healthy subjects (I/I 27.7%, I/D 47.7%, D/D 24.6% and 34.7% I/I, 36.0% I/D, 29.3% D/D, respectively) ( $P > 0.05$ ). t-PA I and D allele frequencies were 51.5% and 48.5% in CP and 52.7% and 47.3% in healthy subjects, respectively. PAI-1 genotype distribution was also similar between CP (18.5% 4G/4G, 44.6% 4G/5G, and 36.9% 5G/5G) and healthy subjects (24.4% 4G/4G, 54.9% 4G/5G, and 20.7% 5G/5G, respectively) ( $P > 0.05$ ). 4G and 5G allele frequencies were not different between groups (40.8% and 59.2% in CP and 51.8% and 48.2% in healthy subjects, respectively).

**Conclusion:** These findings suggest that t-PA or PAI-1 gene polymorphisms are not associated with susceptibility to CP in Turkish population.

165

### Evaluation of papillary blood flow using laser Doppler flowmetry

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**Objective:** Previous investigations have shown that there is an interaction between gingival blood flow and gingival health. The purpose of this study was to compare the papillary blood flow at sites treated with bridge the blood flow at untreated sites.

**Design:** Twenty persons with resin faced, fixed partial dentures were enrolled in the study. The contralateral natural teeth of the site symmetrical to that of the restorations were used as controls. The blood flow was measured from the middle point of the papilla from both from test and control sites by laser Doppler flowmetry (LDF). The plaque index, papillary bleeding index, and probing depth measurements were recorded.

**Results:** There was a statistically significant difference between the test and control groups in papillary blood flow measurements ( $P < 0.05$ ). In contrast, no significant difference between two sites' clinical indices was found ( $P > 0.05$ ).

**Conclusions:** It should be emphasized that there is an important relationship between resin-faced, fixed partial dentures and papillary blood flow. Blood flow measurements provide information about the microcirculation of the tissues. It seems that it is necessary to evaluate, in detail, the effects of fixed bridges on proximal periodontal health conditions.

**Key words:** fixed partial dentures, papillary blood flow, laser Doppler flowmetry.

166

### Clinical and microbiological monitoring from Portuguese subjects group with and without periodontal diseases

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The infectious nature of periodontal disease has been demonstrated in many studies, with specific bacterial, such as *Actinobacillus actinomycetemcomitans* (A.a.), *Porphyromonas gingivalis* (P.g.), *Prevotella intermedia* (P.i.) and *Tannerella forsythia* (T.f.). The subject of this study was to evaluate the presence of these putative periodontal pathogens from patients with and without periodontal diseases. 57 patients were selected based on clinical diagnostic: 33

chronic periodontitis, 4 aggressive periodontitis, 12 gingivitis and 8 periodontally healthy. Clinical examination included conventional periodontal measurements. These bacteria were determined by culture and presumptive identification was obtained by colonial and microscopic morphology, pigment formation, U.V. fluorescence, C.A.A.M, M.U.G and catalase. From the periodontitis patients, 26 (70.2%), were positive for at least one of the tested bacteria. The simultaneous occurrence of A. a., P. i., P. g. and T. f. were observed in 3 (75%) with aggressive periodontitis. The presence exclusive of A. a. was detected from 2 (6%) with chronic periodontitis, 1 (25%) with aggressive periodontitis and 3 (25%) with gingivitis. From chronic periodontitis patients, the simultaneous presence of P. g/A. a. occurred in two cases and P. g/A. a in 11, P. i or T. f. were detected in 18 cases. The obtained results suggest some consistency between clinical diagnosis and microbiological data, particularly in respect to aggressive periodontitis.

167

### FC $\alpha$ RI and FC $\gamma$ RIIB gene polymorphisms in Caucasian periodontitis patients

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The IgA and IgG Fc receptors have been implicated in the pathogenesis of periodontitis, because increased levels are features of this inflammatory disease. The FC $\gamma$ RIIB T (+695)-C single nucleotide polymorphism (SNP) in exon 5 (Ile 232  $\rightarrow$  Thr) and the novel SNP Fc $\alpha$ RI A (+324)-G in exon 3 have been associated with aggressive periodontitis (AGP) in Japanese. To determine whether an association with periodontitis susceptibility in Caucasians exists, Fc $\alpha$ RI and FC $\gamma$ RIIB SNP's were examined in patients with AGP ( $n = 45$ ) and chronic periodontitis (CP,  $n = 78$ ), and in two groups of age-matched healthy controls, <35 years (CY,  $n = 45$ ) and >55 years (CO,  $n = 21$ ). Clinical parameters were assessed, blood samples were taken and DNA was isolated. Genotypes were determined by means of allele-specific PCR, as previously described (Yasuda et al. 2003, Kaneko et al. 2004). The frequency of the genotypes between groups was analysed (Fisher's exact test). A significant difference was observed in the distribution of the polymorphic allele C of the FC $\gamma$ RIIB-232 I/T allele (exon 5) between the groups ( $P \leq 0.03$ ), whereas the higher frequency (37.3%) was detected in the CP group and the lowest frequency (11.4%) in the CY group. No significant difference in the distribution of the Fc $\alpha$ RI A (+324)-G SNP between the groups was present. This is the first report of these Fc receptor SNP's in Caucasians with periodontitis. These results document a possible association of the FC $\gamma$ RIIB-232 I/T with chronic periodontitis in Caucasians.

168

### Distribution of HLA class I antigens in a group of Spanish subjects with chronic or aggressive periodontitis

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The aim of this study was to investigate the distribution of HLA-A and HLA-B antigens in a group of Spanish patients with chronic periodontitis (CP) or aggressive periodontitis (AgP) and to compare the frequency distribution of the different alleles between both groups. Subjects included in the study were recruited among patients who were treated at the Complutense University, Madrid. Based on clinical diagnosis using standard criteria patients were divided into two groups, CP ( $n = 17$ ) or AgP ( $n = 37$ ). Twenty mL blood samples were obtained from each patient and typed for



HLA class I antigens. HLA-A and HLA-B loci were determined by a lymphocytotoxicity test (One Lambda Inc, Canoga Park, CA). Patient profile of both groups was similar for age, sex, and tobacco. Distribution of HLA type I antigens was similar to the one observed in the Spanish population. HLA-A2 allele was the most frequently found with 72.9% in the CP group and 58.8% in the AgP. The HLA-B44 and -B35 allele were detected in 37.8% and 21.6% respectively in the CP group and 41.2% and 29.4% respectively in the AgP group. No statistical significant difference was found in alleles frequencies between both groups. Discrepancies between the data of the present investigation and previous studies may be explained by differences in patient selection criteria, ethnic and geographical origin as well confounding factors like age, sex and tobacco.

169

### Mannose-binding lectin (MBL) gene polymorphisms in relation to periodontitis

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**Background:** Mannose-binding lectin (MBL) is an important component of the innate immunity, activating the complement system. Plasma levels are influenced by polymorphisms in the MBL gene.

**Objectives:** To investigate polymorphisms in the MBL gene in relation to periodontitis.

**Methods:** Ninety four periodontitis patients (mean age  $44 \pm 10$  years) and 69 controls (mean age  $40 \pm 13$  years) of Caucasian origin participated in the study. MBL plasma levels were available and smoking status was recorded. Genotyping was performed by allelic discrimination analysis after PCR.

**Results:** To date polymorphisms at loci -550(H/L) and -221(X/Y) were determined. The prevalence of H/H, H/L, L/L genotypes was 52%, 32%, 16% for patients compared to 36%, 53%, 11% for controls respectively ( $P = 0.033$ ). The L allele carriage among patients was 48% vs. 64% among controls ( $P = 0.053$ ). MBL plasma levels were significantly lower in subjects carrying allele H compared to subjects carrying allele L ( $P = 0.012$ ). The prevalence of X/X, X/Y, Y/Y genotypes was 61%, 32%, 7% for patients and 71%, 26%, 3% for controls respectively ( $P = 0.271$ ). The Y allele carriage among patients was 39% vs. 29% in controls ( $P = 0.187$ ).

**Conclusions:** At least one polymorphism in the MBL gene is associated with susceptibility for periodontitis; H/H genotype was more prevalent in patients and was associated with lower MBL plasma levels. Thus, possibly, in periodontitis MBL genetic variation may lead to a reduced function of the MBL-induced complement pathway.

170

### Role of transporter P-glycoprotein gene polymorphism in drug-induced gingival hyperplasia

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**Background:** Gingival overgrowth is a common side effect of calcium antagonists. Several lines of evidence point to a modulation of inflammatory processes. Since the calcium antagonists act as inhibitors of P-glycoprotein (Pgp), the gene product of MDR1, and inflammation may modify Pgp expression, we analysed the MDR1 polymorphisms as risk factors for gingival overgrowth.

**Methods:** Periodontal, laboratory and anamnestic data and use of calcium antagonists were assessed in a cross-sectional epidemiological investigation ( $n = 1.484$ ). MDR1 polymorphism in exon 21-G2677T/A was determined and Pgp expression detected.

**Results:** P-glycoprotein is expressed in the endothelial layers of blood vessels obtained from healthy or inflamed gingiva. Subjects treated with calcium antagonists had significantly deeper gingival pockets than their drug-free counterparts ( $P < 0.0001$ ). This drug-related effect was associated with the MDR1 2677 G/G-TA genotypes ( $P < 0.001$ ) but not with the variants T/TA. The effect was proved by regression analysis adjusting for the risk factors of periodontitis age, sex, smoking, and education ( $P < 0.0001$ ) and was associated with elevated C-reactive protein. The association was also confirmed in a matched-pair analysis comprising 93 subjects using calcium antagonists and 186 without ( $P < 0.0001$ ). **Conclusion:** Calcium antagonists lead to increased pocket depth which is associated with the MDR1 G2677 polymorphism. The genotype may modify the inflammatory response to the drugs.

171

### Clinical characteristics associated with the presence of *A. actinomycetemcomitans* – *p. gingivalis*

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The aim of the study was to evaluate the clinical characteristics associated with the presence of *A. actinomycetemcomitans* – *P. gingivalis* at the patient and site level. The investigation was based on the databank of a commercial laboratory that provides microbial analysis services (IAI, Zuchwill, Switzerland). Subgingival plaque samples were collected in patients with various periodontal conditions. *A. a.* and *P. g.* levels were determined using oligonucleotide probes. A total of 33 259 samples from 10 946 patients were included in the analysis. The database was stratified according to bacterial levels using three thresholds, e.g. below detection,  $<$  median,  $\geq$  median, and then analysed for frequency distribution. *A. a.* – *P. g.* was not detected in 28.2% of the samples whereas 12.7% of the samples were positive for both species (values  $<$  median,  $\geq$  median). High level of both *A. a.* – *P. g.* (values  $\geq$  median) was detected in only 3.0% of the samples, in patients with a mean age  $47.1 \pm 11.4$  years and in sites with probing depth  $7.6 \pm 2.0$  mm. Distribution of these samples increased with probing depth and patient age. When exploring the effect of *A. a.* or *P. g.* levels on the distribution of samples according to age and probing depth, results showed that *A. a.* levels did not influence the distribution of samples with high *P. g.* In contrast, distribution of samples with high *A. a.* was dependent on *P. g.* levels. Results suggest that characteristics of sites with high *A. a.* – *P. g.* or high *P. g.* alone are similar.

172

### The influence of traumatic occlusion on progression of marginal periodontitis

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The aim of this study was to confirm the direct correlation between traumatic occlusion and progression of periodontitis. In order to clarify the role of traumatic occlusion in etiology of periodontal disease 54 patients were observed. Clinical examination revealed periodontitis (inflammation, gingival recession, periodontal pockets) and traumatic occlusion caused by high crowns or restorations on particular teeth (pain, periodontal abscess, increased mobility). The radiographic signs confirm clinical observation: horizontal bone destruction and vertical destruction on a particular tooth. The following clinical parameters were pooled for analysis: the mean infrabony defects depth (IBD), horizontal bone destruction, gingival recession, loss of attachment (LA), found traumatic occlusion (IBD/S) and duration of traumatic occlusion. Depending on duration (3, 6, 12, 24 months) of traumatic occlusion on single tooth the IBD was

4, 5, 6 and 8 mm, respectively ( $P < 0.001$  for trend) which was also the case for LA on single tooth (7, 9, 10, 12 mm; respectively;  $P < 0.001$  for trend). Persistent traumatic occlusion is an important factor in the etiology of periodontal disease.

173

### The effects of hormonal changes on periodontal tissues and gingival crevicular fluid prostaglandine E<sub>2</sub> levels during pregnancy and post-partum

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In a previous clinical trial we demonstrated the necessity of periodontal therapy in pregnancy. The present study was designed to analyse the inflammatory changes in gingival tissues on a group of pregnant subjects during pregnancy and post-partum with a control group of non-pregnant subjects. A total of 40 females with gingivitis were included in the study. The test group was made up of 20 pregnant subjects in their first trimesters of pregnancy; while control group was formed 20 non-pregnant subjects. Initial periodontal therapy was repeated 3 months intervally, both in control and test groups. Clinical measurements including PI, SBI, PPD and CAL were recorded and GCF samples were obtained at baseline and 3, 6, 9 months after therapy. Following the collection of samples GCF levels of prostaglandine E<sub>2</sub> were evaluated using enzyme immunoassay technique. Statistical evaluation was performed by Mann-Whitney U-test and Wilcoxon signed rank test. Significant improvements were noted in both clinical and biochemical parameters following initial periodontal therapy in control group while no improvement achieved in the test group. However, all clinical and biochemical scores were decreased significantly after the end of pregnancy. So it can be concluded that there is a strong hormonal factor concerning pregnancy; enabled us to achieve the expected improvement in clinical and biochemical parameters. Also, levels of PGE<sub>2</sub> in GCF might be used as a marker of gingival inflammation.

174

### The lack of association of toll-like receptor 2 and 4 gene polymorphisms with generalized aggressive periodontitis

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**Aim:** Toll-like receptors (TLRs) recognize exogenous ligands such as lipopolysaccharide and bacterial lipoprotein during immune response to pathogens. The aim of the present study was to investigate whether TLR2 and TLR4 gene polymorphisms are related to susceptibility to generalized aggressive periodontitis (G-AgP).

**Methods:** A total of 162 subjects were included in the present study. Genomic DNA was obtained from the peripheral blood of 79 patients G-AgP, and 83 periodontally healthy subjects. The TLR2 gene Arg753Gly polymorphism and TLR4 gene Asp299Gly and Thr399Ile polymorphisms were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

**Results:** There was no significant difference in the distribution of TLR2 and TLR4 genotypes and allele frequencies between G-AgP patients and healthy subjects ( $P > 0.05$ ). The 753Gly allele was found in 3.8% of the G-AgP patients as compared to 3.6% in the healthy group. The frequency of 299Gly and 399Ile allele was 2.5% and 1.3% in G-AgP patients. For the healthy subjects, the allele

frequency was 1.8% for 299Gly and 0.6% for 399Ile allele. The G-AgP patients and healthy subjects did not show homozygosity for the TLR2 and TLR4 mutant alleles.

**Conclusion:** These results show that TLR2 and TLR4 gene polymorphisms are not related to susceptibility to generalized aggressive periodontitis in Turkish subjects.

175

### Gene polymorphisms of tissue plasminogen activator and plasminogen activator inhibitor-1 in patients with generalized aggressive periodontitis

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**Aim:** Tissue plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1) are major regulators of plasmin generation. The aim of this study was to investigate t-PA and PAI-1 gene polymorphisms in relation to susceptibility to generalized aggressive periodontitis (G-AgP).

**Methods:** The study population consisted of 162 subjects. Genomic DNA was obtained from peripheral blood of 79 G-AgP patients and 83 periodontally healthy subjects. 4G/5G polymorphism in the promoter region of PAI-1 gene and Alu-repeat insertion/deletion (I/D) polymorphism in intron 8 of t-PA gene were genotyped by polymerase chain reaction and endonuclease digestion.

**Results:** The genotype distributions and allele frequencies of t-PA and PAI-1 genes were similar between G-AgP and healthy subjects ( $P > 0.05$ ). The distribution of t-PA genotypes in G-AgP patients was 30.0% D/D, 47.1% I/D, and 22.9% I/I, and was 29.9%, D/D, 36.0% I/D, and 34.7% I/I in healthy subjects. The I allele was found in 46.4% of G-AgP patients and in 52.7% of healthy subjects, while D allele was 53.6% in G-AgP and 47.3% in healthy subjects. PAI-1 genotype distribution in G-AgP was 27.0% 4G/4G, 45.9% 4G/5G, and 27.0% 5G/5G, while 24.4% 4G/4G, 54.9% 4G/5G, and 20.7% 5G/5G in healthy subjects. 4G and 5G allele frequencies in G-AgP were 50.0% and 50.0%, and 51.8% and 48.2% in healthy subjects.

**Conclusion:** These data suggest that tPA and PAI-1 gene polymorphisms are not related to susceptibility to G-AgP in Turkish subjects.

176

### Polymorphisms in the CD14 and IL-6 genes and increased extent of periodontal disease

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The aim of the study was to examine whether genetic polymorphism in certain cytokine and receptor molecule genes is associated with periodontal disease. Patients with periodontitis ( $n = 51$ , age range 22–61 years) were recruited to the study. Probing pocket depth (pd), attachment loss (al) and alveolar bone loss (bl) were recorded. The following cytokine and receptor gene polymorphisms were studied using PCR technique: CD14, IL-6, TNF- $\alpha$ , IL-10, IL-1 $\alpha$ , IL-1 $\beta$  and TLR-4. The frequencies of various genotypes in the periodontitis patients were compared to the frequencies in a reference population ( $n = 178$ ). The polymorphism data were related to the periodontal parameters. No statistically significant differences could be found in the frequencies of the genotypes between the periodontitis and the reference groups. The extent of periodontal disease was increased in subjects with the T containing genotype of the CD14<sup>-159</sup> and, GG genotype of the IL-6<sup>-174</sup>. The extent of advanced periodontal disease was highest among carriers of a combination composed of the T

containing genotype of the CD14<sup>-159</sup> and the GG genotype of the CD14<sup>-159</sup> with significant differences in the percentages of sites with  $pd \geq 6$  mm, ( $P = 0.015$ ),  $al \geq 6$  mm ( $P = 0.009$ ) and  $bl \geq 8$  mm ( $P = 0.042$ ) in this specific combination genotype

when compared to the rest of the periodontitis group. We conclude that the T containing genotype of the CD14<sup>-159</sup> and the GG genotype of the IL-6<sup>-174</sup> are associated with higher extent of periodontal disease.

## Posters: Periodontal therapy

177

### Analgesic efficacy of diclofenac vs. ibuprofen after periodontal resective surgery

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**Objective:** This study examined the effect of the diclofenac 50 mg as a postoperative analgesic vs. ibuprofen 600 mg on periodontal resective surgery.

**Materials and methods:** Thirty patients suffering chronic periodontal disease were enrolled in a randomised clinical double-blind controlled single-centre study. The baseline examination recorded probing depth, attachment level, mobility, PI, BI, and furcal involvement. After S/RP all periodontal sites that exhibited signs of periodontal disease were submitted to periodontal resective surgery of similar characteristic. There were performed at least two surgeries per subject. The recorded variables were: pain intensity and the need for rescue medication during 1 week. Magnitude of post-surgery pain was assessed using a VAS questionnaire. The results will be presented and statistically analysed.

178

### Long-term follow-up clinical trial of the efficacy of a sonic powered toothbrush in periodontal patients

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**Aim:** The aim of the follow-up study was to determine the long-term safety and plaque removal efficacy of a manual (Oral B® 35) and a sonic powered toothbrush (Sonicare® Elite® Pro) in periodontal maintenance patients.

**Methods:** A previous randomised, single blind, crossover clinical study showed the superior efficacy of the sonic powered toothbrush over the manual toothbrush (0.76; 0.50–1.03,  $P < 0.001$ ). Eighteen months after completion of this previous study, 34 out of 37 patients took part in an examiner-blinded follow-up exam. The full-mouth plaque scores (PI; Silness and Loe, 1973), the gingival index (GI; Loe and Silness, 1963), the sulcus fluid flow rate (SFFR, Periotron 6000) were recorded. The intraoral soft-tissue exam was documented.

**Results:** All 34 patients completed the study. Patients continuously using the sonic powered toothbrush (26) reported stable superior results over the manual toothbrush users (8) in reducing plaque (PI) and inflammation (GI) ( $P < 0.05$ ). No changes in SFFR were detected. No evidence of soft tissue trauma was seen in both groups.

**Conclusion:** Based on the findings of this long-term follow-up clinical trial, the action of the sonic powered toothbrush is more effective in plaque removal and inflammation reduction. The sonic powered toothbrush is an effective tool for maintaining an acceptable level of oral healthcare in periodontal patients.

179

### Oral health status and the evaluation of tooth brushing skill in students with mental disability

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The aim of this study was to determine dental caries and gingival status and to maintain more effective tooth brushing skill in children and young adults with mental disability by using electrical toothbrush.

**Methods:** The target population included 'trainable' subjects with IQ level between 25–45 who attended a special education and practise school. 49 students, aged between 9–17 years, were examined and their caries and gingival status were determined. Then, 10 students were chosen in order to practice a training programme about tooth brushing by electrical toothbrush for four times. In this group the tooth brushing success was determined by using a tooth discoloration method, before and after training procedure. A data collection from to include only buccal surfaces of 12 teeth from different quadrants of the mouth. Data were analysed using Wilcoxon non-parametric method.

**Results:** At the end of the examinations the findings were following: DMF/T = 4.67, DF/S = 6.47, GI = 0.42, the prevalence of calculus = 12%. The number of coloured surfaces was no changed before and after toothbrush training ( $P \leq 0.05$ ). Most of the students did not have the ability to brush all tooth surfaces by themselves and they were not able to brush the postdental area. To conclude, in mentally retarded individuals, it was thought that regularly maintaining tooth brushing practices under observation and getting support of the family and teachers would play an important role in improving tooth health.

180

### Periodontal status of Behçet's disease patients in comparison with healthy individuals

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**Objective:** The presence of painful oral ulcers in Behçet's Disease (BD) is a significant risk factor for an increase in plaque accumulation because of the limitation of oral hygiene applications. The aim of our study was to investigate the oral health and systemic conditions of BD patients compared to healthy individuals.

**Materials and methods:** The material of study was consisted of 201 BD patients diagnosed according to the International Study Group for Behçet's Disease criteria, and 200 healthy controls. Plaque index (PI), pocket depth (PD), clinical attachment level (CAL) and bleeding on probing (BOP) measurements for the clinical periodontal status, and C-reactive protein (CRP) measurements for the systemic condition were obtained. The difference between BD and healthy individuals were tested with *t*-test analysis.

**Results:** The mean values for PI, PD, CAL and BOP for control group were  $1.46 \pm 0.68$ ,  $2.31 \pm 0.33$ ,  $1.79 \pm 0.54$ ,  $15.52 \pm 13.95$ , respectively. The same values for the BD group were  $1.52 \pm 0.56$ ,

## Posters: Periodontal therapy

2.51 ± 0.41, 1.81 ± 0.75 and 25.39 ± 20.1, respectively. The mean CRP values for the control and BD patients were 0.413 ± 0.64 and 8.91 ± 16.27, respectively. There were significant differences for PD ( $P < 0.001$ ), BOP ( $P < 0.001$ ) and CRP ( $P < 0.001$ ) values between BD and control groups.

**Conclusion:** According to the results of our study, the periodontal status was observed to be impaired in patients with BD compared to healthy individuals.

181

### Epidemiological study on the periodontal status and treatment need in a Romanian population sample

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**Aim:** Evaluation of the periodontal status and the treatment need in a national patient sample.

**Materials and methods:** The sample consisted in 8145 patients (4020 men, 4125 women), utilizing: CPI, according to WHO methodology.

**Results:** Results obtained are resumed as follows: (i) at 15–25 years old group there's 25%, periodontal impairment, gingival inflammation being prevalent; (ii) at 26–35 years old group there's 40% (CPI1 – 22%, CPI2 – 17%), (iii) between 36–45 years of age there's 46% prevalence of the periodontal disease, calculus and mild periodontitis (CPI2 – 22.5%, CPI3 – 3%); (iv) for the 46–65 years age group the prevalence of the periodontal disease is in average of 57% with moderate periodontitis (CPI2 – 24%, CPI3 – 5%); (v) for the group of 65 years and above there's severe periodontitis in 62% of the patients and edentation (12.5% – out sextant). When assessing the treatment needs, there is a great difference between rural (43%) and urban patients (36%).

**Conclusions:** In order to obtain periodontal health it is necessary to adapt the national, regional and local programs to the WHO objectives according to the local particularities.

182

### Drug concentrations in gingival crevicular fluid as a risk factor for gingival overgrowth in patients medicated with phenytoin or nifedipine

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Gingival overgrowth is a known side effect of phenytoin (Phy) and nifedipine (Nif) use. However pathogenesis of this side effect is unclear. This study was conducted to determine whether there is an association between Phy and Nif concentrations in gingival crevicular fluid (GCF) and the degree of gingival overgrowth in patients using these drugs. 18 patients medicated with Phy and 18 patients medicated with Nif took part in the study. Vertical and horizontal gingival overgrowth, plaque, gingival and bleeding indices were recorded on each patient. Blood and GCF samples were also collected. Plasma and GCF drug concentrations were determined by high performance liquid chromatography. Nine of the Phy using patients and nine of the Nif using patients demonstrated clinically significant overgrowth and were thus designated responders. In both groups, no significant difference was seen between responders and nonresponders with regard to GCF drug concentration, plaque index, gingival index and bleeding index. There was a significant correlation between plasma Phy concentrations and gingival overgrowth; and also significant correlation between GCF Phy concentration and gingival overgrowth. In Nif group, significant correlations were detected between gingival index and horizontal gingival overgrowth; and between bleeding index and vertical gingival overgrowth. The results of this study suggest that, there is no apparent

relationship between gingival overgrowth and Phy and Nif concentrations in GCF.

183

### The evaluation of alveolar bone fractures taking together out with the tooth in during extraction (a histological study)

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The purpose of this study was to evaluate periodontal structures in the fractures of alveolar bone taking together out with the tooth during extraction, and whether there was any ankylosis or the role of iatrogenic factors in these fractures. This study was carried out on the extracted teeth together with alveolar fractures obtained from 134 individuals not having a systemic disorder. The collected samples were blocked in order to histological investigate. In histologic sections obtained from 134 samples, while ankylosis was observed in 14.8% of the samples, periodontal area was found normal 44.8% of samples. Ankylosis was found upper jaw (80.0%) rather than lower jaw (20.0%). The ankylosis was generally found between the cemento-enamel-junction (CEJ) and 1/3 crown of the root length (90.0%). According to obtained data, only 11.5% of individuals were found gingival recession. Plaque (93.3%) and gingivitis (88.5%) at the highest rate were determined. The occurrence probing depths of 4 mm or more was low ( $n = 15$ , 11.5%). Also, relationship between sex and periodontal pocket was not statistically significant ( $P > 0.05$ ). The fact that alveolar fractures were seen in periodontium having normal structure makes us think that there was an iatrogenic factor in extractions. When we consider the problems lost bone caused and for its treatment required time and money, one should not forget how much important a peace of alveolar bone is.

184

### The 18-months effect of an oscillating-rotating power toothbrush on recession

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**Objective:** To observe the recession changes after 12 months clinical use of an oscillating-rotating power toothbrush and the ADA reference manual toothbrush.

**Methods:** Healthy subjects were appointed to either brushing with the power toothbrush (D17U, Oral-B Laboratories,  $n = 55$ ) or the ADA reference toothbrush ( $n = 54$ ) according to a prospective randomised, controlled, single-blind, parallel design. Participants were asked to brush their teeth twice daily for 2 min each with standard fluoride toothpaste. Attachment loss [mm] and probing pocket depths [mm] were measured at six sites per tooth to the nearest mm by one calibrated examiner at baseline, after 6, 12 and 18 months. Recessions were calculated as differences between CAL and PPD at every site.

**Results:** On buccal surfaces, recessions were statistically significant reduced from 1.58 ± 0.65 mm to 0.68 ± 0.76 mm in the oscillating-rotating toothbrush group ( $P < 0.001$ , paired  $t$ -test) and from 1.28 ± 0.43 mm to 0.54 ± 0.62 mm in the manual toothbrush group ( $P < 0.001$ ). This related to a recession decrease of 0.89 ± 0.91 mm in the oscillating-rotating toothbrush group and of 0.73 ± 0.73 mm in the manual toothbrush group (n.s.,  $t$ -test).

**Conclusions:** Both toothbrushes significantly reduced recessions on buccal surfaces. This study was supported by Oral-B Laboratories.

185

**All that glistens is not gold: quantitative and qualitative analysis of systematic reviews in periodontology**

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**Aim:** The aim of this study was to identify all published systematic reviews and meta-analyses in periodontology, and to make a qualitative assessment of their level of evidence.

**Materials and methods:** A systematic search was performed in the following electronic databases: Cochrane Library, PubMed, Embase, Medpilot ([www.medpilot.de](http://www.medpilot.de)), the dental journals of the Deutsche Ärzte-Verlag ([www.dzz.de](http://www.dzz.de)) and those of the Quintessenz-Verlag ([www.quintessenz.de](http://www.quintessenz.de)). Additionally, two independent reviewers handsearched selected German-language journals: *Schweizer monatszeitschrift für Zahnmedizin*, *Deutsche zahnärztliche zeitschrift*, *Stomatologie*, *Zahnmedizinische mitteilungen* and *Quintessenz parodontologie*. All identified systematic reviews were analysed according to a modified version of the QUORUM statement. The modification, which was made in collaboration with the German Cochrane Centre, involved (i) the specification of the eight given questions to dental aspects, and (ii) the introduction of a point system for each of the specified questions. Every review could yield 0–24 points (0–3 points for each question). Reviews with 18–24 points were rated as having high quality (7–13 points: acceptable quality; 0–6 points: poor quality).

**Result and Conclusion:** Eighty-three systematic reviews were identified: three were of high, 66 of acceptable, and 14 of poor quality. Thus, periodontologists should be aware of the fact that high-quality systematic reviews are scarce.

186

**Choice of antibiotic after sampling by culture and real time PCR**C. Verner\*<sup>1</sup>, A. Daniel<sup>1</sup> and M. Sixou<sup>2</sup> et al.<sup>1</sup>Faculty of dentistry, Nantes, France <sup>2</sup>Toulouse, France

**Objectives:** This study compare the choice of antibiotics according to the two samplings protocols on the same patients: Real Time PCR which refers to the literature and the bacterial culture which makes possible to realize an antibiotics sensitivity test.

**Materials and methods:** Seventy two samplings from 18 patients suffering of aggressive periodontitis have been studied. Sampling has been done at same time and into the same pocket. Comparison of data of the two methods, according to the quality and quantity of pathogens found, has been carried out. An analysis by an antibiotics susceptibility testing was done for the culture.

**Results:** For the quality and quantity of pathogens found, the real time PCR technic presents results closed to the bacterial culture. But there is an agreement of the choice of antibiotic only for 11% of the cases and 5% an intermediate sensitivity was found. If we mix two antibiotics, a correlation was obtained in 44% of the cases and an intermediate sensitivity in 34%. However the cultures show a resistance in 83% to the metronidazole and 33% an intermediate resistance to the spiramicine.

**Discussion:** The variation of sensitivity is important for aggressive periodontitis.

**Conclusion:** There is an important difference between the two methods and in particular for the metronidazole few for other antibiotics. Real time PCR cannot substitute an analysis by an antibiotics susceptibility testing. We have to choose one of these methods according to our clinical objectives.

187

**Survival of furcation-involved molars after resective treatment**

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**Aims:** To enhance the prognosis of teeth with advanced furcation involvement, resective treatment options like root resection or hemisection can be indicated. The goal of this study was to describe the clinical outcome of furcation involved teeth that underwent resective therapy.

**Methods:** In 11 patients, 15 teeth (1 upper premolar, 12 upper molars and 2 lower molars) with advanced furcation involvement (grade II and III) were treated with resective techniques (1 trisection, 2 hemisections and 12 root resections) and consequently restored with fillings (2), single crowns (7) or double crowns (6). All patients agreed to participate in a regular recall system.

**Results:** During the follow-up period (minimum: 20 months, maximum: 132 months), 2 out of 15 resected teeth (13.3%) had to be extracted because of fractures of the remaining roots. The remaining 13 teeth showed a stable clinical outcome without signs of gingival or periodontal -inflammation.

**Conclusion:** If furcation involvement has advanced to grade II or III, resective treatment options should be considered as possible therapeutic strategies. Regular periodontal maintenance and a sufficient coronal restoration of the root resected teeth are important preconditions for long-term survival.

188

**The use of  $\beta$  tricalcium phosphate vs. bovine bone matrix in the treatment of deep intra – osseous defects**

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The aim of this study was to evaluate the effectiveness of pure  $\beta$  phase tricalcium phosphate (p TCP, Cerasorb<sup>®</sup>, Curasan, Germany) as compared to bovine bone matrix (Bio-oss<sup>®</sup> Geistlich Pharma, Switzerland) in the surgical treatment of periodontal osseous defects. Patients with paired intrabony defects and probing depths measuring  $\geq 5$  mm who have been treated for chronic periodontitis were selected. A split-mouth design was utilized. Sixteen intrabony defects were treated with  $\beta$  pTCP (experimental) and compared with 16 contra-lateral defects treated with bovine bone matrix (control). Pocket probing depth (PPD), epithelial attachment level (EAL), width of attached gingiva (WAG) were recorded at the baseline, 3 and 6 months after surgery. In the experimental group, PPD amounted to  $5.73 \pm 1.07$  mm before surgery, and decreased to  $2.50 \pm 0.42$  mm 6 months following surgery, while in the control group PPD decreased from  $5.45 \pm 0.94$  mm pre-surgery to  $2.45 \pm 0.46$  mm post-surgery. Six months following surgery the average gain in EAL was recorded  $1.64 \pm 0.68$  mm for the experimental group and  $1.50 \pm 0.80$  mm was found for the control group. Differences between groups (in favour to experimental group) were also found, but they didn't reach statistical significance ( $P > 0.05$ ). The present study concluded that the use of  $\beta$ TCP (Cerasorb<sup>®</sup>) or bovine bone matrix is equally effective in the treatment of deep periodontal defects.

189

# **Randomized split-mouth study on non-surgical therapy with the chlorhexidine xanthan-based gel chlosite®**

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**Objectives:** To compare effects of a xanthan-based chlorhexidine-gel delivered into periodontal pockets during initial therapy with the xanthan placebo.

**Methods:** Twenty patients with periodontitis underwent examination at baseline and after 6 weeks: PI, BOP, PD, CAL at 6 sites/tooth. Maximal-values/quadrant and mean-overall-values of PD and CAL were considered. Patients received One-Stage-Full-Mouth-Disinfection. Each quadrant of same arch received single subgingival application of chlorhexidine-xanthan-gel (Chlosite®, Ghimas, Italy) (CHL) or the xanthan placebo (XP). Patients used CHX 0.2% mouthwashes 6 weeks. Wilcoxon test was used.

**Results:** Both therapies resulted in significant improvements. In 'max-values/quadrant' of CHL, PD changed from  $8.60 \pm 2.08$  to  $5.50 \pm 2.13$ , CAL changed from  $9.42 \pm 2.38$  to  $6.62 \pm 2.12$ , while in the XP group PD changed from  $8.90 \pm 2.63$  to  $5.55 \pm 2.37$ , CAL changed from  $9.57 \pm 2.56$  to  $6.77 \pm 2.45$ . In the 'max-values/quadrant' of XP, treatment resulted in no CAL gains and slight PD reductions than CHL (ns). In 'mean-overall-values' of CHL, PD changed from  $4.61 \pm 0.83$  to  $3.10 \pm 0.58$  and CAL changed from  $5.07 \pm 0.85$  to  $3.81 \pm 0.70$ , while in XP group PD changed from  $4.85 \pm 1.05$  to  $3.26 \pm 0.74$  and CAL changed from  $5.31 \pm 1.12$  to  $4.17 \pm 0.90$  (all differences significant at  $P < 0.0001$ ). In the 'mean-overall-values', CHL resulted in slightly higher CAL gains (ns) and PD reductions (ns) than XP.

**Conclusions:** Following both approaches, there were no significant clinical improvements in terms of PD reductions and CAL gain.

190

# **Gingival crevicular fluid-free oxygen radicals level in patients with generalized aggressive periodontitis before and after initial therapy**

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Polymorphonuclear leukocytes become activated at the presence of inflammation and release free oxygen radicals (FR) which results in the oxidative destruction of cellular membranes and responsible for serious tissue damage. The aim of this preliminary study was to investigate the change between pre- and post-therapy level of FR in gingival crevicular fluid (GCF) in patients with generalized aggressive periodontitis (GAgP). Fourteen GAgP patients and 10 healthy individuals were participated. At least one single rooted tooth at each quadrant with a probing pocket depth (PPD)  $\geq 3$  mm and bone loss was selected for the patient group. Plaque index (PI), sulcus bleeding index (SBI), PPD and relative attachment level (RAL) were measured before and 12 weeks after initial therapy. GCF samples were obtained at same time points and FR levels were further analysed by luminol-enhanced chemiluminescence assay. Reductions in PI, SBI, PPD and gain in RAL were statistically significant compared to their baseline values ( $P < 0.0001$ ). The level of GCF-FR in the GAgP group was significantly decreased from  $355.6 \pm 82.4$  AUC to  $320.6 \pm 62.6$  AUC ( $P < 0.01$ ) while the level in the control group was  $114.2 \pm 18.5$  AUC. Our findings suggest that initial periodontal therapy caused reduction of GCF-FR levels in GAgP patients, although healthy control levels could not be achieved. Further studies with adjunctive antibiotics/antioxidants are needed to clarify the change in FR levels.

191

# **Plaque inhibition with 0.12% chlorhexidine spray in comparison to 0.2% spray and 0.2% mouthwash**

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**Aim:** To test whether 0.12% chlorhexidine (CHX) spray is as effective as 0.2% CHX spray and 0.2% CHX mouthwash in a 3-day 'de novo' plaque formation model.

**Materials and methods:** The study had a single blind, randomized 3-group parallel study design. 90 volunteers were enrolled in the study and received a thorough dental prophylaxis at the beginning of the test period. Subjects were randomly divided into three equal groups. They were instructed to use their assigned product only and requested to refrain from all forms of mechanical oral hygiene during the 3 day experimental period. After 3 days the plaque growth was assessed according to the Quigley and Hein plaque index (PI) at 6 sites per tooth.

**Results:** After 3 days the CHX mouthwash group had a mean whole PI of 1.17 (SD 0.38) compared with a mean PI of 1.41 for the 0.2% CHX spray (SD 0.33) and a PI of 1.49 (SD 0.42) for the 0.12% CHX spray. The difference between the mouthwash and the two sprays was statistically significant. The two sprays did not differ significantly from each other.

**Conclusion:** Within the limitations of this study, the twice daily applications of CHX spray both in concentrations of 0.2% and 0.12% inhibits 'de novo' plaque formation, although not to the level of 0.2% CHX chlorhexidine mouthwash.

192

# **Evaluation at 6 months of the healing of intrabony defects following treatment with collagen composite matrix. A controlled clinical study**

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**Purpose:** To compare clinically treatment of deep intrabony defects with collagen composite matrix (CCM) to access flap (AF) surgery, in early evaluation 6 months after therapy.

**Methods:** Thirty one patients with chronic periodontitis, with 58 intrabony defects in total, were randomly treated either with CCM (PONENTI, Bucharest, Romania) (test) or with AF surgery (control). Gingival parameters, soft tissue measurements were made at baseline and 6 months after therapy.

**Results:** No differences in investigated parameters were observed at baseline between groups. Healing was uneventful in all patients. 6 months after, test group showed a reduction in mean probing depth (PD) from  $8.00 \pm 1.76$  to  $4.70 \pm 1.80$  mm ( $P < 0.0001$ ) and a change in mean clinical attachment level (CAL) from  $10.07 \pm 3.60$  to  $7.67 \pm 4.10$  mm ( $P < 0.0001$ ). In control group, mean PD was reduced from  $8.61 \pm 1.31$  to  $5.86 \pm 1.94$  mm ( $P < 0.0001$ ) and mean CAL changed from  $10.32 \pm 2.07$  to  $8.32 \pm 2.79$  mm ( $P < 0.0001$ ). The test treatment resulted in higher PD reduction ( $P = 0.03$ ) and CAL gains (ns) than the control one. In the test group and in the control group, 14 and 11 of sites respectively gained at least 3 mm of CAL. In control group, a CAL gain of 6 mm was measured in 1 defect.

**Conclusions:** Within the limits of the present study, it can be concluded that: at 6 months after surgery both therapies resulted in significant PD reductions and CAL gains; evaluation at 6 months of the treatment with CCM resulted in statistically higher PD reductions but non-significant CAL gains than treatment with AF.

193

### Six months and one year results following treatment of intrabony defects with oily calcium hydroxide suspension and enamel matrix proteins

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**Background:** Treatment with enamel matrix proteins (EMD) or oily Calcium hydroxide suspension (OCHS) has been shown to enhance periodontal regeneration. However, until now there are limited data on the medium-term results following these treatment modalities.

**Aim:** Aim of the present clinical study was to present the 6-months and 1-year results following treatment of intrabony defects with EMD and OCHS.

**Materials and methods:** Twelve patients with a total of 22 intrabony defects of a probing depth of at least 6 mm, were randomly treated with one of the treatments. Following parameters were evaluated prior to surgery, at 6 months and at 1 year after: plaque index, gingival index, bleeding on probing, probing pocket depth (PPD), gingival recession (GR), and clinical attachment level (CAL). No statistically significant differences in any of the parameters were observed at baseline between the two groups.

**Results:** The sites treated with OCHS demonstrated a mean CAL gain of  $4.57 \pm 1.65$  mm ( $P < 0.001$ ) and of  $4.14 \pm 1.79$  mm ( $P < 0.001$ ) at 6 months and at 1 year respectively. Sites treated with EMD showed a mean CAL gain of  $4.62 \pm 2.77$  mm ( $P = 0.01$ ) at 6 months and of  $4.75 \pm 2.49$  mm ( $P = 0.01$ ) at 6 months and 1 year, no statistical significant differences between the two treatments were found in terms of PPD and CAL.

**Conclusion:** Within the limits of the present study, it may be concluded that short-term clinical results following treatment with OCHS and EMD can be maintained over a period of 1 year.

194

### The application of bovine pericardium for treatment of gingival recessions

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Gingival recessions were treated by various approaches. The aim of these procedures was not only the coverage of exposed roots but also the enhancement of gingival thickness. Thickness is assumed to be one of the factors for long term stability. Several authors used connective tissue grafts gained from the palate as an autogenous transplant. Recent approaches used acellular freeze dried human dermis as a homologue transplant to avoid a second site surgery. Beside the width of keratinized gingiva similar results could be obtained. In this presentation a new method of increasing gingival thickness by implantation of acellular deproteinised soluble degraded bovine pericardium (Tutoplast®) will be shown. The special preparation of this material maintains collagen in its original 3-D structure. 21 patients (15 female, 6 male), all nonsmokers, with 93 Miller class I or II recessions were treated with a coronal positioned split thickness flap with the bovine material underneath. Recession, CAL where documented at baseline, after 3 and 6 months Clinical results will show this material's ability to achieve the coverage of gingival recessions, to improve thickness and beside this to regenerate aesthetics. Histological and immunohistologic results demonstrate the increase of tissue thickness and the replacement by connective tissue over time. The membrane seems to act as a scaffold. Further investigations will have to compare the outcome of this method to usual techniques.

195

### Effectiveness of vector® in supportive periodontal therapy

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The aim of the present study was to evaluate the effectiveness of the Vector ultrasonic device in supportive periodontal therapy. 41 periodontal maintenance patients were treated in a split-mouth design with either Vector ultrasonic device (Dürr Dental, Bietigheim-Bissingen/Germany) (test) or scaling and root planing (SRP) using hand instruments (Gracey curettes, Hu-Friedy, Chicago, USA). Treatment was performed without local anaesthesia. Following parameters were evaluated at baseline and at 6 months: Plaque index (PI), gingival index (GI), probing depth (PD) and clinical attachment level (CAL). Patient's pain sensibility was measured using a numerical analogue scale registered from 1-10. Mean PD decreased in the test group from  $6.13 \pm 0.81$  mm to  $4.08 \pm 0.72$  mm ( $P \leq 0.001$ ) and in the control group from  $6.06 \pm 0.69$  mm to  $3.96 \pm 0.76$  mm ( $P \leq 0.001$ ). Mean CAL decreased in the test group from  $7.56 \pm 1.16$  mm to  $6.04 \pm 1.06$  mm ( $P \leq 0.001$ ) and in the control group from  $7.45 \pm 0.93$  mm to  $6.00 \pm 1.17$  mm ( $P \leq 0.001$ ). No significant differences between the two groups were found at 6 months. A statistically significant pain sensibility reduction was found when Vector was compared to hand instruments ( $P \leq 0.001$ ). It was concluded that: (i) both therapies led to significant clinical improvements and, (ii) without local anaesthesia, treatment with Vector seems to be more acceptable for patients than treatment with hand instruments.

196

### Effectiveness of tetracycline impregnated bone graft combined with membrane

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Infection of guided tissue regeneration and bone graft treated sites has been one of the common complications which can compromise healing. The aim of this study was to evaluate clinical and microbiological effect of XT-Resolut® membrane (XT-RM) in combination with tetracycline HCL impregnated PepGen P-15® bone graft (ABM-P15). Eleven defects with probing pocket depth (PPD) 36 mm and intrabony defect depth 34 mm was included. All patients were prescribed systemic tetracycline HCL for 2 weeks. Plaque index (PI), sulcus bleeding index (SBI) and microbiological samples were taken at baseline and 2, 4, 8, 12, 24 weeks and other clinical parameters were recorded at 0 and 24 weeks postsurgery. Reduction in PI and SBI at 2 and 4 weeks postoperatively and gingival recession ( $1.63 \pm 1.02$ ), reduction in PPD ( $3.72 \pm 1.36$ ), gain in relative attachment level ( $2.09 \pm 1.30$ ) were all statistically significant compared to their baseline values ( $P < 0.01$ ). The mean number of total viable counts also decreased at 2 and 4 weeks followed by a slight increase at 8, 12 and 24 weeks, but did not return to baseline level. The proportions of obligate anaerobes decreased from a baseline value of 25.77% to 3.23% at week 2, to 10.13% at week 4 ( $P < 0.001$ ), to 16.8% at week 8 ( $P < 0.05$ ) and increased at week 24 (29.05%). These results suggest that clinical improvements could be obtained and periodontopathogens could be controlled during initial phase of healing, however this effect was of short duration.

197

### Non-surgical periodontal therapy with adjunctive use of photodynamic therapy

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This single-blind, controlled clinical trial evaluated the adjunctive use of Helbo® Photodynamic Therapy (PDT) in periodontitis patients during initial periodontal therapy. 24 patients were randomly distributed to a test and control group. Full mouth plaque scores (FMPS), bleeding on probing (BOP), probing pocket depth (PPD), clinical attachment level (CAL) were evaluated and microbial sampling was performed at baseline and 3 months. *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *T. forsythensis* and *T. denticola* were analysed by PCR technique. All patients received conventional initial periodontal therapy and the test group received additionally a session of PDT. No differences were observed at baseline between groups. At moderately deep sites (PD 4–6 mm) mean CAL changed from  $5.6 \pm 1.2$  mm to  $4.8 \pm 1.4$  mm in control group and from  $5.8 \pm 1.4$  mm to  $4.8 \pm 1.3$  mm in the test group. At deep sites (PD  $\geq 7$  mm) mean CAL changed from  $7.6 \pm 0.9$  mm to  $6.6 \pm 1.3$  mm in the control group and from  $8.8 \pm 0.9$  mm to  $6.5 \pm 1.5$  mm in the test group. At 3 months, all parameters improved significantly ( $P < 0.05$ ) compared to baseline, but no statistically significant differences were observed between groups. A slight tendency for additional CAL gain was observed at deep sites in the test group. The microbiological analysis revealed that PDT facilitates further bacterial reduction. These preliminary results suggest that PDT might have a potential to improve the outcome of non-surgical periodontal therapy.

198

### The long-term effect of periodontal treatment cooperated with staff of rehabilitation facilities in adults with intellectual disability

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The purpose of this study was to evaluate the effects of supportive periodontal therapy (SPT) for patients with intellectual disability performed for 22 years. The study population consisted of 27 patients (14 males and 13 females, average 33.1 years old at the first visit) who lived in the rehabilitation facility for persons with intellectual disabilities. At first, staff members were educated the importance of plaque control and toothbrushing techniques by periodontists. Then the staff instructed the persons with intellectual disability in toothbrushing techniques as daily training. After having received initial preparation for 2 years, the patients visited the dental clinic for SPT on average of every 6 months during 22 years. SPT included repeated oral hygiene instruction and debridements. Clinical examination and measurements of gingival index (GI), periodontal pocket rate (PoR), plaque control record (PCR) and number of missing teeth were evaluated at the baseline, the termination of initial treatment and each SPT visit. A significant improvement was observed in GI, PoR and PCR following the initial treatment. During the 22 years of follow-up, all three measurements; GI, PoR, and PCR; were lower than those at the baseline. The average of missing teeth during 22 years was 0.96 per patient. The 51.8% of the patients did not lose any teeth during 22 years of follow-up. These results suggested that SPT was effective to maintain oral health of the study group.

199

### Desensitizing effect of lasers with and without sodium fluoride varnish

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The aim of the study was to evaluate and compare the efficacy of CO<sub>2</sub> and Er:YAG lasers alone and in combination with topical fluoride gel in the management of dentin hypersensitivity. The study was conducted on 50 patients divided into five groups who had clinically elicitable hypersensitivity in the upper anterior region. Following the pretreatment assessment of hypersensitivity, the selected teeth (94 teeth in each group) in all groups received CO<sub>2</sub> laser (1 W, CW, 15 sec), Er:YAG laser (30 Hz, 60 mJ, 15 sec), CO<sub>2</sub> or Er:YAG laser plus topical fluoride gel (F) (CO<sub>2</sub> + F) (Er:YAG + F) or F alone application. Recordings were assessed before treatment and 1 week, 1 month, 6 months after treatment. Within each test group there was a significant reduction in hypersensitivity before and 1 week, 1 month and 6 months after treatment ( $P < 0.001$ ). All the groups showed significant reduction in hypersensitivity at each follow-up examination when compared to the F group. This significance was higher in the CO<sub>2</sub> and CO<sub>2</sub> + F groups ( $P < 0.001$ ) especially at the 1st week compared to the Er:YAG ( $P < 0.05$ ) and Er:YAG + F ( $P < 0.01$ ) groups. The combination of CO<sub>2</sub> and Er:YAG lasers with fluoride gel seem to show an impressive efficacy, when compared to fluoride gel alone, in treating dentin hypersensitivity. However, no superiority was found in terms of desensitization between CO<sub>2</sub>, Er:YAG, CO<sub>2</sub> + F and Er:YAG + F groups.

200

### Comparison of galvano-ceramic and metal-ceramic crowns on clinical periodontal parameters: evaluation after 24 months, preliminary data

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Galvano-ceramic crowns (GCC) are considered as biocompatible, stable and esthetic prosthetic restorations. Few data are available on their clinical effects on periodontal tissues.

**Objectives:** To test the impact of two crown systems on clinical and inflammatory responses of periodontal tissues.

**Methods:** In a prospective, blinded randomised clinical trial, GCC (AGC®, Wieland, Germany) and metal-ceramic crowns (CMC, Degunorm®, Degudent, Germany) were compared in 64 periodontally healthy patients (split-mouth design). This preliminary report presents the first 24-months data for 21 patients. Clinical parameters (GI, PLI, PPD) were taken from 6 sites/tooth by one blinded examiner. Gingival crevicular fluid flow rate (GCF) was determined by Periotron® 6000 (Harco, USA). Non parametric Wilcoxon test was used for statistical analyses.

**Results:** Obvious clinical inflammation was only rarely observed. PLI (0.29 vs. 0.14,  $P = 0.01$ ) and GI (0.59 vs. 0.33,  $P < 0.01$ ) were statistically significant lower at GCC sites after 24 months. PPD were not different and gingival recessions were not observed. GCF was statistically lower at GCC sites at 6 months ( $P = 0.02$ ) after insertion.

**Conclusion:** After 24-months, periodontal tissues adjacent to GCC show significant better clinical parameters. However, the differences were small in absolute numbers. GCC may contribute to the maintenance of gingival health at prosthetic sites. Supported in part by DFG grant 325/3 and Wieland, Germany.



201

### Effects of 0.2% chlorhexidine gluconate to the plaque accumulation on silk suture materials in oral mucosa: a scanning electron microscope study

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**Aim:** To evaluate the effect of chlorhexidine gluconate (CHX) to the plaque accumulation on silk sutures by scanning electron microscope (SEM).

**Method:** After flap operation of mandibular posterior teeth, 14 patients were randomly divided into two groups. 0.2% CHX was used in the test group and saline for the control group. The sutures were removed after 7 days and kept in 70% alcohol and processed for SEM. The material was critical-point dried using dry ice method and observed in a field emission SEM. The contamination of the suture surfaces was scored and the Mann-Whitney *U*-test was used for statistical analysis.

**Results:** Scanning electron microscope observations showed that all of the silk sutures were encrusted with plaque accumulation on the surface and in the inter-filamental spaces. The surface of silk sutures in the control group was completely contaminated by debris along the surface. Significant differences were found between the test and the control groups ( $P < 0.01$ ). The amount of the contamination was significantly lower in the test group. Higher magnification of SEM showed numerous microorganisms which were predominantly rod-shaped bacteria and this debris to be composed mainly of clusters of spherical organisms with some filamentous structures.

**Conclusion:** The results of this study suggested that the CHX group had less contaminated surface area than the control group. SEM observations strongly indicated the effect of CHX in the reduction of plaque accumulation on silk sutures.

202

### Microbiological and clinical effects of chitosan mouthrinses on plaque formation: a pilot study

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Various antiseptic substances have been assorted in mouthrinses and dentifrice preparations to improve outcome of mechanical oral hygiene procedure. Chitosan (Ch) is a derivative of chitin, a natural biopolymer, which is biologically safe, biodegradable, non-toxic and can be formulated in a variety of forms in dentistry. The aims of this study were to evaluate the microbiological and clinical effects on plaque inhibition of Ch mouthrinses and to compare with 0.2% chlorhexidine (CHX). 36 gingivitis patients were recruited. Following clinical examination and recorded PI, GI, PD measures, the volunteers were given oral hygiene instructions, scaling and professional mechanical tooth cleaning. After the final PTC, volunteers were allocated to 1 of 3 treatment groups. Group A: rinsed twice daily for 60 sec each time with Ch solution, group B: rinsed twice daily for 60 sec each time with 0.2% CHX, group C: rinsed twice daily for 60 sec each time with Ch + 0.2% CHX. Subgingival plaque samples were collected from each subject on Day 0 and after 1, 2 and 4 days. And this sample assayed for *S. mutans*, *C. albicans* and *enterococci*. After 4 days of non-brushing, there was no significant differences in plaque accumulation between groups. No significant differences were seen in *S. mutans* and *enterococci* levels among the groups; however *C. albicans* levels were statistically significant between all groups. It was concluded that Ch mouthrinses may be recommended in reducing and delaying the bacterial accumulation.

203

### Preliminary evaluation of the efficacy of a topical anaesthetic on pain and unpleasantness during periodontal treatment

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Periodontitis is the result of complex interrelationships between infectious agents and host factors. Their treatment consists of elimination of the bacterial biofilm and supra and subgingival calculus. The main goals are to reduce the inflammatory and to obtain a biocompatible surface of the tooth. The patient could feel pain during probing or scaling and root planning, which requires the use or of the anaesthesia by injection. This injection with the insertion of the needle in soft tissues can be painful and produce anxiety for the patient. Its duration of action can be also too long. Furthermore, the patient can have unpleasant feelings and numbness. The recent projections into galenic made it possible to develop a eutectic mixture containing prilocaïne and lidocaïne (Oraqix®). The rheological properties of this mixture confer to him a liquid state at low temperature - what makes it possible to condition it in the form of carpule, before use - and in the viscous gel form in the periodontal pocket. In this preliminary study we tested Oraqix® on 40 patients. We evaluate it in term of facility of use, effectiveness, time of action, the duration of the anaesthesia obtained, and eventual side effects. Application of Oraqix® reduced both pain intensity and unpleasantness. Generally, the patients accepted the anaesthetic procedure well. Oraqix may be recommended as a simple pharmacologic strategy to reduce pain and unpleasantness during periodontal treatments.

204

### Coverage of gingival recessions with connective tissue grafts and platelet rich plasma

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Connective tissue grafts (CTG) are accepted as golden standard for coverage of gingival recessions. However, new procedures are developed to overcome problems like a second surgical site and graft size. Platelet rich plasma (PRP) obtained from patients' blood is an autologous source of several growth factors. PRP improves bone and periodontal regeneration. The purpose of the present study was to compare the efficacy of CTG and collagen sponges soaked with PRP in root coverage procedures. 24 patients were enrolled to the study. Test group was treated with PRP activated by calcium and blood obtained from operation field as a source of thrombin. PRP soaked collagen sponges were placed under full thickness flaps. The control group was treated by CTG combined with partial thickness flap. After 6 months clinical parameters were compared to baseline values. Postoperative healing at PRP sites was uneventful and results were esthetically pleasing Root coverage was 84% with PRP and 85% with CTG at 6th month. Statistically significant difference was not observed between the groups. CTG sites had a significant increase in keratinised tissue width as opposed to PRP sites. The results of this study demonstrated that PRP which does not possess cross infection or antigenicity concerns can be prepared at required size and used for treatment of gingival recessions.

205

# Antimicrobial susceptibility of three periodontal pathogens recovered from a chronic periodontitis sample with a high intake of antibiotics

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The aim was to determine the antimicrobial susceptibility of *Fusobacterium nucleatum*, *Prevotella intermedia* and *Porphyromona gingivalis* isolated from periodontitis patients. Twenty-five patients diagnosed with generalized moderate-advanced chronic periodontitis were recruited according to specific criteria. All patients filled out a questionnaire about antibiotic intake. The deepest pocket in each quadrant was sampled. Pooled samples were mixed, diluted and plated on enriched brucella agar plates. Identification of the bacteria was performed based on: colony morphology, gram staining, aero-tolerance and biochemical reactions (RapidAna II, Remel). The bacteria were subjected to antimicrobial testing using: amoxicilin, tetracycline, doxycycline, azithromicin and metronidazole (*E*-test, ABBiodisk). The minimal inhibitory concentrations obtained were compared to crevicular fluid concentrations to determine resistance. Amoxicilin-resistant species were tested for  $\beta$ -lactamase production. Forty-four percent of the patients used antibiotics without any medical prescription. The presence of eleven species could be confirmed: four *F. nucleatum*, five *P. intermedia* and two *P. gingivalis*. All strains were resistant to metronidazole, five were resistant to tetracycline and azithromicin, two were resistant to amoxicilin and doxycycline. The strains resistant to amoxicilin were positive for  $\beta$ -lactamase. Antimicrobial resistance was a common phenomenon for the bacterial samples analysed.

206

# A retrospective longitudinal study on early onset periodontitis

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The aim of the study was to examine the long-term result of periodontal treatment in EOP patients.

**Materials and methods:** Twenty nine EOP patients were re-examined 13–15 years after end of treatment. The patients were re-examined and retrospective data were recorded from the files. The number of appointments for periodontal treatment during the maintenance care was registered. In 16 of the patients the presence of *Actinobacillus actinomycetemcomitans* (*A.a*) were monitored at the appointment for re-examination.

**Result:** PII, GI, the number of deep pockets and bone loss sites were significantly reduced during periodontal treatment. At the time for re-examination the periodontal status were still better compared to the primary status, but compared to the status at the beginning of the period of maintenance care it showed a higher mean number of pockets and a higher mean number of bone loss sites. The individuals with deteriorated status had had significantly fewer appointments of maintenance care compared to the individuals with unchanged or improved periodontal status. 8 of 16 patients harboured (*A. a*) in their deepest pockets at the time of re-examination. The occurrence of *A. a* was not associated with progression of the disease.

**Conclusion:** The periodontal health for this group remained better than before treatment. For the individual patient the present periodontal health is associated with the number of yearly maintenance care.

207

# The effects of a 0.1% chlorhexidine mouthwash on plaque and gingival indices in patients with gingivitis: a 3-month study

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**Aim:** To compare the clinical efficacy and tolerability of a 0.1% chlorhexidine (CHX) mouthrinse during a 3-month trial using a placebo and a 0.2% CHX solutions as controls.

**Materials and methods:** A prospective, parallel, randomised clinical trial was designed to evaluate the clinical effects of the test mouthrinse. 37 patients were assessed at baseline, and days 7, 14, 28 and 84. Clinical parameters were assessed by full mouth modified gingival index, plaque index, discoloration index and bleeding on probing. Data was analysed using the Dunnett's, Fisher and *t*-Student tests.

**Results:** The 0.1% CHX and 0.2% CHX groups showed higher reductions in Plaque Index after 7, 14, 28 and 84 days ( $P < 0.05$ ; excepted 0.1% CHX after 84 days,  $P = 0.06$ ), as compared with the placebo group. The same was true for the modified Gingival Index ( $P < 0.05$ ; excepted 0.1% CHX after 28 days and after 84 days,  $P = 0.11$ ). The discoloration index demonstrated a significant higher level of staining for the 0.2% CHX group after 84 days, as compared with placebo, while no significant differences were found between the 0.1% CHX and the placebo group.

**Conclusions:** The 0.1% chlorhexidine solution can be use as an adjunctive treatment for gingivitis. The test solution was more acceptable for patients because of its lower discoloration of teeth and taste alteration.

Supported by Pierre-Fabre.

208

# The clinical impact of PRP and TCPB in the treatment of infrabony defects

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It is conceivable that concentrated growing factors within platelet rich plasma PRP up-regulates cellular activity and subsequently promotes periodontal regeneration in vivo. In combination with TCPB (RTR<sup>®</sup>) activated cytokine cocktail from PRP should extensively enhance periodontal tissue regeneration. The objective of this study was to compare the clinical effectiveness of two regenerative procedures for intrabony defects: a combination of PRP/TCPB/GTR vs. a combination of PRP/TCPB. 15 patients took part in the study. Interproximal bony defects were surgically treated with a combination of PRP/TCPB/GTR or PRP/TCPB. The primary outcomes of the study included changes in probing depth, attachment level, and defect fill as revealed by re-entry surgeries at 6 months post-treatment. 6 months after surgery both treatment modalities resulted in significant probing depth reduction and clinical attachment gain compared to baseline values. Clinical attachment level improvement was  $3.55 \pm 2.52$  mm for the PRP/TCPB group and  $3.68 \pm 2.35$  mm for the PRP/TCPB/GTR group. Re-entry surgeries evaluated similar defect fill for both treatment groups (PRP/TCPB group:  $3.88 \pm 2.55$  mm, PRP/TCPB/GTR group:  $4.05 \pm 2.33$  mm). Differences between clinical results obtained in the two treatment groups showed no statistically significance. Surgical procedure of this study resulted in highly effective and clinically equivalent changes with no statistically significance when comparisons were made between test and control group.

209

**A 980 nm diode laser effect on clinical and microbial parameters of aggressive periodontitis**

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The aim was to compare the effect of scaling and root planing (SRP) to SRP plus diode laser treatment (SRP + LAS), to LAS or to no treatment (CRL) on clinical and bacterial parameters of aggressive periodontitis (AgP). 30 AgP patients were assessed for PD, CAL and BOP. Four subgingival plaque samples were randomly obtained from each individual, one in each quadrant. Following baseline (BL) sampling, each quadrant randomly received SRP, SRP + LAS, LAS, CRL. A 980 nm diode laser (SmilePro980TM, Biolitec) was used in a continuous mode and a 2 W power setting. Plaque samples were collected from the same site in each quadrant 6 weeks (R1), 12 weeks (R2) and 6 months (R3) post treatment. Clinical parameters were recorded at the same time intervals. The level of *P. g.*, *B. f.*, *A. a.*, *T. d.* as well as total bacterial load (TBL) was evaluated using DNA probes (IAI Pado Test®). Bacterial counts, PD, CAL and BOP were significantly decreased following SRP, SRP + LAS, LAS and didn't reach BL levels at R3. SRP + LAS showed lower bacterial levels compared to SRP or LAS at R1, R2 and R3. *P. g.*, *B. f.*, *T. d.* and TBL exhibited a significant time by treatment interaction effect ( $P < 0.001$ ) showing that each treatment had a different performance on time. SRP + LAS mean levels of all bacteria at R3 were never higher than the corresponding levels of SRP or LAS at R1. In conclusion, SRP + LAS seem to have a superior effect on both clinical and bacterial parameters of AgP over the 6-month monitoring period.

210

**Effects of platelet riched plasma (PRP) on soft tissue wound healing**

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The main purpose of this study was to evaluate the effects of PRP (platelet riched plasma) on clinical effects of soft tissue wound healing in early healing period. In this split-mouth study 16 patients were enrolled. Ten patients with bilateral areas of insufficient attached gingiva in lower jaw were included. Free gingival graft (FGG) was placed on control site, while the other (experimental) site was treated with FGG in combination with PRP. Six patients, each contributing a pair of Miller Class I or II buccal gingival recessions, were treated. In each patient one randomly chosen defect received connective tissue graft (CTG) with coronally advanced flap, while the contralateral defect received a CTG in combination with PRP. Clinical effects were evaluated on the 7th, 14th and 21st day post surgery by early healing index(EHI). Mean EHI values recorded on the 7th day were 2.8 in FGG group, 3.9 in FGG + PRP group and 2.9 in CTG group and 4.1 in CTG + PRP group. Results achieved on the 14th day were 3.5 in FGG group, 4.8 in FGG + PRP group and 3.4 in CTG group and 4.7 in CTG + PRP group, while the results detected on 21st day were 4.5 in FGG group, 4.9 in FGG + PRP group and 4.4 in CTG group and 4.9 in CTG + PRP group. According to the results of this study, we can conclude that use of PRP stimulate and provides superior wound healing response in early healing period(first 2 weeks post surgery).After this period, there are no significant clinical effects on wound healing process and treatment outcomes.

211

**Treatment efficacy of palatal connective tissue graft vs. allogenic freeze-dried dermis graft applied for root coverage in buccal gingival recessions**

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**Background:** The subepithelial connective tissue graft (SCTG) is currently the golden standard in the treatment of gingival recession when the objective is root coverage. Recently, the use of allogenic freeze-dried dermis (AFDD) has been proposed as an alternative grafting material, but data on its efficacy are still limited. The objective of this study was to compare the SCTG and AFDD procedures in the treatment of gingival recessions as it relates to clinical soft tissue coverage, post-operative pain and esthetic outcome.

**Materials and methods:** Forty Miller class I or II bilateral symmetrical areas of gingival recession were treated in 11 patients. All clinical measurements were performed for the selected teeth 2 weeks after initial therapy (baseline) and again 6, 12 and 20 weeks after surgery. Clinical photographs were taken at the same visits. Patients were asked to evaluate post-operative discomfort separately on each side one week after the procedure.

**Results:** Both procedures gave comparable results in terms of clinical root coverage (both vertically and horizontally), increase in keratinized gingiva, as well as matching adjacent tissue colour and consistency. However, the use of the AFDD resulted in reduced postoperative pain and generally fewer postoperative complications.

**Conclusions:** Our study demonstrated that the AFDD could serve as a good alternative to SCTG for root coverage procedures with the added advantage of fewer post-operative complications.

212

**Access flap vs. papilla preservation technique (PPT) combined with emdogain-TS®**

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The aim of the present study was to present the 1½ year results following treatment of intrabony defects with Access Flap vs. PPT combined with Emdogain-TS® (EMD-TS®). Twenty-five patients with a total of 70 intrabony defects were treated by Access Flap (35 defects) and PPT (35 defects) combined with EMD-TS®. The following clinical parameters were evaluated at the baseline and at 1½ years after treatment: probing depth (PD), gingival recession (GR), and clinical attachment level (CAL). The primary outcome variable was CAL. The measurements were made at 6 sites per tooth: mesiobuccal (mv), buccal (v), distobuccal (db), mesiolingual (ml), lingual (l) and distolingual (dl). The cemento-enamel junction (CEJ) was used as reference point. In case the CEJ was not visible, a restoration margin was used for these measurements. The sites treated with papilla preservation technique combined with EMD-TS® demonstrated mean CAL decrease from  $7.9 \pm 1.1$  mm to  $4.0 \pm 0.8$  mm ( $P < 0.001$ ) at 1½ years. The sites treated with Access Flap combined with EMD-TS® demonstrated mean CAL decrease from  $8.5 \pm 2.5$  mm to  $4.8 \pm 2.0$  mm ( $P < 0.002$ ) at 1½ years. It can be concluded that the treatment of intrabony defects with papilla preservation technique combined with EMD-TS® as well as Access Flap combined with EMD-TS® results in significant improvement of the investigated clinical parameters. As our results show there was no significant difference between the data of both investigated groups.

213

### Moxifloxacin as an adjunctive antibiotic in treatment of severe chronic periodontitis – a pilot study

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**Objectives:** The aim was to perform a pilot study using moxifloxacin in treatment of severe periodontitis.

**Materials and methods:** The randomised study included 35 patients with severe chronic periodontitis. All the patients underwent a systematic periodontal therapy (oral hygiene, deep scaling and root planing, supportive therapy), but in 17 randomly selected patients additionally 400 mg moxifloxacin / d over 7 d were applied. Clinical parameters, the microflora, IL-8 and the activity of granulocyte elastase were determined in sites with an attachment loss > 5 mm immediately before periodontal therapy as well as 3 and 9 months later.

**Results:** The clinical outcome in both groups was similar. In both groups a reduction of IL-8 in crevicular fluid was observed; the decrease of granulocyte elastase activity was higher in the moxifloxacin group (80% reduction) than without antibiotics (55%). After treatment less *T. denticola* and *T. forsythia* were found, but only in the moxifloxacin group a significant reduction of *P. gingivalis* ( $>10^5$  before treatment in 40.9% of the samples, 9 months later in 7.2%) and leukotoxin-positive *A. actinomycetemcomitans* ( $>10^5$  before treatment in 12.4% of the samples, 9 months later in 2.4%) was detected.

**Conclusions:** The results of the study indicate the necessity of a larger clinical trial to evaluate moxifloxacin as adjunctive antibiotic in treatment of periodontitis.

214

### Comparison of epithelial proliferative activity following surgical therapy with bioactive glass and flap surgery

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**Background:** Bioactive glass (BG) graft material has been suggested as having an ability to enhance regenerative type of healing of periodontal lesions. The aim of this study was to compare proliferative activity of epithelium following surgical therapy with BG and flap surgery.

**Materials and methods:** Ten chronic periodontitis patients (mean age:  $41.9 \pm 2.34$ ) with paired intrabony defects were randomly assigned surgical therapy with BG (test) or flap surgery (control). Gingival biopsies were taken at surgery (baseline) and 8 week follow-up evaluation visit. After histological processing, proliferating cell nuclear antigen (PCNA) expression was determined in immunohistochemically-stained sections and epithelial thickness was measured in hematoxylin and eosin-stained sections.

**Results:** At postoperative 8 week, PCNA expression and epithelial thickness were significantly increased in both treatment groups compared to the baseline values ( $P < 0.05$ ). There was no significant difference between baseline values of two groups ( $P > 0.05$ ) while at 8 weeks, increase in PCNA expression of control group was significantly greater than that of test group ( $P < 0.05$ ).

**Conclusions:** The results of this study revealed that proliferative activity of epithelium increased in both treatment modalities. Within the limits of this study, it was suggested that healing by long junctional epithelium might be more prominent following flap surgery compared to surgical therapy with BG.

215

### Levels of platelet activating factor in gingival tissue following periodontal surgical therapy

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**Background:** The role of platelet activating factor (PAF), a potent inflammatory phospholipid mediator, on healing after periodontal surgery remains unclear. The aim of this study was to determine levels of PAF in gingival tissues collected from sites that have undergone guided tissue regeneration (GTR) and flap surgery.

**Materials and methods:** Using split mouth design, 20 intrabony defects were randomly assigned treatments with GTR (test) or flap surgery (control). Gingival tissue samples were obtained at surgery (baseline) and 6 month follow-up evaluation visit. One half of each sample was used for histomorphometric analysis that included the measurements of number and diameter of blood vessel profiles (BVP), the other half of sample was used for analysis of PAF levels by high performance liquid chromatographic method.

**Results:** Platelet activating factor levels and diameter of blood vessel profiles were significantly decreased ( $P < 0.001$ ), number of BVP was significantly increased ( $P < 0.05$ ) in both treatment modalities compared to baseline values. Postoperative number of BVP were significantly higher in test group ( $P < 0.001$ ), whereas there was no significant difference in postoperative PAF levels between the two groups ( $P > 0.05$ ). A statistically significant negative correlation was found between PAF levels and the number of BVP ( $P < 0.001$ ).

**Conclusion:** Decrease in PAF levels and increase in the number of blood vessels might be indicators of resolution of inflammation, and periodontal repair and regeneration.

216

### The effect of periodontitis on biting abilities

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Periodontitis is a destructive disease which causes the loss of tooth-supporting tissues. The loss of bone may affect the biting abilities (bite force, occlusal contact area). The aim of this study is to evaluate the biting abilities in periodontitis patients and in healthy control group. The study group consisted of 20 chronic periodontitis patients in whom the mean probing pocket depth and probing attachment level was 3.21 mm and 4.16 mm, respectively. The periodontitis patients had  $\geq 7$  sites with  $\geq 5$  mm probing depths. Bone loss observed in periapical radiographs taken with parallel technique confirmed clinical diagnosis. Twenty subjects with healthy periodontium served as control. Biting abilities were measured with a pressure sensitive sheet and analysed with an image scanner following initial periodontal treatment (scaling and root planning). Independent samples *t*-test showed statistically significant differences between the groups for bite force ( $P = 0.006$ ) and occlusalcontact area ( $P = 0.033$ ) measurements at the 95% confidence level. In the light of these findings, this study suggests that the loss of bone support may cause a decrease in bite force and occlusal contact area. Therefore, using regenerative techniques to regain lost periodontium may help to improve biting abilities.

217

### Comparative study of salivary chlorhexidine concentrations after oral application of different pharmaceutical forms

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**Introduction and Aims:** Chlorhexidine concentration in the mouth may vary according to the pharmaceutical form involved. The

present study compares the salivary chlorhexidine concentration after the application of three different pharmaceutical forms.

**Materials and methods:** Forty healthy subjects over 18 years old were included in a randomized, cross-over, comparative study to analyze the evolution of salivary chlorhexidine concentration after a single oral application of three different pharma. Salivary samples were collected at baseline and 5 min and 1, 2, 6, 12, 24, 36 and 48 h after application. Study variables: mean chlorhexidine concentration at each control time point, maximum concentration, clearance rate and area under the concentration-time curve. The study was authorized by the Clinical Research Ethics Committee of Virgen de la Arrixaca Hospital.

**Results:** Chlorhexidine was still detected in saliva at the last control (48 h) with all three products. The mouthrinse and bioadhesive gel afforded significantly higher chlorhexidine concentrations than the spray formulation ( $P = 0.005$  and  $P = 0.001$ , respectively). In the case of the gel, the initial concentrations decreased faster than when the mouthrinse was used ( $P = 0.004$  after 1 h and  $P = 0.012$  after 2 h).

**Conclusions:** Forty-eight hours after a single application of chlorhexidine, the drug is still detectable in saliva, although the evolution of chlorhexidine concentration varies according to the pharmaceutical form used.

218

### Repeated subgingival chlorhexidine varnish administration in persistent periodontal pockets

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**Background:** Studies have indicated that the outcome of scaling and root planing (SRP) may benefit from the adjunctive subgingival application of a chlorhexidine (CHX) varnish. The aim of this study was to investigate the clinical impact of the latter at persistent pockets.

**Materials and methods:** Fifteen chronic periodontitis patients having completed root debridement phase at least 3 months prior to the study, participated in this intra-subject parallel study. Per patient at least four persistent non-adjacent bleeding pockets  $\geq 5$  mm were selected and randomized at split mouth level to one of two strategies. Control sites were scaled and root planed. If pockets remained deeper than 3 mm, SRP was repeated at 1 and 3 months. Test sites received the same treatment; yet, they were additionally disinfected using a CHX varnish. Clinical response parameters were recorded at baseline, after 1, 3 and 6 months. A site-based analysis was performed by means of repeated measures ANOVA.

**Results:** Both strategies were effective at improving the clinical status. At one month, test sites expressed a significant additive pocket reduction of 0.39 mm ( $P = 0.035$ ) and a trend towards a higher clinical attachment gain ( $P = 0.074$ ). However, beneficial effects were not maintained.

**Conclusions:** The application of a CHX varnish as an adjunct to SRP in persistent pockets can be considered; yet, when patients are well-compliant following an intensive supportive care program, this agent does not provide a lasting benefit.

219

### Bacterial contamination of e-PTFE membranes following regenerative periodontal therapy with a synthetic bone-graft and PRP

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**Objective:** The objective of this study was to assess the influence of the bacterial contamination of e-PTFE barriers used in GTR-therapy of intrabony defects on the healing.

**Methods:** Thirty eight patients (19/19) each with one deep intrabony defect were treated with a b -TCP (Cerasorb) + PRP + e-PTFE (test) or b -TCP + e-PTFE (control). PPD and CAL were recorded at baseline and at 6 months. Membranes were removed after 5–6 weeks and microbiological examination performed. The presence of 6 selected periopathogenic species: *A. actinomycetemcomitans* (*A. a.*), *P. gingivalis* (*P. g.*), *P. intermedia* (*P. i.*), *F. nucleatum* (*F. n.*), *Actinomyces spp* (*A. spp.*), *P. micros* (*P. m.*) were identified by cultures.

**Results:** In the test group *P. g.* was not detected. The number of pathogenic isolates cultured from the retrieved membranes was: *A. a.* 3 (16%), *P. i.* 6 (31%), *F. n.* 5 (26%), *A. spp.* 6 (31%), *P. m.* 12 (63%). The CAL gains measured at 6 months. In the presence of periodontal pathogens were: *A. a.* 54%, *P. i.* 56%, *F. n.* 62%, *A. spp.*, 62%, *P. m.* 53%. In the control group *P. i.* was not detected and was positive for 1 *A. a.* (5.3%), 2 *P. g.* (12%), 10 *F. n.* (53%), 5 *A. spp.* (26%), 13 *P. m.* (68%). The CAL gains obtained at 6 months in the presence of periodontal pathogens were: *A. a.* 50%, *F. n.* 52%, *P. g.* 60%, *A. spp.*, 55%, *P. m.* 54%.

**Conclusion:** Mean CAL gain was higher in the test group even in the presence of *A. a.*, *P. i.*, *F. n.* although the CAL gain showed marked individual variations.

220

### Clinical evaluation of platelet rich plasma and a synthetic bone graft material in the treatment of intrabony defects

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The purpose of this study was to evaluate the effect of bioactive glass graft material (BGGM) with and without PRP on clinical healing of intrabony defects in humans. Twenty nine intrabony defects were randomly treated with either PRP/BGGM ( $n = 15$ ) or BGGM ( $n = 14$ ) alone. Nine months after surgery, significant reductions were observed on plaque index (PI), gingival index (GI), bleeding on probing (BOP), probing depth (PD), clinical attachment level (CAL), the distance between cemento-enamel junction (CEJ) and base of the defect (ESJ-BD) and intrabony defect depth (IDD) in both groups. The values of gingival recession (GR) and the distance between CEJ crest of the defect (ESC-CD) were not statistically significant. PD reduction was  $3.60 \pm \text{mm}$  in the PRP/BGGM group and  $3.29 \pm 1.68$  mm in BGGM group. Clinical attachment gain was  $3.13 \pm 1.77$  mm in the PRP/BGGM and  $2.86 \pm 1.56$  mm in BGGM group. IDD was reduced  $3.47 \pm 0.53$  mm and  $3.36 \pm 0.55$  mm in the test and control groups, respectively. None of the differences between two treatment modalities were statistically significant. The results of this study suggest that both PRP/BGGM combination and BGGM alone are effective in the regenerative treatment of intrabony defects in patients with severe periodontitis. The results also showed that using PRP with BGGM has no additional benefit in the reduction of pocket depth, clinical attachment gain and defect fill.

221

### Clinical and microbiological effects of different antimicrobials on generalized aggressive periodontitis

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**Aim:** To evaluate and compare the effects of adjunctive metronidazole plus amoxicillin, doxycycline and metronidazole on clinical and microbiological parameters in patients with generalized aggressive periodontitis.

**Materials and methods:** Forty three patients participated in this Randomized Clinical Trial divided into four groups. Six weeks

## Posters: Periodontal therapy

after scaling and root planning (SRP), groups 1, 2 and 3 received adjunctive metronidazole plus amoxicillin, doxycycline and metronidazole respectively and group 4 acted as controls. Clinical recordings concerning probing depth, probing attachment level and bleeding on probing were performed at baseline, 6 weeks after SRP and 6 months from baseline. Subgingival samples were analysed using the 'checkerboard' DNA-DNA hybridization for *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Tannerella forsythensis* and *Treponema denticola*.

**Results:** All treatments resulted in improvement of clinical parameters (ANOVA  $P > 0.05$ ). Systemic administration of metronidazole plus amoxicillin or metronidazole resulted in statistically significant greater reduction of the proportion of sites  $> 6$  mm than scaling root-planing ( $z$ -test,  $P < 0.05$ ). These antimicrobials yielded a significant effect on levels of important periodontal pathogens for 6 months.

**Conclusions:** Adjunctive metronidazole plus amoxicillin or metronidazole alone (when *A. actinomycetemcomitans* is not involved) is effective in deep pockets of aggressive periodontitis patients.

222

### Comparative clinical study of guided tissue regeneration and subepithelial connective tissue graft in the treatment of gingival recession

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The aim of this study was to compare the clinical efficacy of two surgical approaches in the treatment of the recession type defects and to determine the influence of smoking on the outcome of these techniques. Seventeen pairs of Miller Class I and II defects were selected in 17 patients (11 non-smokers, 6 smokers). The defects were randomly assigned to the test (GTR using a bioabsorbable membrane) or control (SCTG combined with a coronally positioned flap). Recession depth (RD), probing depth (PD), clinical attachment level (CAL), recession width (RW), width of keratinized tissue (KT) were assessed at baseline and 6, 9, 12 months postsurgery. Both techniques yielded significant improvements in terms of RD and RW decrease, CAL and KT gain compared to baseline values. There were no significant differences between the two groups in RD, CAL, RW either at baseline or postoperative evaluations. The percentage of root coverage (RC) achieved 80.04% for GTR and 89.82% for SCTG on the 1 year postsurgery ( $P = 0.212$ ). Smokers showed significantly less percentage of RC compared to non-smokers in GTR group (45.26% vs. 87.67%, respectively;  $P = 0.018$ ). However, in SCTG group, smoking was not associated with a statistically significant decrease in percentage of RC (80.66% for smoking, 95.83% for nonsmoking;  $P = 0.750$ ). The results indicate that both surgical approaches are effective in addressing root coverage. Treatment outcomes following GTR in gingival recession defects are impaired in smokers.

223

### Clinical and biochemical evaluation of systemic doxycycline as an adjunct to non-surgical periodontal therapy of chronic periodontitis

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**Background:** Periodontal breakdown is a consequence of the action of bacterial and host derived proteinases and mediators. Ideal therapy must address both components by applying standard mechanical procedures and systemic pharmacotherapeutics as bacteria reducing and host modulating adjuncts. The present

study was planned to evaluate the adjunctive efficacy of systemic doxycycline clinically and biochemically.

**Methods:** Twenty chronic periodontitis patients were placed in two groups. The control group received initial periodontal therapy, while the test group additionally received systemic doxycycline for 2 weeks. PI, SBI, probing pocket depth (PPD) and relative attachment level (RAL) recordings and GCF samples were obtained initially and at 1st, 3rd and 6th months after therapy. GCF levels of MMP-8 and PGE<sub>2</sub> were evaluated by enzyme immunoassay. Mann-Whitney  $U$  and Wilcoxon signed rank tests were used for statistical analysis.

**Results:** Both groups showed significant improvements in all parameters ( $P < 0.05$ ). Initially the mean PI value was higher in the control group ( $P < 0.05$ ) but the intergroup differences were not significant ever after ( $P > 0.05$ ). There were no significant intergroup differences regarding mean SBI scores and mean decrease in PPD, RAL, MMP-8 and PGE<sub>2</sub> level ( $P > 0.05$ ).

**Conclusion:** The use of systemic doxycycline demonstrated no clinical or biochemical beneficial effect. Standard mechanical procedures are sufficient for the successful therapy of chronic periodontitis.

224

### Clinical and biochemical efficacy of short-term systemic ibuprofen in non-surgical periodontal therapy of chronic periodontitis

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**Background:** Ideal periodontal therapy must simultaneously target periodontopathogens and the destructive host response. Therefore, management of the disease may require additional interventions such as the use of nonsteroidal anti-inflammatory drugs that may modulate the host response. The present study was designed to evaluate the adjunctive efficacy of systemic ibuprofen clinically and biochemically.

**Methods:** Twenty chronic periodontitis patients were randomly assigned to two groups. The control group received initial periodontal therapy, while the test group additionally received systemic ibuprofen for 2 weeks. PI, SBI, probing pocket depth (PPD) and relative attachment level (RAL) recordings and GCF samples were obtained at the beginning and at 1st, 3rd and 6th months following therapy. GCF levels of MMP-8 and PGE<sub>2</sub> were evaluated with enzyme immunoassay. Results were analysed by Wilcoxon signed rank test for treatment modalities and Mann-Whitney  $U$ -test for intergroup changes.

**Results:** Both groups showed significant improvements in all parameters after therapy ( $P < 0.05$ ). Neither mean PI scores, nor the improvements in SBI, PPD, RAL, GCF MMP-8 and PGE<sub>2</sub> levels demonstrated statistical significance between groups ( $P > 0.05$ ).

**Conclusion:** The use of systemic ibuprofen demonstrated no beneficial effect on the clinical or biochemical outcome of periodontal therapy. Standard mechanical periodontal procedures are sufficient for the successful treatment of chronic periodontitis.

225

### Root coverage with enamel matrix derivatives with and without connective tissue graft – a modified surgical technique: 1-, 2- and 6-year-results

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Recently, the enamel matrix derivatives (EMD) in combination with or without connective tissue graft (CTG) were applied more frequently in the therapy of gingival recession with different

surgical techniques as a consequence of better clinical and histological results in case of periodontal regeneration. Aim of this clinical study was to evaluate the coverage of gingival recession defects with EMD with or without CTG.

**Methods:** Twenty five patients (16 f, 9 m) from 16 to 58 years of age with 92 gingival recessions (Miller classes I, II) and with at least 4.0 mm of clinical attachment loss were treated with a modified surgical technique for the root coverage by CTG with EMD ( $n = 45$ ) and only EMD ( $n = 47$ ). Vertical recession defect, probing depth, clinical attachment level, dehiscence depth, the keratinized gingiva and recession coverage were recorded before surgery and at 1, 2 and 6 years.

**Results:** The average pre-surgical recession depths were 4.4 mm ( $\pm 1.3$ ) (EMD + CTG) vs. 3.2 mm ( $\pm 1.1$ ) (EMD). Both treatment modalities led to a significant decrease in recession and to an attachment gain. Mean root coverage 1-year postoperatively was 92.7% (EMD + CTG) vs. 96.3% (EMD) and 2-year postoperatively was 92.1% vs. 94.3% as well as 6-year postoperatively was 88.4% vs. 85.7%.

**Conclusion:** The results of this study demonstrate that the applied modified surgical techniques are safe without any postoperative complications and predictable with better clinical outcomes at the donor and recipient sites.

226

### Evaluation of a non-staining chlorhexidine mouthwash. A short-term clinical trial

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**Background:** Chlorhexidine (CHX) is known for its antiseptic and antiplaque properties in the treatment on periodontal diseases. It induces stain formation on tooth surfaces as local side-effect. Aim of the present study was to evaluate the potential reduction of staining formation of a mouthwash containing CHX and Anti Discoloration System (ADS), in non-surgical periodontal therapy.

**Materials and methods:** Sixty patients in general good health conditions and suffering from moderate chronic i were enrolled. Full Mouth Bleeding Score (FMBS), Full Mouth Plaque Score (FMPS) and Full Mouth Staining Score (FMSS) were registered at baseline. All subjects received oral hygiene instructions and underwent scaling and root planing in 2-4 appointments. All the patients were randomly divided into two groups, test and control, and were prescribed to rinse for 1 min twice a day for three weeks with 0.2% CHX mouthwash respectively containing and non-containing an Anti Discoloration System. FMBS, FMPS and FMSS were again registered after 21 days and compared to baseline data.

**Results:** At the term of the analysis, CHX with and without ADS system showed similar effectiveness in term of inflammation inhibition (FMBS reduction) and antiplaque property (FMPS). FMSS was statistically less in test group compared with control group.

**Conclusion:** The CHX + ADS seemed to be more effective in inhibiting stain formation on tooth surfaces than CHX alone, maintaining similar antiseptic activity.

227

### Reliability of clinical and paraclinical parameters in different study populations during an experimental gingivitis study

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**Objectives:** In designing a CCT to assess the efficacy of oral antiseptics the selection of parameters and participants is a basic principle. The aim of this experimental gingivitis study was to

determine whether correlations exist between different gingivitis parameters in distinctive populations.

**Methods:** Three populations were selected depending on the oral hygiene level of the participants (excellent vs. modest). Population A (excellent) consisted of 39 dental students, B (modest) of 38 participants from a local population, and C was a mixed population (A + B,  $n = 77$ ). During the experimental gingivitis study the participants rinsed with either a placebo or 0.2% chlorhexidine (CHX). After 21 days, Gingival Index (GI), Modified Gingival Index (MGI), Bleeding on Probing (BOP), gingival crevicular fluid (GCF), and colony forming units (CFU) were assessed. The association between the parameters was tested using the Pearson correlation coefficients.

**Results:** Statistically significant correlations were observed between GI and MGI in all groups and populations. GI and BOP as well as MGI and BOP correlated in group C. GI and GCF correlated only in group A after rinsing with CHX as did MGI and GCF. No correlations were found between GI and CFU.

**Conclusion:** Gingival Index and MGI are the most reliable parameters regardless of the composition of the study population. For the use of BOP during experimental gingivitis, however, a mixed population is preferred.

Supported by GABA International AG.

228

### Effects of co-administration of azithromycin during periodontal treatment for early onset periodontitis

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**Purpose:** We examined the clinical usefulness of azithromycin (AZM) that is potentially effective in eliminating biofilm during basic periodontal therapy in patients with early onset periodontitis (EOP).

**Materials and methods:** Five patients were clinically diagnosed as having EOP, and gave consent to participate in co-administration of AZM were recruited in the study. AZM was administered (500 mg/day, three days) during the period of basic periodontal therapy. Tests were conducted at presentation and also at the completion of basic therapy, and the changes were compared and analysed. The test items were; (i) periodontal pocket (PD); (ii) bleeding on probing (BOP) and (iii) pathological periodontal pocket rate proportion of periodontal pockets of 4 mm or above (PoR).

**Results and Conclusion:** Co-administration of AZM significantly ( $P < 0.05$ ) increased the improvement rates of PD, BOP and PoR. Significant improvement of clinical symptoms was observed within a short treatment period. Multiple regression analysis confirmed significant improvement of PD and BOP by AZM co-administration, but the improvement of PoR did not reach statistical significance. These results probably reflect improvement of gingival swelling as a result of regression of inflammation, and suggest the necessity of approaching deep pockets such as infrabony pockets. These results indicate that co-administration of AZM augments the therapeutic effects of basic periodontal therapy on EOP patients.

229

### The effect of coronally advanced flap and allogenic acellular dermal matrix with and without enamel matrix proteins on gingival recession defects

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**Aims:** This study was aimed to compare the clinical outcomes of root coverage procedures, using a Coronally Advanced Flap

(CAF) in combination with Acellular Dermal Matrix (ADM) with and without the application of Enamel Matrix Derivatives (EMD). **Material and methods:** A total of 22 recession defects (CI I or II Miller) in eight patients were selected and randomly allocated to test and control groups. In the test group a combination of CAF, ADM and EMD was used whilst the control defects were treated only by CAF and ADM. Recession depth (RD), recession width (RW), width of keratinized tissue (KT), clinical attachment level (CAL) and the position of mucogingival junction (MGJ) were measured at the base line, 2 and 4 months after the treatments. The data were analysed by student *t*-test and one-way ANOVA.

**Results:** Both of the treatments led to a significant root coverage in the recession sites ( $P < 0.001$ ). However there was no significant difference in any of the parameters between the study groups. Two months after the surgeries, mean root coverage for CAF + ADM + EMD and CAF + ADM was  $82.75 \pm 22\%$  and  $88.89 \pm 22\%$  respectively. However 4 months after surgery, these values were changed to  $80.89 \pm 22\%$  and  $90.00 \pm 21\%$  respectively.

**Conclusion:** Using EMD does not improve the clinical efficacy of CAF + ADM in root coverage. However, histological studies recommended to reveal any benefit that may arise by application of EMD in combination with ADM and CAF in this procedures.

230

### Vertical strip gingival graft: a new technique for gingival augmentation, a pilot study

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There are numerous methods for widening keratinized attached gingiva. This study is to present 'Vertical Strip Gingival Graft Technique' as an attempt to improve methods of gingival augmentation. After bed preparation of 12 patients with no attached gingiva more than 0.5 mm, free gingival graft was divided in two pieces and each part was fixed in one end of the bed. In this method, an area between two grafts was remained uncovered. Clinical Parameters were measured before surgery, 6 and 12 weeks after operation. Results showed significant increase in the width of attached gingiva ( $P < 0.01$ ). The mean distance of stent to mucogingival line between baseline and six week's data; and baseline and 12 week's also showed significant changes ( $P = 0.001$ ). With respect to the limitation of this study, by application of this technique wider recipient site with less donor tissue can gain keratinized coverage.

231

### Ibuprofen arginine (SPEDIFEN®) for pain control during scaling & root planning – a randomized, triple-blind, placebo-controlled, parallel-group trial

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**Background:** Fear of pain often keeps patients from complying with a periodontal maintenance program. Anti-inflammatory agents have proven effective for alleviating postsurgical dental pain, but clinical trials testing their efficacy for managing pain associated with non-surgical periodontal treatment are lacking.

**Aim:** To investigate the analgesic efficacy and tolerability of ibuprofen arginine in patients with periodontitis during and after professional dental hygiene.

**Methods:** In this randomized, triple-blind, placebo-controlled, parallel-group trial, 64 patients with chronic periodontitis received either 800 mg ibuprofen arginine or placebo 30 min before treatment. Numeric pain and electronic visual analog scales ranging from 0 to 100 were employed.

**Results:** Average pain levels during treatment were lower following ibuprofen arginine (quartiles: 0.5, 4.5, 11) compared to placebo (4, 16, 26), corresponding to a percentage reduction in median pain of 72% ( $P = 0.023$ ). The median maximum pain was 28 (interquartile range 10–50) following placebo and 10 (4–31) following ibuprofen arginine ( $P = 0.065$ ).

**Conclusions:** In patients with mild to moderate chronic periodontitis, ibuprofen arginine was safe and superior to placebo for alleviating pain during non-surgical periodontal treatment. Its painless administration and rapid onset of action make it well suitable for pain management in a general dental office.

232

### The application of photodynamic therapy in the periodontology

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The goal of this double-blind randomized trial was to examine whether the Photodynamic Therapy (PDT) leads to a clinical healing, which is comparable with the mechanical root debridement in patients with persistent periodontal pockets in the supportive periodontal therapy (SPT).

**Methods:** Sixty-seven chronic periodontitis patients, who were enrolled at least 1 year in SPT and with at least three teeth with probing depth (PD)  $\geq 5$  mm, were randomized in two treatment groups. One group (29) has been treated with ultrasonic device (US) and the other group (25) with PDT. We measured the probing depth (PD), loss of attachment and the bleeding on probing (BOP) on teeth with periodontal at six sites with an electronic probe. The samples in the PDT-group were lasered with PDT agent 0.5 min per site. The activation of the agent was carried out by application of photo sensor in the pocket. The control teeth were debrided with US.

**Results:** Both treatment methods were successful in reduction of pocket depth from 5.27 mm to 4.49 mm by using US ( $P < 0.001$ ) and from 5.33 mm to 4.73 mm by using PDT respectively ( $P < 0.001$ ) for the baseline pockets  $> 4$  mm. There were no statistically significant differences between both groups in the reduction of the PD for baseline pockets  $> 4$  mm ( $P = 0.203$ ), for all pockets ( $P = 0.717$ ), nor for attachment loss of baseline pockets  $> 4$  mm ( $P = 0.517$ ) or for all pockets ( $P = 0.627$ ). Hence, PDT may offer a meaningful alternative treatment approach for conventional root surface debridement.

233

### Reconstruction of periodontal defects using a bovine-derived xenograft

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**Initial Background:** For a long time the gold standard in GTR of severe periodontal defects has been a two-step procedure using



titanium reinforced membranes. Biodegradable barriers had been only used for small defects. The presentation describes a series of more than 900 regeneratively treated periodontal defects.

**Materials and Methods:** A bovine-derived xenograft has been used for defect reconstruction in combination with a collagen membrane or autogenous tissues alone for coverage depending on the defect morphology. In a retrospective study more than 900 regeneratively treated infrabony defects had been followed-up. One, 2 and 3 years postoperative data (PPD, CAL-gain, tooth mobility) have been surveyed.

**Results:** The overview of a 5-year period between 2000 and 2005 demonstrates that a successful outcome revealing PPD reduction, CAL gain and reduction of tooth mobility can be achieved in more than 96%.

**Conclusion:** A one-stage procedure using a xenograft provides the advantage of avoiding surgical intervention in any donor sites for the patient's comfort depending on the defect morphology, less recession and esthetic predictability. It can be shown that a one-stage procedure using deproteinized bovine derived xenograft is an option to get predictable long-term results in the treatment of severe periodontal defects following the guidelines of the surgical protocol.

234

#### Adjunctive effect of a systemic biphosphonate in non-surgical therapy of advanced generalized chronic periodontitis: a randomized clinical trial

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Aim of the study was to assess the adjunctive clinical effect of systemic neridronate in non-surgical treatment (PT) of advanced generalized chronic periodontitis (GCP). Sixty GCP patients were randomly assigned to two treatment groups. Thirty subjects in test group (TG) received PT with adjunctive neridronate (12.5 i.m. per week/3 months). Control group (CG) received PT only. Clinical parameters were evaluated by a calibrated masked examiner at baseline and 3-months and a masked therapist performed PT. Groups were balanced at baseline and all clinical parameters improved at 3 months in both groups. None of the patients in the TG showed oral necrotic lesions. No differences were noted in terms of bleeding score between the groups ( $P = 0.933$ ). In moderate pockets (4–6 mm) no differences were noticed between groups (95%CI: -0.4–0.2;  $P = 0.646$ ). In deep pockets ( $\geq 7$  mm), PPD reduction was 3.0 mm (2.4–3.4) in CG and 2.7 mm (2.0–3.4) in TG (-0.5–1.2;  $P = 0.414$ ). Mean clinical attachment level (CAL) gain in PPD  $\geq 7$  mm was 2.8 (2.2–3.4) in CG and 2.2 (1.5–2.9) in TG (-0.3–1.4;  $P = 0.210$ ). CAL gains  $\geq 2$  mm at 25% of sites in test patients compared with 19% in placebo ( $P = 0.25$ ). PPD reductions  $\geq 2$  mm were observed in 25% of sites in TG and 24% in CG. A total of 76% of pockets with PPD  $\geq 5$  mm at baseline were  $\leq 4$  mm at 6 months in TG. This compared with 70% in the CG ( $P = 0.4$ ). Adjunctive use of systemic neridronate did not result in additional improvements of periodontal clinical conditions in GCP patients, 3 months after PT.

235

#### The effect of locally delivered chlorhexidine (PERIOCHIP®) on chronic periodontitis patients

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**Background:** The impact of locally delivered chlorhexidine on clinical and microbiological parameters of chronic periodontitis requires further documentation.

**Aim:** Aim of the present study was to investigate the effects of Periochip® as an adjunct to mechanical treatment of chronic periodontitis.

**Materials and methods:** Fifty chronic periodontitis patients were randomized in two groups. The test group ( $n = 25$ ) received scaling-root planning and adjunctive Periochip® in four pockets  $> 5$  mm. The control group ( $n = 25$ ) received scaling-root planning only. Clinical indices (PD, CAL, BOP and PI) were assessed at bsl, 3 and 6 months after completion of treatment. Subgingival samples from preselected pockets were analysed at bsl, 3 weeks, 3 and 6 months after treatment for presence and levels of 10 bacterial species using DNA-DNA 'checkerboard' hybridization.

**Results:** Data were analysed with both the patient and the site as the observational unit (Mann-Whitney and Wilcoxon tests,  $P \leq 0.05$ ). Both treatments resulted in improvement of clinical indices and non statistically significant differences were observed between the two groups at any timepoint. No major differences were observed between the two groups concerning levels of key periodontal pathogens.

**Discussion/conclusion:** Data from the present study suggest that Periochip® as an adjunctive treatment does not enhance clinical and microbiological effects of scaling-root planning at the initial treatment phase of chronic periodontitis patients.

236

#### Effect of full-mouth disinfection in the treatment of drug-induced gingival overgrowth

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**Aim:** Retrospective evaluation of the clinical effect of non-surgical periodontal therapy according the concept of full-mouth disinfection (FMD) in patients with drug-induced gingival overgrowth (GO).

**Material and Method:** Ten patients (seven female, 32–73 years of age) medicated with cyclosporine and/or calcium channel blocker received oral hygiene instruction, professional tooth cleaning followed by scaling and root planning of all four quadrants within 24 h. Patients rinsed with 0.12% CHX solution and brushed with 1% CHX gel for the next 2 weeks. The clinical situation was re-evaluated approximately 4 months after FMD. After completion of FMD eight patients were assigned to supportive periodontal therapy (SPT) and monitored for a mean of 24 months. Plaque control record (PCR), gingival bleeding index (GBI), pocket depth (PD) were recorded at baseline, re-evaluation and the last SPT visit. PD sites were stratified as follows:  $\leq 4$  mm, 5–6 mm and  $\geq 7$  mm.

**Results:** The number of sites with PD  $\leq 4$  mm was increased significantly after FMD (56.5–97.4%). Concomitantly sites with 5–6 mm and  $\geq 7$  mm decreased in the same interval (27.1–2.2%, 16.4–0.4% respectively). PCR and GBI also declined significantly compared to baseline. All clinical variables remained stable over SPT. Further surgical therapy was required only in one case.

**Conclusions:** FMD and regular SPT were effective in resolving inflammation and reducing the need for surgical treatment in patients with drug-induced GO.

237

### Increasing the effectiveness of periodontal supportive therapy by chlorhexidine or hydrogen-peroxide gel applied in night guard splints

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**Introduction:** The aim of the supportive therapy is to maintain long-term beneficial periodontal therapeutic effects. The maintenance of high-level of oral hygiene requires a highly motivated patient. In cases of decreased patient collaboration the use of local chemical plaque control can still have an adjuvant effect. The nature of the chemical agents, the patient's compliance, the cost and the time span of the treatment without side effects are all subjects that need to be considered.

**Materials and methods:** Changes in plaque and gingival indices were followed at non-smoking patients, who were enrolled in periodontal supportive therapy after scaling and root planning (SRP) treatment. Patients were divided into three groups of ten. In Group I SRP was followed with no adjuvant therapy. In Groups II and III 1% chlorhexidine or a 3% hydrogen-peroxide gel was given in a custom made foil-based plastic night guard splint. Night guards were in function for 30 min once a day for 21 consecutive days. Differences between the treatment results were analysed using the Wilcoxon signed-rank test.

**Results:** In Groups II and III the plaque and gingival indices significantly decreased after 3 weeks but not at the Group I.

**Discussion:** These antiseptic agents applied in the foil-based plastic night guard splint could be used effectively in gingivitis and plaque control during the short-term supportive therapy.

238

### A dental ultrasonic scaler augmented with a smart surface recognition feature – *in vivo* application

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**Background:** We recently introduced a surface detection system based on a conventional dental ultrasonic scaler designed to discriminate cementum and dental calculus, which is the prerequisite for complete and thorough calculus removal. After successful *in vitro* testing, the results of the first *in vivo* application will be presented in this study.

**Methods:** The root surfaces of 63 periodontally involved teeth were systematically scanned with the detection scaler *in vivo*. All positions of the supragingival portion of the insert and the corresponding results of the detection system were synchronously saved as separate video or data stream files on a computer disk. After extraction, size and prevalence of calculus of all scanned root surfaces were evaluated and the instrument's tip was repositioned onto the then extracted teeth for detection accuracy evaluation.

**Results:** The calculus surface prevalence of 22.3% corresponded to 43 individual regions, out of which 39 were correctly classified. According to the mean size of the calculus spots, the non-calculus surface was virtually assorted to 152 individual 'regions', out of which 123 were correctly classified. Thus, calculus and cementum were discriminated with a sensitivity of 90% and a specificity of 80%.

**Conclusion:** We show the possibility to classify subgingival calculus under *in vivo* conditions with a smart dental piezoceramic scaler. This device may help to find the optimal time point to stop scaling during a treatment procedure.

239

### Factors for tooth loss in periodontal treated patients (long-term results 10 years after periodontal therapy)

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**Objectives:** Assessment of factors contributing to tooth loss 10 years after initiation of periodontal treatment.

**Methods:** Ninety-three adult patients were recruited for the study. Inclusion criteria were full-mouth radiographs from beginning of therapy and completion of anti-infective therapy. Clinical examinations including probing parameters, Plaque and Gingival Index, samples for interleukin-1 haplotype (IL-1) test, and a detailed questionnaire on e.g. smoking habits were obtained by the same examiner. Periodontal conditions were surveyed according to the criteria of the Swiss Dental Association. Medical files were searched for initial diagnosis, frequent supportive periodontal therapy (SPT) and prognosis index.

**Results:** The following means of tooth loss per patient within the 10 years of observation were observed: gender (male/female):  $1.5 \pm 3.5/1.7 \pm 3.9$ ; smoking (no/yes):  $1.1 \pm 1.8/3.1 \pm 6.5$ ; prognosis (A/B, C):  $1.4 \pm 2.4/2.7 \pm 4.9$ ; initial diagnosis (moderate/severe chronic, aggressive periodontitis):  $0.6 \pm 1.4/2.0 \pm 4.2$ ; regular maintenance (yes/no):  $0.45 \pm 0.8/3.1 \pm 5.1$ ; IL-1 (no/yes):  $0.95 \pm 4.7/2.1 \pm 1.3$ . Stepwise linear regression analysis identified irregular SPT and IL-1 haplotype to be associated with an increased rate of tooth loss ( $P < 0.005$ ).

**Conclusions:** Some criteria seem to interact with tooth loss whereas only regular SPT and absence of IL-1 haplotype could be proven to be statistically significant in prevention of tooth loss.

240

### Adjunctive low-dose doxycycline in severe chronic periodontitis: effect on clinical parameters and crevicular fluid transforming growth factor- $\beta$ 1

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**Objectives:** Present study evaluated the effect of adjunctive low-dose doxycycline (LDD) on clinical parameters and gingival crevicular fluid (GCF) transforming growth factor-beta1 (TGF- $\beta$ 1) levels in severe chronic periodontitis over 12-month period.

**Methods:** Thirty-five patients with severe periodontitis and 11 healthy subjects were included. Following first session of non-surgical therapy at baseline patients randomized to take either LDD bid or placebo bid for 3 months. Recording of probing depth (PD), clinical attachment level, papilla bleeding index and plaque index and GCF sampling were performed at baseline, 3, 6, 9 and 12 months. TGF- $\beta$ 1 was analysed by ELISA. Significant changes over time and between groups were assessed by Friedman, Wilcoxon and Mann-Whitney tests.

**Results:** Thirteen patients in both groups completed the trial. Clinical parameters significantly improved following both therapies ( $P < 0.01$ ). Adjunctive LDD was more effective in reducing PD of initially deep pockets ( $\geq 7$  mm) by  $\geq 3$  mm at 6 and 9 months and by  $\geq 4$  mm at 6 months than adjunctive placebo ( $P < 0.05$ ). Sites with moderate PD (4–6 mm) at baseline had lower PD in LDD group at 9-month compared to placebo group ( $P < 0.05$ ). GCF TGF- $\beta$ 1 levels of LDD group was significantly higher than baseline ( $P < 0.01$ ) and placebo group ( $P < 0.017$ ) at 3 months.

**Conclusion:** These data ensures further data for beneficial effects of non-surgical therapy supplemented with LDD in the long-term management of severe chronic periodontitis.

241

# Reliability of plaque – and gingivitis parameters in different study populations – an experimental gingivitis study

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**Objectives:** Several indices are recognized as reliable for scoring plaque and gingivitis during evaluation of antiplaque procedures. The aim of this study was to determine whether correlations exist between plaque parameters as well as between plaque- and gingivitis-parameters in distinctive populations.

**Methods:** Three study populations were selected depending on the oral hygiene level of the participants. Pop. A (excellent) consisted of 39 dental students; B (modest) of 38 participants from a local population and C was a mixed pop. (A + B). During a 21-day experimental gingivitis study using parallel group design the participants rinsed with either a placebo or 0.2% chlorhexidine (CHX). After 21 days, two different plaque indices (PII, QHI) were evaluated. Furthermore the Gingival Index by Loe (GI), and the Mod.Gingival Index by Lobene (MGI) were assessed. For statistical analysis the Pearson correlation coefficient was used.

**Results:** Statistical significant correlations were observed between PII and QHI in all groups and populations. A correlation between plaque indices and gingivitis parameters was only shown for population A, and for the CHX-groups in population B and C.

**Conclusions:** For assessment of plaque accumulation PII and QHI are the most reliable parameters regardless of the composition of the study population. For associations between plaque accumulation and inflammation, population with excellent oral hygiene are advisable.

242

# Use of platelet-rich plasma in periodontal surgery – a prospective randomised double blind clinical trial

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Platelet rich plasma (PRP) has been used in the treatment of intrabony defects. Studies have examined the significance of pure-phase  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) as bone substitute material with good results. The aim of this prospective controlled randomized trial was to evaluate the effect of PRP in bone regeneration. Twenty-two patients with periodontitis showing contralateral intrabony defects were included. Defects were randomized to  $\beta$ -TCP (Cerasorb®) in combination with PRP (test) or alone (control). PPD, CAL, and relative attachment level (RAL) were assessed before and after surgery. Defect dimensions were recorded at surgery and during re-entry at 6 months, with the vertical depth of the defect as primary outcome variable. An early healing index (EHI) was assessed 3 days, 1, 2 and 4 weeks after surgery. Both treatments led to clinical improvements. The median reductions of PPD and CAL at test sites were 0.8 mm and 0.28 mm, and at control sites 0.4 mm and 0.13 mm, respectively. The median reduction of open vertical depth was 1.9 mm (interquartile intervals: 0.75 mm, 2.5 mm) at test sites, compared with 2.6 mm (1.8 mm, 3.5 mm) at control sites. The EHI showed a reduction

from grade 3 after 3 days to grade 1 after 4 weeks. No significant differences between test and control sites were found ( $P = 0.8$ , Wilcoxon). PRP + TCP had no additional effect.  $\beta$ -TCP (Cerasorb®) is a satisfactory graft material in periodontal surgery even without PRP.

**Acknowledgement:** This study was supported by curasan AG, Germany.

243

# Impact of non-surgical periodontal treatment on oral malodor in a Turkish children population with gingival inflammation

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Oral malodor affects a large proportion of population and may cause a significant social or psychological handicap to those suffering from it. Oral malodor has a positive correlation with the accumulation of bacterial plaque in the oral cavity. The aim of the present study was to two hold; firstly to determine whether oral malodor and periodontal disease parameters are associated with one another in 150 Turk subjects (mean age  $9.1 \pm 2.7$ ; ages ranging from 7 to 12); secondly to investigate the impacts of non-surgical periodontal treatment on oral malodor. Parameters measured included whole mouth odor judge scoring, Halimeter, saliva pH scores, gingival index, plaque index and probing depth before and after the treatment procedures. Odor judge scores were significantly associated with Halimeter. However, gingival index, plaque index, probing depth was significantly associated with odor judge scores or Halimeter scores. The statistical analysis revealed that periodontal treatments presented a significant reduction ( $P < 0.001$ ) in VSC formation. Results suggest that in the population studied, periodontal health and oral malodor are associated with one another.

244

# Ultrasonic vs. curette scaling in transcanine region: a comparison of clinical and microbiological outcome

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**Aim:** This study evaluates the clinical and microbiological findings during mechanical scaling with ultrasonic scalers or hand curetts. The effect of diode laser on periodontal microflora was evaluated. **Methods:** Voluntary 40 subjects where participated in this randomised split-mouth designed study where one side was treated with ultrasonic scaler (SG – ultrasonic group) and the other side with hand curette (CG – curette group). Measurements of PD, BOP, GI and PI at  $t = 0, 6$  and 12 months were recorded. Microbiological samples were taken at baseline, during the surgery and 1 month after and were analysed by cultivation and molecular micro-IDent assay (Hain Life science, Nehren, Germany). Periodontal tissue was treated by the diode laser at half of all periodontal open flap surgery.

**Results:** Clinical parameters of PD, BOP, GI and PI significantly decreased within both groups with no differences comparing the SG and CG. At baseline *B. forsythus* was detected in 93.3%, *P. gingivalis* and *T. denticola* in 66.7%, *A. actinomycetemcomitans* in 60% and *P. intermedia* in 33.3% of all patients. The presence of pathogens were significantly reduced in samples taken during surgery as well as 1 month after. In addition the samples of sites treated with diode laser show significant reduction of pathogens comparing to non laser treated sites.

**Conclusions:** The clinical and microbiological outcome of SG show no significant differences compare to CG in transcanine region.

245

### Evaluation of free gingival grafts for augmentation of keratinized gingiva

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**Aim:** Aim of this study was to assess free gingival graft (FGG) as means for long-term augmentation of gingiva and possibly root coverage in a specialized clinic setting.

**Material and methods:** Totally 71 teeth in 17 patients (15 female, two male, mean age  $27.1 \pm 7.5$  years) were treated by means of FGG during a three-year specialist training. All patients had gingival recession (GR) on at least one tooth, were non-smokers, otherwise healthy, with good oral hygiene, all of them signing written consent. Measured parameters included: initial gingival recession (iGR), postoperative (pGR), initial width of keratinized gingiva (iKG), postoperative width of keratinized gingiva (pKG), and thickness of FGG. Since all FGGs were placed in areas previously included in mucosal tissue, the initial thickness of keratinized gingiva was considered zero. Operating resident performed all preoperative measurements, while residents not aware of investigations, during recall session, recorded postoperative values.

**Results:** Mean iGR was  $2.49 \pm 1.41$  (range 0–6 mm), mean iKG was  $2.35 \pm 0.85$  mm (range 0–5 mm). Statistical analysis included Pearson's correlation and Spearman correlation coefficient ( $\phi$ ), which was for GR 0.72 ( $P < 0.01$ ), and for KG 0.33 ( $P < 0.01$ ). There was statistically significant reduction of GR, as well as augmentation of KG.

**Conclusion:** FGG is a predictable, simple procedure for augmentation of keratinized gingiva with long-standing results, and, in a smaller extent, for root coverage.

246

### Comparison of new and 3-month-old brush heads for plaque removal using a powered toothbrush

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**Background:** It is recommended that powered toothbrush (PTB) heads should be replaced every 3 months.

**Aim:** Compare effectiveness of new and 3-month-old brush heads in plaque removal using a counter rotational PTB and assess effect of bristle wear on plaque removal.

**Method:** Single examiner blind, randomized study in which 33 adults attended the clinic on two occasions following 48 h periods of no oral hygiene. Following plaque scoring, subjects brushed for 2 min with a new brush head at one visit and a brush they had used for 3 month at the other visit (random allocation). Plaque was re-scored and percent reductions in plaque scores calculated. Bristle wear of the 3-month-old brushes was determined by measuring brush surface areas (BSA).

**Results:** Mean plaque score ( $\pm$ SD)% reductions with new brush heads were not significantly different from those achieved with the 3-month-old brushes for total plaque (new =  $37.6 \pm 18.2$ ; 3-month-old =  $36.7 \pm 18.8$ ); ( $P = 0.8$ , Paired  $t$ -test) and proximal plaque (new =  $30.9 \pm 19.2$ ; 3-month-old =  $0.5 \pm 18.9$ ) ( $P = 0.9$ ). BSA ranged from 0–135% (mean 26.9%; SD  $\pm$  31.4%). Compared with new brushes in the same subjects, no significant differences were found for plaque score reductions for 3-month-old brushes with minor (BSA 0–10%,

$n = 14$ ,  $P = 0.74$ ), moderate (BSA 11–40%,  $n = 11$ ,  $P = 0.57$ ) or marked wear (BSA 41–135%,  $n = 8$ ,  $P = 0.14$ ).

**Conclusion:** Counter rotational PTB's with 3-month-old brush heads exhibiting various degrees of wear were as effective as new brushes in plaque removal. (NSW Dental Board Grant).

247

### Treatment of mandibular class II furcations with enamel matrix proteins and bone grafts

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The aim of this study was to evaluate the clinical and radiographic outcome of combined application of enamel matrix proteins (EMD) with bovine derived xenograft (BDX)(EMD + BDX) or bioactive glass (BG)(EMD + BG) in mandibular Class II furcations. Fourteen chronic periodontitis patients presenting a total of 44 buccal mandibular Class II furcation involvements were included in this split mouth study. Prior to surgery and at 10 months plaque and sulcus bleeding indices, probing depth (PD), marginal soft tissue, relative attachment, probing and radiographic bone levels in vertical direction were measured. Probing bone level was also measured in horizontal direction. Both treatment modalities resulted in statistically significant clinical improvements, with no significant differences between the groups. Considering the vertical measurements, in the EMD + BDX group  $2.71 \pm 0.32$  mm of PD reduction,  $2.19 \pm 0.26$  mm of attachment gain,  $0.39 \pm 0.99$  mm of recession were obtained whereas in the EMD + BG group  $2.58 \pm 0.45$  mm of PD reduction,  $2.05 \pm 0.34$  mm of attachment gain,  $0.32 \pm 0.97$  mm of recession were found. The mean vertical clinical and radiographic bone gain was  $1.70 \pm 0.65$  mm and  $1.66 \pm 0.48$  mm in the EMD + BDX group whereas in the EMD + BG group, these values were  $1.95 \pm 0.36$  mm and  $1.48 \pm 0.40$  mm respectively. The mean horizontal bone gain was  $3.78 \pm 0.46$  mm and  $3.76 \pm 0.42$  mm in the EMD + BDX and EMD + BG groups respectively. Both combination therapies resulted in similar promising improvements.

248

### Effect of periodontal treatment on clinical symptoms of Behçet's disease

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**Objective:** Recurrent painful oral ulceration in the mouth of a Behçet's disease (BD) is a major restriction upon regular oral hygiene habits. It was reported that plaque, bleeding and periodontal indices were found higher in active Behçet's disease patients compared to the control group. The aim of our study was to evaluate the effect of alterations in the periodontal status of BD patients on the clinical symptoms of BD with and without periodontal treatment.

**Materials and methods:** The study material was consisted of 29 periodontally treated and 31 untreated BD patients. The plaque (PI), bleeding on probing (BOP), probing depth (PD) and clinical attachment levels (CAL) values, and the frequency of occurrence and the healing time of oral ulcers before and 3 months after periodontal treatment were recorded. The differences between the groups were tested by using  $t$ -test and Wilcoxon Signed Rank tests.

**Results:** The mean values for the frequency of occurrence of oral ulcer at the beginning and 3 months after for treated and untreated groups were  $7.42 \pm 7.5$  and  $7 \pm 7.89$ , and  $7.93 \pm 5.81$  and  $4.1 \pm 3.44$ , respectively ( $P < 0.01$ ). The mean values for the healing time for treated and untreated groups were  $6.26 \pm 4.22$  and  $6.75 \pm 4.18$ , and  $7.06 \pm 2.96$  and  $5.58 \pm 2.64$ , respectively ( $P < 0.001$ ).

**Conclusion:** Within the limits of our study, significant contribution of the periodontal treatment on the frequency of occurrence and healing time of oral ulcer was observed in BD patients.

249

### Autogenous cortical bone particles and enamel matrix derivative in the treatment of deep intraosseous defects: a 12-month case series

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The aim of the present study was to investigate the effectiveness of a regenerative procedure based on supracrestal soft tissue preservation in association with combined autogenous cortical bone particles (ACBP)/enamel matrix derivative (EMD) application in the treatment of non-self supporting periodontal intraosseous defects. Thirteen consecutively-treated patients, seven females and six males, aged 30–65 years, two smokers, were included. A total of 14 deep, 1–2 wall intraosseous defects were selected. Pocket probing depth (PPD), clinical attachment level (CAL), and gingival recession (REC), at baseline and 12 months after surgery, were recorded. PPD amounted to  $9.1 \pm 1.6$  mm before surgery, and decreased to  $4.0 \pm 1.4$  mm post-surgery (PPD reduction:  $5.1 \pm 1.7$  mm,  $P < 0.0000$ ). CAL varied from  $10.3 \pm 1.5$  mm pre-surgery to  $5.4 \pm 1.7$  mm post-surgery, with a CAL gain of  $4.9 \pm 1.8$  mm ( $P < 0.0000$ ). REC shifted from  $1.1 \pm 0.9$  mm at baseline to  $1.4 \pm 1.1$  mm after surgery, REC change being  $0.3 \pm 0.8$  mm ( $P > 0.05$ ). Our results supported the effectiveness of a regenerative procedure based on supracrestal soft tissue preservation combined with ACBP/EMD in clinically and statistically improving soft tissue conditions of non-self supporting periodontal intraosseous defects.

250

### Free gingival grafting and gingival margin over 7 years

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**Aims:** (i) To assess whether the areas with small amounts of attached gingiva are more prone to gingival recession and loss of clinical attachment level (CAL) than areas with gingival grafting to increase the width of attached gingival and (ii) whether gingival grafting prevents recession and loss of CAL.

**Patients and methods:** Twenty females and six males, mean age 33 years at baseline (BL), with midvestibular singular or multiple gingival recessions in the mandible had undergone 28 free gingival grafting from palatal donor sites to increase the width of attached gingiva. A group treated by gingival grafts consisted of 98 teeth at BL and 35 teeth after 7 years. A control group was composed of 35 teeth at BL and also 7 years later. Using periodontal probe we assessed the following parameters: probing depth (PD), CAL, gingival margin regarding cemento-enamel junction, width of keratinized gingiva and bleeding on probing (BOP). The statistical analysis of differences in each parameter between the 7 years and BL was performed using Wilcoxon signed-rank test and for BOP using McNemar's test.

**Results/Conclusion:** We found out: (i) statistically significant gain of CAL and reduction of recession on test group ( $P < 0.05$ ); (ii) significant reduction of PD ( $P < 0.05$ ) and non significant BOP on both groups. The areas with small amount of attached gingiva seems to be more prone to gingival recession in spite of non significant change of CAL.

251

### Effect of adjunctive local or systemic metronidazole on mmp-8 and timp-1 levels in gingival crevice fluid in chronic periodontitis patients

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The aim of this study was to evaluate the effects of initial periodontal treatment combined with local/systemic use of metronidazole on clinical parameters and gingival crevice fluid (GCF) levels of matrix metalloproteinase-8 (MMP-8) and tissue inhibitor of metalloproteinase-1 (TIMP-1) in chronic periodontitis patients. Out of 30 subjects, 10 received scaling and root planning (SRP), 10 adjunctive local metronidazole (SRP + LM) at 0 to 7 days and 10 adjunctive systemic metronidazole for 10 days (SRP + SM). Plaque index (PI), sulcus bleeding index (SBI), probing depth (PD) and relative attachment level (RAL) were recorded, GCF samples were collected before and 49 days after treatments. MMP-8 and TIMP-1 were assessed by enzyme linked immunosorbent assay. Significant improvements in PI, SBI, PD were observed in all groups ( $P < 0.01$ ), only SRP + LM group showed significant attachment gain ( $P < 0.05$ ). The difference in attachment gain between the SRP and SRP + LM groups was significant ( $P < 0.05$ ). Marked decrease in MMP-8 level and increase in TIMP-1 level were detected in all groups while the SRP-LM group only showed a significant change in the TIMP-1 ( $P < 0.05$ ). The results indicate that initial periodontal treatment with and without use of local/systemic metronidazole improved clinical parameters and GCF levels of MMP-8 and TIMP-1 in chronic periodontitis patients. Furthermore, local administration of metronidazole may have an additional effect on attachment gain and TIMP-1 level change.

252

### Surgical and non-surgical treatment of periodontal diseases: two modalities of treatment

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**Aim:** A clinical trial was performed to compare the outcomes of non surgical and surgical therapies in subjects with a recurrent periodontal disease.

**Material and methods:** As basic therapy, 32 patients included in this trial had previously been treated by a non surgical root planning (RP). At active therapy, remaining inflammatory pockets were treated by a split mouth design and each quadrant was randomly assigned to one of both therapies: surgical modified Widman flap (SU) and RP. After active therapy all subjects were provided with supportive periodontal therapy (SPT) every 3 months, for 2 years. The clinical parameters evaluated were: reduction in probing pockets depth (rPPD) with an electronic Florida probe, gain in clinical attachment level (gCAL), bleeding on probing index EASTMAN (BoPI) and plaque index O'Leary (PI).

**Results:** Both therapies were found effective for the treatment of periodontitis in deep ( $\geq 7$  mm) and moderate pockets (5–6 mm); in smokers (S), formerly smokers (FS) and non smokers (NS). At deep pockets, SU seemed to be more effective than RP in terms of gCAL and rPPD. At moderate pockets, gCAL seemed to be more effective with RP. The S group showed less marked results. The FS and NS groups showed the same results. PI and BoPI were significantly reduced with both therapies.

**Conclusion:** Both SU and RP therapies seem to be effective methods for the treatment of recurrent periodontitis. In the treatment of deep pockets, SU therapy resulted in greater rPPD and gCAL.

253

# **Does emdogain® improve the result of coronally advanced flap in the treatment of multiple gingival recessions? a prospective split mouth study**

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**Background:** The effectiveness of coronally advanced flap (CAF) in the treatment of gingival recessions has been largely demonstrated in literature. Some studies have already tested the use of Emdogain® (EMD) in combination with various surgical techniques for achieving root coverage.

**Objectives:** The aim of this study was to assess the efficacy of EMD in improving the results of CAF in the treatment of multiple gingival recessions.

**Methods:** This intra-individual test has been performed on 10 patients that presented at least two bilateral and comparable recession-type defects (Miller Class I and II) affecting adjacent teeth. Surgical root coverage was performed as a bilateral simultaneous CAF procedure, with the adjunct of EMD for the test site and without EMD in the control site, in accordance with the randomization procedure. Clinical parameters were recorded at baseline and 6 months after surgery by a blinded examiner and included: recession length and width, keratinized tissue, probing depth and papilla height.

**Results:** At the 6 months evaluation, according to follow-up data available for eight of 10 cases, the test group showed better results in term of recession length reduction and tissue appearance. Both treatment procedures displayed good results with significant root coverage gain (70–80%).

**Conclusions:** According to these data, the use of EMD seems to improve the efficacy of the CAF procedure for root coverage in multiple recession treatment.

254

# **Local periodontal therapy with subgingival controlled release of chlorhexidine – microbiological effects**

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Studies on controlled release of chlorhexidine (PerioChip®) in periodontal pockets have shown an effect on microbiota before treatment. The aim of this randomized, prospective, double-blinded, placebo-controlled study was to analyse the effect of the local periodontal therapy with the adjunctive use of PerioChip® on microbiota before, during and after active treatment. 24 adults with severe chronic periodontitis (≥12 teeth with PPD ≥5 mm) were randomized in two groups ( $n = 12$ ). After a prophylaxis phase, in the test group (PC) PerioChips® and in the control group (PL) placebo chips were inserted in pockets with PPD ≥5 mm. After 10 days, SRP was done and new chips were placed. Plaque samples were taken from the deepest pocket of each quadrant at baseline (T0), before SRP (T1a) and 1 month after SRP (T2). Bacterial counts of A. a., P. g., P. i., T. f. and T. d. were assessed by RT-PCR. In the PC group, a reduction of bacterial counts of T. f. and T. d. was found between T0 and T1a (mean:  $1.4 \times 10^5$  bacteria/10 µl for T. f. and  $7.3 \times 10^3$  for T. d.). In the PL group, an increase of T. f. and T. d. was found for the same time (mean:  $2.1 \times 10^5$  for T. f. and  $7.4 \times 10^3$  for T. d.). The difference between groups was significant ( $P < 0.01$ , U test,  $\alpha$  level:  $P < 0.05$ ). Similar results were shown for T. d. between T0 and T2 ( $P < 0.02$ ). These results demonstrate that PerioChip® reduces periodontal pathogens before and after subgingival mechanical treatment.

**Acknowledgement:** This study was supported by DEXCEL®PHARMA, GmbH, Germany.

255

# **Evaluation of the benefits of using a power toothbrush during initial phase of periodontal therapy**

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**Background:** Studies evaluating the efficacy of oral hygiene procedures typically focus on either prevention, or maintenance after periodontal therapy. Little is known about the specific benefit of a power toothbrush during therapy.

**Objective:** To evaluate the clinical efficacy of power as compared to manual toothbrushing in patients undergoing the initial phase of periodontal therapy.

**Materials and methods:** This was an examiner masked, randomized two-arm parallel study involving 70 adults (age 23–81), with untreated periodontitis. After an initial supragingival debridement, subjects were assigned to tooth brushing with either a manual (M) or a power toothbrush (P). Subjects returned after 7, 14 (D14) and 28 (D28) days.

**Results:** In the subjects of group P the mean Plaque Index (PII) was significantly lower at D28 than in those of group M ( $P = 0.23$ ,  $M = 0.41$ ,  $P = 0.006$ ). These subjects also had a significantly lower mean number of sites remaining with a PII > 1 (D14:  $P = 3.9$ ,  $M = 11.3$ ,  $P = 0.018$ ; D28:  $P = 5.8$ ,  $M = 13.4$ ,  $P = 0.005$ ), and a significantly lower mean BOP (D14:  $P = 0.29$ ,  $M = 0.38$ ,  $P = 0.017$ ; D28:  $P = 0.30$ ,  $M = 0.37$ ,  $P = 0.034$ ). Differences in the mean Gingival Index, the number of sites with a GI > 1, mean recession, mean pocket probing depth and the number of pockets > 4 mm were not significant.

**Conclusion:** Subjects using a power toothbrush during initial treatment reduced supragingival plaque to lower levels than subjects using a manual brush.

**Acknowledgement:** Supported by Oral-B Laboratories.

256

# **Success of tunnel preparations in molars with class III furcation involvement**

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**Objective:** Retrospective evaluation of success after tunnel preparation of class III furcation molars.

**Methods:** In 41 patients (29 female, mean age  $54.8 \pm 10.8$  years) tunnel preparations were rendered to a total of 56 molars from 1992–2000. Before and 1–13 years after therapy examinations took place. A multilevel regression analysis was used to identify factors influencing the survival time of tunnel preparations: sex, jaw (maxilla/mandible), molar type (1st/2nd molar), smoking, participation in supportive periodontal therapy (SPT).

**Results:** Six tunnel preparations were performed in the maxilla, 50 in the mandible. Forty tunnel preparations were done at 1st, 15 at 2nd, and 1 at 3rd molars. Eight tunnel preparations were lost during the observation period: seven in the mandible. For the lost tunnel preparations a mean survival time of  $40.8 \pm 18.6$  months was observed. For those tunnel preparations that were still in function a mean survival time of  $53.1 \pm 22.9$  months was calculated. Multilevel regression analysis identified frequent SPT as positive ( $P = 0.08$ ) and smoking ( $P = 0.08$ ) as negative prognostic factors for tunnel survival.

**Conclusion:** After an observation period of  $50.7 \pm 22.6$  months 48 of 56 tunnel preparations were still in function (86%).

257

### Adjunctive systemic amoxicillin and metronidazole with scaling and root planning for treatment of severe chronic periodontitis

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**Objectives:** The aim was to study the effect of systemic metronidazole and amoxicillin as an adjunct to mechanical therapy in patients with chronic periodontitis.

**Methods:** Altogether 50 adult patients with untreated periodontitis were randomly assigned to receive full-mouth scaling and root planning with systemic metronidazole plus amoxicillin (T-group) or scaling and root planning with placebo (P-group). Clinical measurements including probing depth (PD), clinical attachment level (CAL), plaque index (PI) and bleeding index (BI) were taken at baseline and 6 to 8 weeks after therapy. The deepest pocket was selected for microbiological testing. Patients received coded study medication of either 500 mg amoxicillin in combination with 250 mg metronidazole or similar appearance placebo, every 8 h for the following 7 days.

**Results:** There was a significant change in PD, CAL, PI and BI in the T-group as compared to the P-group after therapy. Parallel to the clinical changes, treatment reduced the number of A. a, P. g and P. i compared with baseline in the T-group. This reduction was significant, but in P-group only p. i colony count reduced significantly. After therapy there was a significant difference between the T-and P-group in the number of patients negative for A. a, P. g and P. i.

**Conclusions:** The significant differences between T-and P-groups support the considerable adjunctive benefits to SRP of amoxicillin and metronidazole combined in the treatment of chronic periodontitis.

258

### Repeated subgingival scaling versus combined subgingival scaling/surgery in young adults with generalized advanced periodontitis – a prospective study

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**Objective:** Current literature supports a concept of non-surgical/antibiotic periodontal therapy. Our prospective split-mouth study compared the clinical results of surgery vs. repeated subgingival scaling without antibiotics in 30–40 years old adults with generalized advanced periodontitis.

**Material and method:** Twenty-six patients ( $37 \pm 3$  years) were treated: Initial exam (1), subgingival scaling, baseline exam (2), randomly assigned 2nd subgingival scaling (control) and periodontal surgery (test), final exam (3) 6 months p. o.. Mean pocket depth (PD) and attachment level (AL) were analysed (SUDAAN).

**Results:** PD decreased from  $4.1 \pm 0.2$  mm (1) to  $3.1 \pm 0.1$  mm (2) and  $2.9 \pm 0.1$  mm (3) for control and from  $4.5 \pm 0.2$  mm (1) to  $3.5 \pm 0.1$  mm (2) and  $3.1 \pm 0.1$  mm (3) for test sites. The decrease 1–3 was highly sign. ( $P < 0.001$ ) for both therapies, PD reduction 2–3 only sign. ( $P = 0.001$ ) for test sites with a sign. ( $P = 0.010$ ) difference between test and control. AL increased  $0.2 \pm 0.1$  mm (2) plus  $0.3 \pm 0.1$  mm (3) for control, and  $0.2 \pm 0.1$  mm (2) plus  $0.2 \pm 0.1$  mm (3) for test sites. Total AL-gain was significant for control ( $P = 0.008$ ) and test (0.012). AL-gain 2–3 was only sign. ( $P = 0.022$ ) for control sites with no significant difference between both therapies.

**Conclusion:** Both treatments reduced PD, gained AL. A 2nd subgingival scaling did not further reduce PD but gain AL.

Changes in PD, AL are in line with results for patients with generalized chronic periodontitis and don't support a general use of antibiotics.

259

### Root surface assessment after treatment with two ultrasonic micro inserts: topographic features

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**Aim:** Comparison of effects on the root surface of two micro insert designs, one curette shape (MIC: H4\* R and L) the other with a round section (MIR: TK2 R and L\* 5)., (Satelec Bordeaux, France).

**Methods:** Forty single human rooted teeth were treated *in vivo* on their proximal surfaces by the same clinician, the same generator at low power using the two inserts. The choice of the insert was randomized. After extraction the teeth were prepared and observed with SEM. Secondary electron was used to qualify the cementum topography. On four areas on each surface the appearance of scratches, alterations, and the cementum appearance (smooth, altered, crackled or dappled) were scored from 0 (no sign) to 3.

**Results:** A total of 320 zones were analysed by statistical generalized model (GENMOD). When MIC were used, all the surfaces appear smoother than when MIR were used. Using MIC, cervical zones appear smoother, less dappled and less crackled than in apical ( $P < 0.05$ ) when on distal we have observed more alterations. No significant differences were observed with MIR.

**Conclusion:** The different micro inserts were effective in the treatment of the root surfaces. Few scratches and alterations appear after ultrasonic debridement. Generally, the surface appears smoother in cervical when treated with MIC. The differences noted on distal and apical reveals that the MIC needs to be use by a trained clinician. This study must be combined with the previous studies, which has shown MIC less aggressive than Gracey.

260

### Clinical and microbiological evaluation of effectivity of a 0.12% chlorhexidine toothpaste

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Chlorhexidine has been known as an effective antimicrobial agent to inhibit supragingival dental biofilm formation. The purpose of this study is to evaluate the effects of a 0.12% chlorhexidine toothpaste on plaque control, gingival inflammation and microbial composition of supragingival biofilm. Eighteen consenting volunteers were divided into two groups (test and control) and used the toothpastes containing chlorhexidine 0.12%, stannous fluoride and a placebo with stannous fluoride only, for 4-week period. Measures of Plaque and Gingival Index, clinical attachment level, clinical probing depth and supragingival plaque samples were collected on days 0, 7, 14 and 21. Polymerase Chain Reaction (PCR) will be used to identify the bacterium periodontopathogen: *Porphyromonas gingivalis*. Our results demonstrated significant statistical associations between Gingival Index and the test toothpaste, and the positivity of the *P.gingivalis* with gingival bleeding.

261

### Bacterial interference in periodontal therapy: a proof of principle?

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**Aims:** The level by which periodontopathogens are re-established subgingivally after root planning is correlated with clinical

## Posters: Implant therapy

improvement and the risk for relapse. Because of the importance of the establishment of a beneficial subgingival microbiota, the aim of his study was to assess quantitative changes in the subgingival microflora after root planning when beneficial bacteria were applied adjunctively.

**Methods:** *Streptococcus mitis*, *S. sanguinis* and *S. salivarius* were selected on their ability to interfere with adhesion of periodontopathogens in in vitro studies. These species were applied after root planning adjunctively in a split mouth beagle dog model. Clinical and microbiological parameters (culture, checkerboard hybridization) were monitored during 12 weeks.

**Results:** Bacterial culturing revealed that adjunctive application of beneficial bacteria after root planning resulted in a significant lower recolonization rate of anaerobic bacteria, *Porphyromonas gingivalis* and *Prevotella intermedia*. The checkerboard analysis confirmed an interference with subgingival recolonization. The interference appeared to be beneficial strain, pathogen and frequency of application depended.

**Conclusions:** This study gives a prove of principle for the hypothesis that a subgingival application of beneficial species after mechanical debridement can prolong the beneficial microbial shift towards a less pathogenic microbiota.

**Acknowledgement:** Supported by NIDCR grant 1 R21 DE015360-01.

262

### The effect of a mouthwash containing extracts of *Calendula officinalis* on plaque and gingivitis

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**Aims:** To evaluate effects of mouthwash (mw) containing extracts of *Calendula officinalis* (Plandula) on prevention of dental plaque formation & subsequent development of gingivitis in young adults.

**Materials and Methods:** Test product; herbal mw containing 0.64% *Calendula officinalis* in 2.56% Plandula mw. Clinical trial; placebo controlled, double blind, crossover design, consisting of two 14-day test periods, separated by 4 weeks washout period. Participants continued on regular non-supervised, self-performed oral hygiene measures, without flossing. Rinsed with 15 ml mw twice daily for 30 s after brushing. On day1, new toothbrush & toothpaste provided. 34 screened subjects aged 19–23 years randomly assigned into two groups. Gp1 started with placebo (distilled water) for 2 weeks followed by 4 weeks washout period & switched to Plandula for 2 weeks. Gp2 started with Plandula. Scores recorded at baseline & after 2 weeks: Plaque Index (PI)

(Silness & Loë, 1964) – records presence/absence of plaque, Gingival Index (GI) (Loë & Silness, 1963) – determines presence/absence of gingival bleeding. Full mouth prophylaxis carried out after each measurement.

**Results:** On plaque; statistically significant PI score difference in means of 23.9% compared to placebo. On gingivitis; statistically significant reduction of 62.3% in mean GI scores compared to placebo.

**Conclusions:** Plandula might have potential of inhibiting plaque accumulation & effective in reducing gingivitis, seemed more effective in reducing gingivitis.

263

### Comparison of ten instruments for subgingival removal of artificial calculus

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This *in vitro* study evaluated the cleaning efficacies of four ultrasonic and two sonic tips, two rotating instruments, the Vector and the manual curette at different areas of the root. The roots of 50 molars were totally covered with artificial calculus. Teeth were fixed in silicone in order to imitate periodontal pockets. All roots were cleaned (maximum 5 min) with each of the following instruments ( $n = 5$ ): DesmoClean, PerioSet, CavitronJet, PiezonMaster, SonicFlex with smooth (SFS)/diamond tips (SFD), SuprassonP-Max with smooth (SMS)/ diamond tips (SMD), VectorSystem and GraceyCurettes. Photographs were taken before and after cleaning from 4 sides. The remaining calculus in relation to the respective surface area was determined. Statistical analysis was carried out (ANOVA, Bonferroni,  $P < 0.05$ ). Cleaning efficacies for buccal or oral surfaces were found to be approx. 80% for PM and SFS, 70% for DC, CJ, SFD, SMS, SMD, GC and 50% for PS and VS. Significant differences ( $P < 0.05$ ) were obtained between PS and GC, DC, CJ, PM, SFS, SFD as well as between VS-PM and VS-SFS. The efficacies for approximal surfaces amounted to between approximately 80% for PM and 50% for PS and VS and did not differ significantly from GC (70%). Significantly more calculus remained using PS compared to DC, PM, SFS, SFD and using VS compared to PM ( $P < 0.01$ ). Regardless of their working methods, most instruments achieved about 70% efficacy, except for PS and VS. Furthermore, their efficacies were not site-specific.

## Posters: Implant therapy

1

### Hyaluronic acid in peri-implant maintenance of immediate function implants. A comparative study with chlorhexidine

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**Background:** The maintenance phase of the prosthetic rehabilitations with immediate function implants, assumes a role as important as the surgical phase, representing a main factor

influencing the success rate. The aim of this prospective clinical trial was to compare the survival and healing of immediate function implants, inside a maintenance protocol using hyaluronic acid (HA) and chlorhexidine (CHX) gels.

**Materials and Methods:** Thirty patients, with four immediate function implants (All-on-Four concept) placed in the mandible (total of 120 implants), were included in two groups (HA and CHX) using only these two chemicals in their daily implant self-care. Both groups were followed for 4 months, with clinical observations on the 10th day, 2 months and 4 months post-surgically.



**Results:** The implant survival rate at 4 months was 100% in both groups. The HA and the CHX produced good results on the maintenance of complete rehabilitations of the edentulous mandible with immediate function implants. Statistically significant differences were found in favour of the HA group in the Modified bleeding index on the 2nd observation ( $P = 0.003$ ). The difference was located mainly in the axial implants placed in the 5th sextant ( $P = 0.05$ ).

**Discussion/Conclusion:** The findings point out that the importance of the maintenance protocol in implantology, and that both chemicals are valid tools for implant maintenance.

## 2

### The question of implant-crown relation

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**Introduction:** Traditional standard protocols have to be considered especially in cases of periodontal-compromised situations. The purpose of this article is to report the outcome and verify the possible negative consequences on long-term-result, with special focus on bone loss or loss of implant in cases of divergent protocols (the length of the crown should not be longer than the length of the implant).

**Material and method:** Twelve patients (41–80 years) received 80 Astra Tech implants (4.5 and 3.5 diameter; 8–11 length) The patient were followed up 2–8 years following the prosteric restoration.(length of the crown > length of the implant).

**Results:** During the observation period of 2–8 years after prosthetic restoration no significant bone resorption could be recorded. No implant was lost during the observation period no inflammation surrounding the soft tissue was found.

**Discussion:** We considered that the Conical Seal Desing brings increased mechanical strength to the implant-abutment interface, the stress at this point is significantly reduced.

**Conclusions:** If we want bone to be retained although a compromised situation exist, dental implants should have an intimate relationship between bone loading and bone structure (biological-biomechanical-stimulation) as example in case of ASTRA TECH implant system (Conical Seal Desing and the Micro Thread). The protocol regarding crown-implant relation should be redefined according to this criteria.

## 3

### Three years clinical experience with the new direct abutment

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**Purpose:** This study reports the clinical and radiographs result, following the use of the commercially pre-prepared Direct Abutment of the Astra Tech implant system.

**Material and Method:** Twenty patient with one or more missing teeth received a total of 60 Astra Tech 4.5 and 3.5 diameter. Implant were placed according to the recommended surgical protocol of the manufacturer. After haling period, the abutment were connected (using a force of 15 Ncm) followed by the standard impression, registration, and laboratory techniques for a ceramometal or allceramic restaurations. Radiographs were taken using a long cone technique on crown cementation and annually thereafter. Pateint were followed up every 6 months to determine clinical stability of the crown and the tissue health.

**Result:** After 3 years, the success rate was 100%. No Implants had been lost, and all restoration remained in function. We found no detectable mobility of the restoration and no loosening or

fracturing of the abutment. Mean distance for the marginal bone loss was 0.2 mm. Excellent soft tissue health was maintained around all the implants.

**Conclusion:** The Direct Abutment of Astra Tech implants system was simple to use and there were no complications. The rectorative and laboratory procedure time and cost was reduced of to 40%. All patient were satisfied with the esthetic outcome.

## 4

### RANKL – OPG concentration in crevicular fluid of patients with and without peri-implantitis

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MD DDS

Peri-implantitis is associated with progressive bone resorption that may lead to implant failure. Receptor activator of NF-kappaB ligand (RANKL) is a member of the TNF-superfamily required for the formation, activation, and survival of osteoclasts. A functional relationship between RANKL expression and peri-implant bone resorption was therefore reasonable to suggest. Here we determined the concentration of soluble RANKL, not bound to its antagonist osteoprotegerin, in crevicular fluid of dental implants. Thirteen patients with symptoms of peri-implantitis and ten patients with fully osseointegrated implants were included in the study. In eight patients with peri-implantitis and seven patients with osseointegrated implants, soluble RANKL was detectable by immunoassay, with a calculated mean value of 288 nM and 427 nM in the crevicular fluid, respectively. Concentration of soluble RANKL did not correlate with the clinical parameters probing depth, bleeding index, and plaque index. These results indicate that peak concentrations of soluble RANKL in crevicular fluid of osseointegrated implants are similar to those observed in peri-implantitis. Local concentration of soluble RANKL may therefore not solely account responsible for progressive bone resorption in peri-implantitis.

## 5

### A clinical study of the peri-implantitis in a long term maintenance after implantation

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Recently, oral implants were evaluated functionally and esthetically as clinical value for oral denture. Still, there are only a few study on the maintenance therapy after treatment. The aim of this study was to evaluate the conditions of peri-implantitis in long terms maintenance. We analysed 93 patients (mean age 48.5 years old, total 455 implants) who have being treated therapy over 3 years. This program started from 1990 to 2004. The patients were regularly measured pocket depth (PD) around implants in four sites about third month. 192 implants (42.2%) were diagnosed peri-implantitis. There was a large number of the deepest pocket of implant in medial and distal side. In the other hand, most shallow pockets were in buccal side. According to these peri-implantitis possibility been within 4.3 years ( $SD = 2.9$ ), and at that moment PD was 5.05 mm ( $SD = 1.80$  mm). The means PD and starting PD of peri-implantitis were significantly greater than healthy surrounding implant tissue. There were many peri-implantitis in maxilla than mandibular, and no significantly difference between incisor and molar. In conclusion, the results showed that the establishment of peri-implantitis has high possibility about 4 years after implantation and the prevalent was higher in maxilla.

6

# **Tactile sensibility of single tooth implants, a clinical, single-blinded, randomised, split-mouth study**

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In the present study the active tactile sensibility of a single-tooth implant was compared with the remaining natural tooth of the contralateral side. The experiment was performed with anaesthesia of the antagonistic natural teeth on both sides. The aim of the study was to get more information about the neurophysiological mechanisms of osseoperception and the meaning of periodontal mechanoreceptors for oral tactile sensibility. Testing the 50% values of tactile sensibility and the equivalence from implant and tooth represented the 'primary endpoint' of the study. The results showed a mean tactile sensibility of  $16 \pm 9 \mu\text{m}$  for the pairs of natural teeth (controls) and  $20 \pm 11 \mu\text{m}$  for the implant region; the intraindividual difference was  $3.5 \pm 7 \mu\text{m}$ . At thresholds of  $\pm 8 \mu\text{m}$ , chosen according to the thickness of the thinnest occlusal foil used which also is  $8 \mu\text{m}$ , the statistical evaluation resulted in an equivalence of the active tactile sensibility when comparing single-tooth implants occluding against natural antagonists with the remaining natural dentition ( $P < 0.01$ , Power  $> 80\%$ ). Comparing the results with other publications, anaesthesia of the antagonists did not impair the tactile sensibility of the implants. The periodontal receptors of the antagonistic teeth do not have that important role for the capacity of oral implants to transmit tactile sensibility. This study confirms the theoretical considerations about osseoperception.

7

# **Short vs. long implants in augmented and non-augmented mandibular and maxillary sites: a systematic review of 22 prospective studies (11 222 implants)**

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Twenty-two prospective clinical trails including 11 multicenter studies were systematically reviewed to evaluate the survival of short and long implants in augmented and non-augmented mandibular and maxillary sites. A total of 11 222 implants in 3647 patients were placed in 38 implant centers in 15 countries all over Europe and the USA. Implants of six different manufactures with five different surfaces were followed up over a period of  $5.3 \pm 4.7$  years. Implant length reached from 5 to 20 mm. A total of 83% of the implants were retained in original jawbone while the remainder were placed in areas previously augmented using onlay graft or sinus lift techniques. Implants placed in non-augmented mandibular bone showed the highest success rate (98.7%), followed by implants in the non-augmented anterior maxilla (98.0%). A 97.6% success rate was calculated for implants in the non-augmented posterior maxilla as well as in augmented mandibular bone. A significantly lower implant survival rate was recorded in augmented posterior (93.8%) and anterior (91.0%) maxillary sites. In original jawbone short and long mandibular implants showed similar survival rates, while a significantly higher failure rate of maxillary implants shorter than 8 mm could be observed. In augmented sites mandibular implants shorter than 8 mm and maxillary implants shorter than 10 mm showed significantly lower survival rates. It can be concluded that the survival of short implants is impaired in the maxilla as well as in augmented bone.

8

# **New protocol for implant surgery: low speed without irrigation bone drilling: in vivo study concerning 40 implants**

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In a previous *in vitro* study\*, low heat elevation was shown after cortical bone drilling, with a low drilling speed without irrigation. The aim of this study is to evaluate this protocol *in vivo* in dental implantology.

**Material and methods:** Forty implants (standard Straumann® implant,  $\phi$  4.1 mm) were set up for 15 patients (seven female, eight male). Every type of bone was concerned: upper jaw, molar area (5), premolar area (11), canine (3), incisor (4); lower jaw, molar area (9), premolar (3). Every drilling step (predrilling, and then 2.2, 2.8 and 3.5 mm diameter drills) was performed with low speed (450 rpm) without irrigation. Standard X-rays were performed after placement of the implants, and then after 6 months and 1-year visit check up.

**Results:** After an average time of 18 months of loading, osseous integration following Albrektsson success criterion's (Int JOMI, 1986) was recorded for all implants. X-rays measurements did not show either craterisation or abnormal osseous lost around implants.

**Conclusion:** In the limit of this study, it can be concluded that implant socket can be performed with success at low drilling speed (450 rpm) without irrigation. This technical approach can provide an easier legibility of the laser marks of the drills, especially in the molar areas whose access is difficult; a step-by-step control of the axe and depth of the drills, and the possibility of easily recovering the whole part of the drilled bone.

\*Reingewirtz et coll, Clin Oral Impl Res, 1997.

9

# **Clinical evaluation of bränemark Ti-unite implant and ITI SLA implant in the post maxillary area with sinus elevation technique**

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Aim of this study was to evaluate the efficacy of Bränemark Ti-Unite implant system and ITI SLA implant system placed in the atrophic posterior maxilla with sinus floor elevation procedure. Eighty patients received placement of Bränemark Ti-Unite implants (195 implants) in their atrophic posterior Maxilla with sinus floor elevation procedure (153 osteotome sinus floor elevation technique and 42 window opening procedure). Fifty patients received placement of ITI SLA implants (83 implants) in their atrophic posterior Maxilla with sinus floor elevation procedure (77 osteotome sinus floor elevation technique and six window opening procedure). Chart review were taken from each patient. The total failed implants were seven and the total implant survival rate was 96.4% in Bränemark Ti-Unite system. The total failed implants were one and the total implant survival rate was 98.8% in ITI SLA system. The implant survival rate with osteotome technique was 96% and 97.6% with window opening in Bränemark Ti-Unite system. The implant survival rate with osteotome technique was 98.7% and 100% with window opening. The implant survival rate with osteotome technique was 96% and 97.6% with window opening in ITI SLA system. The results of this evaluation show that the placement of Bränemark Ti-Unite system as well as ITI

SLA system is a reasonable treatment option for patients with the atrophic posterior maxillary area.

10

# Treatment of peri-implantar gingivitis in patients with and without history of rheumatoid arthritis: a pilot study

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**Aim:** The aim of this study was to treat peri-implantar gingivitis (PIG) in patients with and without Rheumatoid Arthritis (RA) and to analyse if there were differences between them.

**Material and Method:** Eighteen patients were selected and divided in two groups: Group (1): Ten patients with no history of RA (five men: age  $55.1 \pm 10.92$  and five women: age  $54.6 \pm 9.87$ ) and Group (2): eight patients (four men: age 58 and four woman: age 56.8). All had, at least, three implants unitary (Straumann) and one or two sites with PIG. Patients of Group (2) were under medical treatment, taking a non-steroid anti-inflammatory COX-2 selective, and both groups were submitted to a prophylactic scaling (monthly) and chlorhexidine gel (0.5%) topic, 1 x day, during 6 months. All patients were examined 2, 4, 6 and 12 months after study for evaluation of clinical parameters – Plaque Index (PI) and bleeding on probing (BoP). Qui-square and Fisher Exact tests were used to analyse the statistical results between groups.

**Results:** The statistical analyse in 2 and 4 months showed similar PI and BoP scores on both groups, however, there were differences statistically significant between 4, 6 and 12 months on BoP ( $P < 0.001$ ) in Group 2.

**Conclusion:** The results shows that patients with RA need shorter intervals between dental recalls for supportive therapy, moreover, biofilms upon prosthetic surfaces cause an inflammation of peri-implantar tissues due to an excessive production of pro-inflammatory cytokines from subjacent connective tissue.

11

# Radiographic changes in immediately restored dental implants in periodontally susceptible patients, 2 year results.

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**Objectives:** Radiographic bone changes (CB) of immediately restored implants (R) was compared to non submerged non restored (NR) and submerged implants (S).

**Materials & Methods:** Treated chronic periodontitis patients received implant therapy. A provisional restoration was immediately provided for R implants. The rest either received healing abutments (NR) or were submerged, 2nd stage surgery performed 6 months later and restored thereafter (S). Two year radiographic bone level changes were compared between the three groups. Results are reported between insertion, 6, 12 and 24 months expressed in mm as mean change  $\pm$  SE.

**Results:** A total of 89 implants were inserted in 18 patients, of which 41 in extraction sites. Thirteen implants failed and were excluded from the analysis. Mean CB change was  $0.845 \pm 0.092$ ,  $1.263 \pm 0.121$ ,  $1.47 \pm 0.122$  and  $0.113 \pm 0.083$  between 0–6, 0–12, 0–24 and 12–24 months, respectively. 0–6-month CB changes of R and NR ( $0.957 \pm 0.12$ ,  $0.654 \pm 0.199$  respectively) and 6–12-month CB changes of S ( $0.792 \pm 0.13$ ) were not significantly different (ANOVA). 12–24-month CB changes in R, S and NR ( $0.188 \pm 0.132$ ,  $0.07 \pm 0.122$  and  $0.007 \pm 0.239$  respectively) were not different between groups ( $P = 0.7197$ ). No difference

was found between R and NR groups for BC in any of the time intervals. No difference was found between extraction and non extraction sites either.

**Conclusions:** (i) BC of immediately restored implants do not differ from non restored implants and (ii) BC of immediately restored implants is within the literature range.

12

# Results of cover screw loosening following surgical placement of implants: a retrospective cohort study in a private office setting.

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The purpose was to assess the sequelae of implant cover screw loosening (CSL) found at the second stage of implant surgery in a private periodontal practice. The prevention and treatment of CSL, and its clinical impact on implant placement will be discussed.

**Methods:** The study included total of 157 implants (115 TiUnite and 42 Osseotite). All implants were placed by one investigator and positioned flush with the crestal bone. Measurements were done on digitized periapical radiographs taken at Stage II surgery prior to placement of the healing abutment. Bone loss around implants with both CSL and non-CSL on the mesial and distal aspects and the distance of the microgap caused by CSL were measured. The distortion factor was calculated to compensate for non-standardized radiographs.

**Results:** The relationship between CSL and early implant exposure was statistically significant. The relationship between CSL and radiographic crestal bone loss was statistically significant. No correlation found between the distance of microgap and crestal bone loss. No significant relationship between CSL and the following: patient's ability to wear prosthesis following Stage I, age, gender, systemic diseases, bone augmentation, use of membrane, implant configuration, implant diameter, and pre-surgical steroids.

**Conclusions:** The findings of this study led to the recommendation of increasing the post-operative visits to identify and treat CSL and/or exposure to limit crestal bone loss.

13

# Prosthetic complications in implant borne reconstructions after an observation period of at least 40 months

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**Aim of the study:** To evaluate the prevalence and type of complications in fixed implant borne reconstructions.

**Material and Methods:** In 105 patients, 172 FPDs (317 prosthetic units) were made on 283 ITI implants (eighty crowns, 92 different types of fixed bridges). Mean evaluation time was  $62.5 \pm 25.3$  months. In 45 cases the reconstruction was screw retained and in 127 cases cemented (Harvard® or Improv®).

**Results:** Complications occurred after a minimum period of 2 months and a maximum period of 100 months (mean:  $35.9 \pm 21.4$  months). Fifty-five prosthetic interventions were needed on 44 reconstructions (25%) of which 88% were in the premolar and molar region. The lowest percentage occurred in crowns (25%), the highest in 3–4 units FPDs ( $P < 0.048$ ). Screw retained reconstructions had significantly more complications than cemented ( $P < 0.001$ ). More complications occurred in patients

## Posters: Implant therapy

with bruxing habits than in the non-bruxing group ( $P < 0.001$ ). Of the clinical interventions, 14% were classified as minor (e.g. polishing), 71% as moderate (10–60 min of chair time but no laboratory costs), 14% as major interventions (more than 1 h chair time and additional laboratory costs). Type and duration of necessary interventions were not different between the bruxing and non-bruxing group. Additional laboratory costs ranged from 28 Euro to 840 Euro.

**Conclusion:** Complication rate was 17% on the prosthetic unit level, 25% on the reconstruction level.

14

### Perforation of cover screws leads to irreversible marginal bone loss

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**Background:** Reports show that bone remodelling around one-stage implants is similar to around two-stage implants, however an unintentional soft tissue perforation above the cover screw of a two-stage implant appears to cause an inflammatory reaction resulting in marginal bone destruction.

**Aim:** This study aimed to determine the consequence of early cover screw exposure on marginal bone.

**Materials and methods:** Sixty AstraTech® implants installed in partially edentulous jaws were compared: 20 implants were placed following a two-stage protocol but showed an early exposure, 20 implants were placed following a two-stage protocol and were exposed after a normal subgingival healing time (3–6 months), and 20 implants were placed following a one-stage surgery. Digital radiographs were taken at implant placement, and after abutment surgery for the two-stage exposed and two-stage submerged group or after 3 months for the one-stage group. Bone loss mesially and distally was measured by an on screen cursor after calibration.

**Results:** Mean bone remodelling was 1.96 mm (range: 0.2–3.2 mm) around two-stage exposed implants, 0.01 mm (range: 0.0–0.3 mm) around two-stage submerged implants and 0.14 mm (range: 0.0–1.2 mm) around one-stage implants.

**Discussion and conclusion:** The unintentional perforation with two-stage implants leads to significant bone destruction, probably caused by the creation of biological width. An intentional perforation (one-stage) is acceptable because the flap design respects the biological width.

15

### Soluble rankl in crevicular fluid of dental implants

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**Background:** Peri-implantitis is associated with progressive bone resorption that may lead to implant failure. Receptor activator of NF-kappaB ligand (RANKL) is a local produced member of the TNF-superfamily required for the formation, activation, and survival of osteoclasts. A functional relationship between RANKL expression and peri-implant bone resorption was therefore reasonable to suggest.

**Objective:** Here we determined the concentration of soluble RANKL, not bound to its antagonist osteoprotegerin, in crevicular fluid of dental implants.

**Methods & Results:** Thirteen patients with symptoms of peri-implantitis and ten patients with fully osseointegrated implants were included in the study. In eight patients with peri-implantitis and seven patients with osseointegrated implants, soluble RANKL

was detectable by immunoassay, with a calculated mean value of 288 nM and 427 nM in the crevicular fluid, respectively. Concentration of soluble RANKL showed no correlation with the clinical parameters probing depth, bleeding index, and plaque index.

**Conclusion:** These results indicate that peak concentrations of soluble RANKL in crevicular fluid of osseointegrated implants are similar to those observed in peri-implantitis. Local concentration of soluble RANKL may therefore not solely account responsible for progressive bone resorption in peri-implantitis.

16

### Evaluation of immediate loading success rate on peri implant tissues in patients with posterior fixed implant-supported prosthesis

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**Introduction:** The aim of this study was to evaluate the clinical response and success rate of immediately loaded implants compared with delay loaded implants.

**Materials & Methods:** Twelve patients were enrolled in this study. A total of 28 Xive implants were placed in these patients and randomly divided into two groups (14 implants in test group and 14 implants in control group). Test group implants were immediately loaded within 24 h after surgical placement with an temporary acrylic resin restoration. Control group implants were left to heal submerged for 3–4 months and then were loaded with FRC restorations. Implants were evaluated at the time of surgery and every 2 months, considering the following parameters: Plaque index, bleeding index, probing depth, mobility, pain and bone resorption. Peri-implant bone resorption was evaluated on periapical radiographs with standardized parallel technique.

**Results:** Survival rates in test and control groups were 87% and 100% respectively. No significant statistical difference was observed between the two groups in PI and PD. No pain, BOP or mobility was observed in all recalls. Mean bone resorption of peri-implant bone height was 0.26 mm ( $\pm 0.32$ ) in test group and 0.98 mm ( $\pm 0.59$ ) in control group. The difference was significant ( $P = 0.011$ ).

**Conclusion:** Peri-implant bone resorption in immediate loading implants was significantly lower than that of delay loading ones.

17

### Periodontal and peri-implant health in patients with poor compliance

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**Background:** Compliance with maintenance program (MP) has been reported as an important factor for periodontal health. The purpose of this study was to determine changes observed in periodontal and peri-implant parameters in a re-established MP for patients with poor compliance.

**Materials and Methods:** Twenty-seven poor compliant patients (13 males, 14 females,  $47 \pm 12$  years old) and 95 implants (six single, 21 fixed partial restorations,  $2.4 \pm 1.6$  years in function) were included. Initial oral health was evaluated with modified Dental Index. 3 months rigid MP was applied for 1 year and gingival health status was evaluated by using plaque index, gingival index (GI), bleeding on probing (BOP), clinical attachment level (CAL) and simplified oral hygiene index (sOHI). Periodontal (PS) and

peri-implant sites (PIS) were compared by using Wilcoxon matched-pairs and step-down Bonferroni method.

**Results:** Initial CAL was approximately 3 mm for both PIS and PS, and remained stable. Plaque accumulation was statistically lower at PIS compared to PS at each appointment ( $P < 0.05$ ). Although not significant, BOP was higher at PIS compared to PS and a slight improvement was observed in sOHI by last appointment ( $P > 0.05$ ). This did not affect GI values.

**Conclusion:** The results suggest that tissue response against plaque accumulation may be different for PIS and PS. Also, despite a re-established MP, changes observed in home care practice are minimal in patients with poor compliance.

18

### Alveolar ridge augmentation using collagen membrane and DFDB in combination with PRP

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**Background:** The purpose of this study is to evaluate a surgical procedure for vertical and horizontal alveolar-ridge reconstruction in the maxilla using demineralized freeze-dried bone (DFDB) graft mixed with platelet-rich plasma PRP associated with a collagen membrane prior the implant placement.

**Methods:** A group of 30 single tooth missing patients, presenting the need for vertical and horizontal bone augmentation of at least 3 mm, were treated before implant placement. For the period of the first surgery, in the group of 15 patients (experimental group), DFDB graft was mixed with activated PRP and placed in wanted position in the region of bone defect, covered with collagen membrane secured with titanium pins. After 4 months, pins were removed and 15 ITI implants were successfully placed. In the Second group of 15 patients (control group) augmentation procedure was performed with DFDB only, prior 15 ITI implant placement.

**Results:** The significant bone level increase was reached in all patients of experimental group. Bone augmentation achieved in control group was on statistically lower level. Abutment connection was obtained 3 months after implant placement. Clinical parameters after prosthetic restoration, confirmed the presence of a healthy peri-implant mucosa.

**Conclusion:** Surgical technique performed in experimental group in combination with ITI implant placement provides supreme functional and aesthetics outcomes in alveolar-ridge augmentation and prosthetic rehabilitation.

19

### Microflora formation around one-stage dental implants – a preliminary report

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**Background:** Limited information is available on initial bacterial colonization of dental implants. This study aims to determine the characteristics of newly forming microflora around one-stage implants.

**Materials and Methods:** Eleven periodontally healthy patients (seven males, four females,  $48 \pm 16$  years old) needing single implant were included. Exclusion criteria were antibiotic uptake within 3 months or simultaneous guided bone regeneration. Subgingival plaque samples were obtained around adjacent teeth and implant prior to and at 2, 4, 8, 12 weeks post implant placement. Polymerase chain reaction was used to detect specific microorganisms. Clinical evaluation included probing depth,

bleeding on probing and, width and thickness of keratinized gingiva.

**Results:** No early healing complications were noted. *Fusobacterium nucleatum* was initially present in nine patients and continued to be part of tooth flora during 12 weeks. Four patients had *Fusobacterium nucleatum* around implant by week 2 and six by week 12. Detection of *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tanarella forsythia* and *Treponema denticola* was rare. Bacteria were not detected in internal part of implant body.

**Conclusion:** The rate of specific bacterial growth around implants is slower than around teeth. Further studies are necessary to better establish the rationale for post-operative care of one-stage implants.

20

### Clinical parameters analyses in implants placed nearly to retained roots

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**Aim:** The aim of this study was to evaluate clinical and radiographic parameters on implants in function and positioned adjacent to roots intentionally retained.

**Materials and Methods:** Eight patients were selected to receive implants (Straumann Implants) in inferior molars with divergent roots, which, at least, one root had extraction indication. After 3 months of healing period, the implants were placed (single-stage surgical protocol in conjunction with controlled immediate loading) on six patients and the clinical parameters (attachment level and probing depth) were analysed at each 90 days, during 1 year, including instructions of oral hygiene and radiographs as control. Nevertheless, in the other two patients the implants were maintained submerged and radiographs were taken as control.

**Results:** All implants succeeded and during the whole study it was not observed any kind of alterations on those clinical parameters analysed, as well as any radiographic alteration on those implants intentionally retained.

**Conclusion:** Implants placed nearly to roots intentionally retained with their prosthesis showed similar aspects, attachment level and probing depth, on mesial and distal sites.

21

### Single tooth replacement in the aesthetic zone with nobel perfect immediate placement and provisionalization

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In the aesthetic zone maintaining the existant and providing stability to the hard and soft tissue architecture of the failing tooth may be important goals of implant therapy. The purpose of this study was to evaluate some clinical outcomes of Nobel Perfect® dental implants placed in fresh extraction sockets with immediate provisionalization.

**Materials and methods:** Six patients, 35–58 years, were treated. Implants were inserted in the extraction sites of upper incisors using a method described by A. Rocci, J. Gottlow, with the exceptions: (i) No attempt was made to fill the gaps between the implant and the alveolae and (ii) The peak of the implant was not always aligned towards the centre of the interproximal contact areas. All implants were placed above the bone crest and received straight abutments and temporary crowns immediately. Clinical

## Posters: Implant therapy

evaluation were made at implant placement and provisionalization and 12 month later. The following variables were recorded: implant success/failure, radiographic osseous change and photographic gingival level.

**Results:** No implants were lost during the 12 months follow-up. Most papilla filled the available interproximal space and minor change at radiographic osseous and photographic gingival levels were observed.

**Discussions/Conclusions:** This technique seems to allow excellent clinical results. With the method there is freedom to avoid deeply submerged implants and apparently demonstrate the potential for preserving the existent osseous and the gingival architecture.

22

### Fixation screws resistance to cuspid distalization

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Mini-implants, miniplates and fixation screws have been used as alternatives for orthodontic anchorage. The purpose of this study was to determine the resistance of fixation screws to cuspid distalization. Thirty 10 mm by 1.4 mm fixation screws (Osseofix, 3i) were placed in 15 patients with no periodontal disease referred for orthodontic treatment. Each patient received two screws, one in the maxilla and one in the mandible. A screw was placed 1 month and the other 2 weeks before the application of forces. A Nance button was used as control on the opposite maxilla. A force of 200 grams was applied weekly for a period of 4 months. Statistical analysis was made with a one way of analysis variance (ANOVA) and a chi square test. At the end of the 4 months, nine screws were lost due to plaque accumulation. All 10 screws placed 1 month before distalization resisted the movement. Of the 11 screws placed 2 weeks before distalization, seven resisted the movement and four did not (two in the mandible and two in the maxilla). No differences were found between test and control, or regarding time of placement or whether screws were placed on cortical or cancellous bone. The fixation screws provide an excellent alternative method for orthodontic anchorage. They are cheaper and easy to install and to remove. Although no statistical difference was found, based on the observations, it is recommended that the orthodontic forces be applied 1 month after placement of the screws.

23

### Postmenopausal females, medication, osteoporosis and implant integration – first cases of a prospective clinical study

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Data on Osseointegration of dental implants in osteoporotic patients are missing and the influence of oral Bisphosphonates (BP) remains unknown. This prospective controlled clinical study tests implant integration and function under load in mandibles of postmenopausal osteoporotic (Tests) vs. age-matched healthy females (Controls) and analyses possible morphological differences in bony structures by Micro-CT. Clinical parameters for plaque (PI), bleeding (BoP), peri-implant crevicular fluid (PCF), probing depth (PD), and attachment level (AL) are obtained at 1 and 3 weeks and 3, 6, 9 months after implant installation. In the test group either BP (Alendronate) or Placebo were randomly administered starting 1 month prior to implantation; in addition, everybody in this group received Ca-VitD substitution. Bone cores were obtained during implant surgery, undecalcified embedded and analysed (Micro-CT). Implant healing lasted for 3 months before loading. This preliminary report presents two exemplary

cases (Test/Control). From the Tests the case with the lowest Bone/Tissue Volume (BV/TV) ratio was chosen, from Controls the one with the highest. Clinical parameters were related to Micro-CT data. Despite approximately 50% difference in BV/TV ratio between Test and Control, an uneventful integration and stability of implants were observed. Three months after insertion PCF value was 44 (for Test) vs. 105 (for Control) Periotron-Units and AL was at 3.5 vs. 3.75 mm, respectively.

24

### Platelet rich plasma in regenerative treatment of infrabony defects around immediate implants in the anterior maxilla

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Platelet rich plasma (PRP) delivers enriched growth factors that could enhance wound healing in intraoral bone defects.

**Objective:** The aim was to evaluate PRP-mediated bone regeneration in immediate oral implantation in conjunction with bone grafts.

**Methods:** Following extraction of teeth in anterior maxilla, 14 implants (Bränemark, MK III TiUnite, Ø 3.75) in seven patients were immediately placed. The first group received PRP with beta-tricalcium phosphate ( $\beta$ -TCP) while the second group was treated with b-TCP alone. Guides were used as referral points to standardize the measurements taken at baseline and after 9 months.

**Results:** All areas showed complete hard tissue fill and none of the implants demonstrated any loss of stability. Bone grafts healed well without any infection. Hard tissue fill was significantly higher in PRP +  $\beta$ -TCP group compared to graft alone (25%;  $P < 0.05$ ). The histological results showed that new bone was formed among particles of  $\beta$ -TCP in both groups, but in PRP +  $\beta$ -TCP group, new bone was denser and presented mature woven bone, more new bone was formed into the micropores of the particles. In addition, there were less  $\beta$ -TCP particles observed confined within new bone.

**Conclusions:** PRP significantly enhances the clinical effects of the bone graft in regenerative treatment of anterior peri-implant defects leading to a denser and mature bone formation.

**Acknowledgement:** Supported by the Research Fund of Istanbul University. Project No 1734/15082001.

25

### Influence of various surface treatments on reosseointegration around contaminated implants

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The similarity of periodontitis and peri-implantitis demands for the utilization of similar principles for the treatment. Different decontamination methods were available cleaning of implant surfaces contaminated with bacteria. The aim of the present study was to evaluate the effects of various decontamination methods on reosseointegration on contaminated implants. Six mongrel dogs were used. The mandibular 1st molars and all premolars were removed bilaterally. Three months later, experimental implants with different surface characters were installed in each sides of the mandible. The implant consisted of two parts; the implant body and an exchangeable intraosseous implant cylinder. After osseointegration, experimental peri-implantitis was induced by cotton ligatures until the bone loss reached the junction of the two segments of the implant. After debridement of the bone defects, three treatment models were performed; (i) contaminated cylinders were removed, pristine cylinders were placed; (ii) contaminated cylinders were cleaned *in situ* with saline and (iii)

contaminated cylinders was removed, cleaned with saline, sterilized by autoclaving. All implants were covered with membranes. After 3 months, histological evaluations were accomplished. The results indicated that *in situ* saline therapy demonstrated a significant difference at SLA surfaces in bone-implant-contact. Treatment of contaminated implants *in situ* with saline resulted in resolution of peri-implantitis and bone fill in defects.

26

### Histological evaluation of wound healing following simulated immediate implant placement

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The gap occurred following immediate implantation around the coronal portion of the implant is expected to be filled by newly regenerated bone tissue. A rich blood supply was an essential requirement to promote rapid and uncomplicated postoperative healing. The purpose of this study was to evaluate the reaction of peri-implant tissue to immediately placed implants into simulated extraction sites following guided bone regeneration. Nine mongrel dogs were used. The premolar teeth on both sides were extracted. After 90 days, four holes were prepared in bone for each edentulous quadrant. Then the coronal part of these holes was enlarged to 4 mm diameter until 3 mm depth. Experimental implants with three different surface characters were placed into three of the holes. One cavity was left empty as control. Total of 54 stable experimental implants were covered by membranes. The dogs were sacrificed at 3, 7, 21, 28, 35, and 49 days after implant surgery. At 3 and 7 days, abundant newly formed blood vessels and immature bone were seen on SLA and TPS implant surfaces. At 21, 28, 36 and 49 days no vascular network was detected and the surface of the bone was a mixture of immature and mature tissue. Within the limits of this study, the results demonstrated that osseointegration occurs around immediately placed titanium implants in simulated extraction sites and the use of barrier membranes will heal in a predictable way.

27

### Function and patient satisfaction in implant therapy

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**Objective:** The aim of our study was to evaluate the function and patient satisfaction in implant therapy including the surgical steps.

**Methods:** In total, 19 patients that received two-step implant therapy with various prosthetic constructions were evaluated by a questionnaire of 26 questions. Out of 26 questions, four were about the comfort, duration, and patient compliance during the surgical phase, 10 questions were related to the patient satisfaction with completed prosthesis, and 12 questions were focused on the situation before implant therapy, comparison between conventional and implant-supported prosthesis. Data was evaluated by chi-square analysis.

**Results:** All patients reported full satisfaction due to implant therapy as the treatment of choice in restoring partial or full edentulism ( $P < 0.05$ ). Restoring function and aesthetics were observed as the main expectancy. No patients reported any peri- and post-surgical pain while 89% of patients reported no discomfort during the surgery. Seven patients had minor post-operative complaints but they reported that these minor issues did not affect their overall satisfaction of the outcome of the therapy. After the completion of prosthetic restorations, 63% of patients felt their implant-supported prosthesis were similar to their natural dentition ( $P < 0.05$ ).

**Conclusion:** The duration of the implant therapy was the major concern and this finding suggests that immediate loading in implant therapy should be chosen when possible.

28

### A 10-year follow-up with astra tech implants

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The present retrospective long-term trial statistically evaluates 1999 Astra Tech implants placed in 607 patients following the Kaplan Meier procedure. Additionally, proximal and distal bone apposition and resorption in 40 patients with implants *in situ* since for least 10 years were examined radiologically, digitalized using Friacom-Dental Office Software, and subsequently evaluated. In order to verify bone level alterations, the distance between referencing point and marginal bone level was measured. The median observation period was 50.6 months. The survival rate after 5 years, remaining unchanged till the end of the observation period ( $> 10$  years), was 97%. The radiological examination of the marginal bone level of 40 patients (163 implants) with implants *in situ* for at least 10 years showed a mean bone loss of 0.4 mm (SD 0.7 mm). The outcome of the present study confirms very good long-term results with the Astra Tech implant system in terms of implant survival rate and marginal bone level. Furthermore, the marginal bone level with a median bone resorption rate of 0.4 mm, observed over a 10-year period, represents a special clinical success with a very good esthetic outcome.

29

### Immediate and early load on zygoma implants: initial results of a 3-year prospective study

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**Background:** The atrophic maxilla presents specific problems for implant placement. Often these patients are candidate for rehabilitation on zygoma implants. Recent publications have demonstrated the predictability of immediate loading of implants in different oral locations. The aim of this study is to report on the use of immediately loaded zygoma implants to rehabilitate severely atrophic maxillae.

**Materials and methods:** The inclusion criteria were the same used for the original two-stage surgery. Forty-six zygoma and 127 regular implants on the residual bone crest were placed in 25 consecutive patients. Then, straight and angulated abutments were placed and occlusal registers as well as impressions were taken during the surgery. A screw retained full arch restoration was selected in 23 patients, two patients received partial cemented rehabilitations. Six patients were rehabilitated using an early, 5 days, load protocol. Nineteen patients followed an immediate, 24 h, loading protocol. The patients were instructed for a soft diet during 4 months. Follow-up controls were performed at 1, 4 and 12 months, thereafter annually.

**Results:** After 7 to 38 months follow-up (mean 16.2 months) the CSR of both zygoma and regular anterior implants, was 100%. CSR of the prostheses was 100%. No complications were observed during the study.

**Conclusions:** Within the limits of this study it is possible to predictably splint zygoma implants with conventional implants utilizing an immediate load protocol.

30

### Guided surgery and immediate screw retained prostheses: 1-year prospective study

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**Background:** Computers have been used to plan implant surgery. Today, through a specific radiological examination and a new computer software, the number, size, depth and position of the implant needed for the restoration, can be planned on a 3D reproduction basis. The system makes flapless surgery possible as well as the fabrication of a screwed prosthesis before surgery. The aim of this study is to illustrate the clinical outcome of the first patients rehabilitated with the above mentioned system.

**Material and Methods:** Fifteen consecutive patients; 14 maxillae and four mandibles, including the learning curve, were rehabilitated by means of 18 full arch screwed prostheses supported by a total of 127 implants. Inclusion criteria were: edentulous of at least one arch, bone enough to place six implants of a minimum length of 10 mm. After a virtual planning using a Procera Ö software, implants were placed according to the positions marked by an specifically produced surgical guide. No mucoperiosteal flap was raised. All the prostheses were screwed immediately after the surgery.

**Results:** Eight implants failed in one smoker and bruxist patient 3 months after the surgery. The CSR of implants and prostheses was 93.8% and 94.9% respectively. Minimum pain or discomfort was experienced.

**Conclusions:** The method enhances accuracy and precision of the surgical procedure, minimises postoperative morbidity. Patient selection is important to insure the success of the procedure.

31

### An up to 5-year prospective clinical study on implants in the zygomatic arch for prosthetic rehabilitation of the atrophic edentulous maxilla

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**Background:** Prosthetic rehabilitation with implant-supported prostheses in the atrophic maxilla usually requires bone augmentation procedures to enable implant placement. However, a rigid anchorage can be achieved by using so called zygomatic implants placed in the zygomatic arch in combination with routine implants placed in residual bone areas. The aim of the present study was to long-term report on the clinical outcome of zygomatic and regular implants for the rehabilitation of the severely atrophic maxilla.

**Materials and methods:** Sixty-nine consecutive patients with severe maxillary atrophy were, during a 5-year period, rehabilitated with a total of 69 fixed full-arch prostheses anchored on 435 implants (131 zygomatic). Fifty-seven bridges were screw-retained and 12 cemented. The patients had been followed for at least 6 months and up to 5 years in loading.

**Results:** CSR of zygoma and regular implants was 100% and 98% respectively. Periotest measurements of zygomatic and regular implants showed a decrease of the PTV value with time. Loosening of prosthetic gold screws was recorded in nine patients. Fracture of one gold screw as well as the prosthesis occurred twice in one patient. Fracture of anterior prosthetic teeth was experienced in 12 patients.

**Conclusions:** The results from the present study show that the use of zygomatic and routine implants represents a predictable alternative to bone grafting in the rehabilitation of the atrophic edentulous maxilla.

32

### Radiographic changes of graft materials after sinus floor elevation

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The aim of this study was radiographically to evaluate the change of the graft materials after Mx sinus floor elevation and the influence by the graft material type on graft height. A total of 59 patients (28 in LA and 31 in CA) who underwent sinus floor elevation composed of lateral (LA) and crestal approach (CA) were radiographically followed for up to about 48 months. Changes in graft height were calculated with respect to implant length (IL) and grafted sinus height (BL). From BL/IL, we evaluated change of the grafted height according to function time, the time of implant placement in LA and influence of the type of graft materials to change of the graft height and the height change of the grafted sinus floor relative to the implant apex. To assess graft remodelling, a novel index (SGRI) was used. Sinus graft height decreased significantly in both approaches in 12 months ( $P < 0.01$ ). Significant difference was not observed between staged and simultaneous approach in graft height change according to time in LA. However, staged approach had more height loss to simultaneous approach in all observed time. In LA, there was significant difference in BL/IL change according to graft type ( $P < 0.05$ ). Autogenous bone had maximum height reduction and MBCP<sup>TM</sup> had minimum height reduction. SGRI was increased statistically significantly from baseline to 3 months and 3 ( $P < 0.05$ ) to 12 months ( $P < 0.01$ ) in both approaches.

33

### Evaluation of clinical performance of Swissplus dental implants in partially edentulous patients: a retrospective analysis

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**Aim:** The aim of this study was to evaluate clinical performance of Swissplus Dental Implant System.

**Materials and methods:** A total of 105 implants which were inserted in 30 patients (16 male, 14 female), between 35 and 66 years of age. A one-stage surgical protocol with delayed loading was used. Abutment connection was performed 3–6 months after implant installation. The patients were followed for at least 1 year after loading. The survival rates were evaluated according to location, diameter and length.

**Results:** Three implants were lost during the 1 year of follow-up. The overall survival rate at 1 year was 98.09%. The corresponding survival rate was 96.07% for straight implants and 98.14 % for tapered implants. The survival rates of anterior and posterior regions of maxilla were 92.8% and 100%, respectively. For anterior and posterior mandible survival rates were 80% and 98.1%, respectively. The mean loss of marginal bone at the implants during the first year in function was 0.33 mm.

**Discussion/Conclusion:** These favourable results showed that SwissPlus Implant System can be used safely in partially edentulous patients. Larger numbers of implants followed for longer periods of time are needed for further investigation. Good bone quality, primer stability adequate loading are important factors for long term maintaining of bone-implant interface structurally and functionally.



34

### Integration and function of dental implants in postmenopausal women with osteoporosis compared to healthy females of matched age. preliminary results

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Postmenopausal osteoporosis is the most common form of this skeletal disease. Prospective studies evaluating the relationship between osteoporosis, medication and integration of dental implants are lacking. This controlled, prospective study in postmenopausal women compares integration and function of dental implants in patients with diagnosis of untreated osteoporosis (tests) to that in age-matched healthy controls. Implants installed in lateral areas of mandibles are allowed to heal for 3 months prior to loading. Clinical parameters for plaque (PI), bleeding (SBI, BoP), peri-implant crevicular fluid (PCF), probing depths (PD), attachment level (AL) and keratinized gingiva (KG) are assessed and analysed at week 1, 3 and 6, 9 months after implant insertion. All members of the test group receive Ca-VitD substitution and according to a random assignment Bisphosphonates (Alendronate) or Placebo. Continuous healing tendency is indicated in both groups by decreasing PCF levels. In control group PCF is significantly higher at 1 week than in other samples ( $P = 0.008$ ;  $0.001$ ;  $0.014$ ;  $0.046$ , respectively); same trend is observed in the test group. No significant changes in mean values from month 3 to month 6 are detected for PD, AL and KG in both groups. At 3-month evaluation differences between tests and controls regarding PCF, PD, AL and KG values are statistically none-significant. At 6 months PCF levels correlated with PD in controls ( $P = 0.041$ ), and with KG levels in tests ( $P = 0.01$ ).

35

### Function and patient satisfaction in implant therapy

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**Objective:** The aim of our study was to evaluate the function and patient satisfaction in implant therapy including the surgical steps. **Methods:** In total, 19 patients that received two-step implant therapy with various prosthetic constructions were evaluated by a questionnaire of 26 questions. Out of 26 questions, four were about the comfort, duration, and patient compliance during the surgical phase, 10 questions were related to the patient satisfaction with completed prosthesis, and 12 questions were focused on the situation before implant therapy, comparison between conventional and implant-supported prosthesis. Data was evaluated by chi-square analysis.

**Results:** All patients reported full satisfaction due to implant therapy as the treatment of choice in restoring partial or full edentulism ( $P < 0.05$ ). Restoring function and aesthetics were observed as the main expectancy. No patients reported any peri- and post-surgical pain while 89% of patients reported no discomfort during the surgery. Seven patients had minor post-operative complaints but they reported that these minor issues did not affect their overall satisfaction of the outcome of the therapy. After the completion of prosthetic restorations, 63% of patients felt their implant-supported prosthesis were similar to their natural dentition ( $P < 0.05$ ).

**Conclusion:** The duration of the implant therapy was the major concern and this finding suggests that immediate loading in implant therapy should be chosen when possible.

36

### Incidence of fracture in endosseous osseointegrated implants

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**Introduction:** The fracture of dental implants osseointegrated is an infrequent complication within the dental treatment. In spite of his low prevalence, it is a clinical situation that bears importance for the difficulty of his treatment.

**Objective:** The objective of our work is to quantify the risk of fractures of implants and knowing the prevalence that we can find this complication at the daily clinic with.

**Materials and Methods:** A bibliographic quest in medical seekers like PubMed and ScienceDirect have been realized. Likewise, we have looked up the bibliographic bottoms of Murcia's University.

**Results:** The incidence of the fractures of implants is very low. In the realized meta-analysis we have found inferior values to the 0.5%.

**Discussion:** It proves to be important to differentiate several types of designs, in terms of his mechanical resistance. Finally, the incidence found for solid threaded implants comes from approximately one fracture for each 220 implants.

37

### Influence of implant surface topography on early osseointegration. A histological study in human jaws.

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This study evaluated the influence of anodized surface on bone-to-implant contact (BIC%), the bone density in e threads area (BA%) as well as bone density outside of the threads area (BD%) in human jaws after 2 months of unloaded healing. Thirteen subjects (mean age  $42.61 \pm 6.15$  years) received two micro-implants (2.5 mm in diameter and 6 mm in length) each during conventional implant surgery either in mandible or maxilla. The micro-implants with commercially pure titanium surfaces (machined) and anodized surfaces served as control and test surfaces, respectively. After 2 months, the micro-implants and the surrounding tissue were removed and prepared for histomorphometric analysis. All micro-implants except for two machined and one oxidized micro-implant surfaces were found to be clinically stable after healing period. Histometric evaluation indicated that the mean of BIC% was  $21.71 \pm 13.11\%$  and  $39.04 \pm 15.75\%$  to machined and oxidized micro-implant surfaces ( $P < 0.05$ ), respectively. The BD% was higher for the oxidized surface, although there was no difference between maxilla and mandible ( $P = 0.99$ ). The anodized surface presented influence on BA% in the type IV bone. Data suggest that the anodized surface presented a higher percentage of bone-to-implant contact when compared to machined surfaces under unloaded conditions after a healing period of 2 months.

38

### Tilted implants. A 5-year retrospective clinical, radiological and periotest study

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**Background:** Tilted implants have been described as alternative to the sinus elevation in patients with severe atrophy of the posterior maxillae. However, few studies provide long-term controlled data on the behaviour of those implants.

**Objective:** To report the long-term clinical outcome of tilted implants compared to axial implants in different oral situations and locations.

**Material and methods:** A total of 161 patients were rehabilitated with 172-implant-supported prosthesis. A total of 828 Branemark System® implants were placed. Out of them, 320 were tilted implants and 508 were placed axially to the occlusal plane. The average follow-up period was 78 months. The radiographic controls included one initial radiograph at the moment of prostheses connection and a final control radiograph. The marginal bone loss between both radiographs was measured. Implant stability was evaluated using the Periotest® device.

**Results:** The 5-year CSR was 100% and 98% for tilted and axial implants, respectively. The CSR of the prosthesis was 100%. The mean annual marginal bone loss was 0.22 mm and 0.18 mm for tilted and axial implants respectively. No significant differences between both groups were found.

**Conclusions:** Within the limits of this sample it is concluded that the tilted implant is a predictable technique.

39

### Zigomatic implants: a new surgical approach

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**Background:** Current literature describes zygoma implant as intrasinus surgical approach, ideally maintaining the sinus membrane intact while gaining access to the zygomatic bone. An anatomical limitation of the technique comes from the presence of a pronounced concavity of the anterior maxillary wall. This forces to move the implant head from the crest to a more palatal position. The aim of this study was to report our experience with zygoma implants utilizing a new surgical approach.

**Materials and methods:** Twenty consecutive patients showing extreme concavities of the maxillary anterior wall were treated with 87 regular and 41 zygoma implants inserted with an extra-sinusal surgical approach that allows the placement of the implant head close to the residual crest. Implants were followed for 6 to 18 months after occlusal loading, mean follow up of 12 months. Inclusion criteria were the same used for zygoma implant placement and the presence of an anterior maxillary concavity precluding the placement of the implant head on the palatal bone closer to 10 mm to the centre of the residual bone crest.

**Results:** CSR of zygoma and regular implants was of 100%. No pain, discomfort or complications related to the extrasinusal path were recorded.

**Conclusions:** Within the confines of this study, the extra-sinusal surgical approach represents a predictable alternative to the intra sinus approach for the rehabilitation of the atrophic edentulous maxilla.

40

### Influence of the diagnostic value of 3D cone-beam tomograms

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Cone beam volume tomograms are more and more accepted for oral diagnosis. A total of 38 digital volume scans were evaluated by five investigators with the score between 1 and 5 for diagnostic findings of anatomical structures. Structures evaluated were canalis mandibularis, foramen mentale, foramen incisivum, nasal and sinus floor and TMJ. The age, the body-mass-index (BMI), amount of metal fillings, superstructures and implants were documented for each patient. The scans were performed by a new 3d radiological device (Sirona, Bensheim, Germany) with a voltage of 90 kV and an energy dose of 28 mAs. BMI and amount of metal material in the oral cavity have no significant influence on visualization of evaluated anatomical structures. A stronger correlation could be

found for the age. The median for all anatomic structures were very good (1) or good (2). The three-dimensional imaging is an appropriate imaging technique even for elder patients to evaluate anatomic structures for implant prosthetic planning.

41

### Peri-implant condition in toothless patients carrying mandibular implant overdentures on bar anchorages – ITI system

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**Aim:** Analyse advantages of using two ITI implants with bar anchorages for retention of inferior overdentures and importance of motivating and instructing patient in oral hygiene. Use plaque index Silness & Loe and calculus index, modified for this treatment. Monitoring 10 years 50 patients treated.

**Material and Methods:** Fifty patients; 28 women, 22 men; 50–82 years, 100 implant, position 32–33 and 42–43. Was evaluated: stability of prosthesis, strength distribution, maintenance of biological support, implant splint, and different index of oral hygiene.

**Results:** Stabilization, implant splint, fixation of prosthesis against horizontal translation forces, was obtained. 22.5% of subjects showed plaque score 0, result of oral hygiene instructions. 90% don't exhibited sulcus bleeding indicating health of peri-implant soft tissues. One patient shown plaque and subgingival calculus, oedema, reddening, bleeding and radiographical bone loss not exceed 2 mm.

**Conclusion:** In patients followed 10 years all conditions of retention, stability and function take place with smaller number of implants that necessary for fixed implant rehabilitation. This is the best treatment when high degree of bony atrophy appears and is impossible to place implants in back sector. In cases of poor hygiene, presence of plaque and calculus on abutments and on bar causes bone loss around implants, being able to cause the failure of this technique.

42

### Immediate occlusal loading of implants in 24 consecutive full-arch cases in the maxilla: 6- to 38-month results

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**Purpose:** The purpose of this study is to evaluate prospectively survival and success rates of implants placed in the complete edentulous maxilla and immediately loaded with a full-arch restoration.

**Materials and methods:** Four male and 20 female patients, aged between 48 and 81 years (mean age 65.7 year), were treated with 289 implants placed in edentulous maxillary bone or extraction sites (mean 12.04 implants per patient). Seventy-two hours after implant placement, a metal-reinforced provisional prosthesis was fabricated and 266 implants were rigidly connected and immediately loaded. Twenty-three implants, which had unsatisfactory primary stability, were maintained unloaded. Success rate of implants was evaluated clinically and radiographically.

**Results:** A total of 289 implants were followed up from a minimum of 6 months to a maximum of 38 months. Of 289 implants placed, 23 implants were not immediately loaded. Three of these unloaded implants in two different patients were removed owing to loss of osseointegration. Of 266 immediately loaded implants, 10 implants, although still osseointegrated, did not fulfil success criteria. Therefore, the absolute success and survival rates were 95.5%

and 98.9%, respectively. Concerning immediate loaded implants, success and survival rates were 96.2% and 100% respectively.

**Conclusion:** A high success rate can be achieved when screw-type osseointegrated implants are immediately loaded with fixed full-arch restorations in the maxilla.

43

### **Papillae height and its relation with osseous crest in single unit rehabilitations with oral implants. A retrospective study**

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**Background:** The presence of the papilla can determine the success of the treatment. Parameters such as the distance from the contact point to the crestal bone have been related with the presence of papillae. Yet, in between presence or absence, several anatomical levels of papillae can be found. The aim of this study is to correlate the different levels of papillae, in the single unit implant rehabilitation, with bone related parameters.

**Materials and methods:** A systematic selection of 259 clinical histories, including at least one single unit implant restoration, was performed. Out of them, 59 patients and 64 implants complied with the following inclusion criteria: single unit implant restoration, the crown having uni- or bi-lateral contact areas, and patient acceptance for participation in this study. The following data were retrieved: implant location; abutment type; oral radiographs; distance of implant-abutment junction (IAJ) to marginal bone crest and to the contact point; distance of the marginal bone crest to contact point; horizontal distance from the implant to the adjacent tooth at IAJ level. Papillae were classified following the Jemt index.

**Results:** Type 2 papillae were present in 45%, followed by type 3 in 32%. Mean distances from contact point to osseous crest in type 2 and 3 papillae were 9 and 6 mm respectively.

**Conclusions:** Within the limits of this study, the results do not show a direct relationship between marginal bone level and papillae height.

44

### **Survival rates of implants with a rough surface geometry in the posterior maxilla and mandible**

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**Introduction:** The predictability of endosseous implants for prosthetic restoration of missing teeth has been well documented. High rates of clinical success have been reported with implants placed in both the maxilla and mandible. However, the survival rate of short implants has been shown to be lower than that of standard size implants. ( $\geq 10$  mm in length). Implants with rough surfaces have been shown to have a high survival rate when placed in various areas of the mandible and maxilla. The purpose of this retrospective study was to determine the survival rate of short implants ( $< 10$  mm in length) with different rough surfaces in the posterior maxilla and mandible.

**Material and methods:** The Department of Periodontology and Implant Dentistry at the New York University Kriscer Dental Center database of rough surfaced implants has been kept up to date by forms reporting implant status at stage I, II and post-loading. Analysing this database we were able to report on 223 short implants placed with a total of six failures.

**Results:** The overall survival rate was of 97.8%.

**Conclusion:** The results of the present research indicate that short implants with a rough surface geometry show survival rates comparable to implants of standard lengths.

45

### **Peri-implant health on epi-crestal placed implants with grid-blasted and low or high temperature etched surfaces**

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Modern implant surfaces show a high quality of osseointegration with a high bone to implant contact. The formation of the crestal implant design is under research to maintain the peri-implant bone and soft tissue. This study compares the crestal bone level on implants with grid blasting surface and two types of etching procedures in hip grafting and radiation patients. The high temperature etched surface shows a much deeper micro-morphology than the low temperature etched surface. The roughness of both surfaces is about  $R_a = 2.6 \mu m$ . Two groups of 25 patients each received a total of 221 implants. A total of 112 implants were placed with the low temperature etched surface with a smooth collar of 1.1 mm and 109 implants were placed with high temperature etched surface with a smooth collar of 0.4 mm. The radiological bone level after 1 year of prosthetic loading was 2.1 mm below the implant shoulder in the low temperature etched group and 0.9 mm in the high temperature etched group. The prevalence of peri-implantitis with bleeding on probing was low with 4.5% of the implants in both groups. These results demonstrate that the reduced machined collar and the solely high temperature etched implant collar stabilize the peri-implant bone formation. Even in cases with higher bone loss the high temperature etched surface showed no increased signs of peri-implantitis or plaque accumulation on the micro-structured surface.

46

### **Would hazards of unloaded and loaded implants differ?**

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**Background and Aims:** Implant life times are commonly stated as time since placement of either the implant or the prosthetic superstructure. Because of its relative shortness in longer follow-up, the functionally unloaded – pre-prosthetic – phase is typically not analysed separately. Pre- and post-prosthetic risks of implant loss and associated risk factors are compared in the Dusseldorf Implant Registry Cohort Studies (DIR-CS).

**Material and methods:** In three cohorts of single-tooth replacement/abutment supplement ( $n = 278$ ), partial edentulism ( $n = 388$ ), and edentulous maxilla/mandible ( $n = 214$ ), patients had been followed for up to 8 years. Risk factors including patient and implant characteristics were assessed with Cox regression for life tables.

**Results:** Annual loss rates in these cohorts were 20.1%, 13.6%, and 19.4%, respectively, for unloaded, and 3.5%, 1.6%, and 2.5%, respectively, for loaded implants. All risk factors, even the well-known higher loss rate of ceramic implants, were demonstrated to depend on whether implants were functionally loaded or not.

**Conclusion:** By their distinct magnitudes of risk and their different risk factors, implant service times before and after placement of prosthesis deserve separate analyses more systematically.

47

### **Can melatonin and growth hormone reverse the effects of aging in bone?**

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**Purpose:** The aim of this study was to investigate the effect of Melatonin and GH on Bone metabolism and density in rats.

**Material and Methods:** Forty female rats were used: eight young and 32 old. The young and eight old were used as control. The remaining

was randomly divided into three groups: eight treated with melatonin (1 mg/kg/day), eight with GH (2 mg/kg/day), and eight with Melatonin plus GH. Rats were killed and blood for an assay of osteocalcin was collected and Tibiae dissected. Osteocalcin levels, morphometric and densitometric parameters mean  $\pm$  SD of each group were calculated. The groups were tested by the analysis of variance (ANOVA) elaborated with the Statgraphics 5.0 statistical software package. Significance for the analysis was set for  $P < 0.05$ .

**Results:** The plasmatic Osteocalcin levels were increased in the GH (267 ng/ml), melatonin (186 ng/ml) and GH-Melatonin Groups (205 ng/ml) when compared with the old control; group (80 ng/ml), with statistically significant differences. The morphometric evaluation showed statistically significant more bone area in the melatonin (5.97 mm<sup>2</sup>), Gh (6.23 mm<sup>2</sup>) and melatonin-Gh groups (5.8 mm<sup>2</sup>) when compared with the old control group (4.4 mm<sup>2</sup>). The densitometric analysis showed a statistically significant more BMD in melatonin (0.136 g/cm<sup>2</sup>), GH (0.14 g/cm<sup>2</sup>), and melatonin-gh groups (0.135 g/cm<sup>2</sup>) when compared with the old control group (0.125 g/cm<sup>2</sup>).

**Conclusion:** GH and Melatonin seem to exert beneficial effects against age-induced changes in bone mass, metabolism and density in rats.

48

### Clinical and radiographic analysis of SLA ITI dental implants placed in the posterior maxilla with the osteotome technique. A prospective study

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Maxillary sinus floor elevation using osteotomes has been successfully used for implant placement when a limited vertical height is available at posterior maxilla.

**Objectives:** (i) To evaluate the clinical outcomes of SLA ITI dental implants placed in the posterior maxilla using the osteotome technique; (ii) to radiographically assess the vertical displacement of the maxillary sinus floor and long-term changes in sinus graft height with this technique.

**Material and methods:** Twenty-two patients with a total placement of 54 implants. Sinus floor elevation was performed with bone chips and tricalcium phosphate as bone fill material. Presurgically and postsurgically at 3 and 12 months digital panoramic radiographies were analysed and clinical outcomes assessed. Implant stability assessment using resonance frequency analysis was recorded at baseline and 3 months.

**Results:** The success rate was 94.2%. The mean preoperative distance between the sinus floor and the crest was 6.4 mm. The mean vertical bone gain assessed radiographically at the centre of the implant was 4.1 mm at surgery but was reduced significantly ( $P < 0.05$ ) to 3.6 mm at 3 months. Implant stability assessment at surgery ranged between 28 and 77 ISQ with a mean value of 62.2 ISQ at 3 months.

**Conclusions:** The osteotome technique provides a way to increase the amount of available bone but the grafted area apical to the implants undergoes remodelling and sinus graft height decreases significantly at 12 months.

49

### Tissue engineering for tooth replacement : preliminary results of a clinical trial with a tissue engineered periodontal ligament covered implant.

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The periodontal ligament (PDL) exerts strong regenerative capacity and could remove clinical limitations of current solution for

the replacement of lost teeth. This Tissue Engineering (TE) solution consists of PDL cells growing around a titanium pin. This ligament will liaise the pin to the jawbone, mimicking natural PDL. Here we report for the first time the preliminary results of a clinical trial in humans of such an implant, so called-ligaplant. PDL cells were extracted and amplified *in vitro*. Bioactivated titanium conical pins were seeded with autologous cells, cultured for eight weeks, and implanted into corresponding patients. Periodontal regeneration was followed by clinical and radiological evaluation. Three of nine ligaplants were present and functional after 22 months. Mechanical tests by twisting and pulling revealed a maximal coronal mobility of ligaplant less than 1 mm above that of the control teeth, and no ankylosis. The felt resistance could be discussed as a ligament connection to the bone. Radiological evaluation revealed bone reformation. Around a ligaplant persisted a radio translucent space showing the presence of a non-mineralized layer. This structure resembling the PDL around a natural root might be formed by the tissue engineered ligament. This observation was in agreement with the mechanical tests. Our study shows that TE PDL covered implants could be an alternative to osseointegration, and would ease tooth replacement in defective or growing bone.

50

### The use of narrow neck implants in the replacement of single or multiple missing mandibular incisors: functional and aesthetic outcomes.

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There are no studies that focus on mid or long-term results of mandibular incisors replacement with narrow diameter implants in partially edentulous patients. The authors have retrospectively evaluated 31 patients treated for single or multiple lower incisor replacement with narrow neck implants with a mean follow-up of 23 months. The patients were divided in three groups: single tooth (ST), multiple unit restoration (MU) and restorations on adjacent implants (AI). Survival and success rates and soft tissue parameters such as modified Plaque Index (mPI), Peri-implant Probing Depth (PPD), Bleeding on Probing (BOP) and the Papilla Index were analysed. Patients and clinicians on Visual Analogue Scales performed subjective evaluation. The implants and prostheses showed a survival rate of 100% and an overall success rate of 94%. The distribution of mPI index outcomes showed better results for the ST group. The AI group showed a statistically significant increase in PPD and in BOP index. The Papilla Index showed a better outcome distribution in ST and MU group. The professional subjective evaluation showed good outcomes for the ST and MU groups and significantly poorer results in the AI group. It may be concluded that the replacement of lower incisors with narrow neck implants leads to favourable functional and aesthetic results in cases of single-tooth or multiple-unit replacement. Worse results are achieved if two adjacent mandibular incisors are replaced with adjacent implants.

51

### Aesthetic outcome of immediate vs. delayed anterior implant placement associated with immediate temporization

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The aim of our prospective study was to compare gingival aspect (papilla and buccal contour) and peri-implant bone remodelling of immediate vs. delayed anterior maxillary implant placement. Sixty-nine Screw-Vent implants (Zimmer dental) were placed in the

anterior region in 57 patients. Thirty-four implants (group A) with immediate placement and 35 implants (group B) with delayed placement (3 months). A temporary crown was immediately placed in all cases. Clinical evaluation and radiographic control (post-op., 1, 3, 6, 12 months) enabled us to analyse gingival variation and bone modification. Implant survival rate at 1 year was similar ( $A = 97.05$ ;  $B = 97.14$ ). Despite a progressive alveolar bone loss in the delayed placement compared to the immediate uncontrolled bone resorption, interproximal bone loss was similar after 1 year ( $A = 0.67$  mm,  $B = 0.7$  mm). The overlying papilla preservation was slightly better in the immediate placement (80% vs. 60%). Regarding the buccal aspect, immediate placement showed more contour modification during the first 6 months, as a result of tissue remodelling. In the delayed placement, a better aesthetic buccal contour was obtained due to the possibility of soft and hard tissue management during implant surgery. In conclusion, despite the multiple advantages of immediate placement (papilla preservation, time saving, patient comfort), tissue variation in immediate placement is less predictable, without a second chance to enhance the aesthetic result by tissue management.

52

### Immediate loading in total edentulous patients: 2-year prospective clinical study

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**Background/Aim:** In edentulous patients, the literature supports immediate placement and immediate load in the mandible/maxilla using cross-arch stabilization of the implants and a fixed passive-fitting prosthesis on multiple implants having verifiable primary stabilization upon placement. The aim of this study was to evaluate the implant and prostheses survival for immediate loading total edentulous patient.

**Materials and Methods:** In 23 consecutive patients, 12 females and 11 males, between 39 and 74 years old, four smokers and 19 non-smokers, were placed 168 implants (83 maximum and 85 mand.) to support 26 full immediate fixed provisional prostheses. Only 159 of the 168 presented ISQm values  $\geq 70$  and these were loaded at least in the first 48 h after implant placement. All 23 patients were rehabilitated with ceramic fused-metal fixed prostheses 4 month after implants placement. Clinical/radiographic parameters were evaluated at 6, 12, 18 and 24 months after implant loading.

**Results:** Two of the 159 immediate loaded implants did not osseointegrate (98.74% implant success rate). The cumulative success rate was 100% for the 26 provisional and definitive prostheses. No other implant was lost during the 2-year follow-up period. 100% prostheses success rate at 2-year follow-up.

**Discussion/Conclusion:** Immediate loading in total edentulous patients seems, for the 26 clinical cases of the study, a predictable technique with 98.74% implant success rate and 100% prosthetic success rate.

53

### Infrared laser-assisted peri-implantitis treatment. Clinical and radiological experience on 27 cases

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The incidence of peri-implantitis is relatively low (Klinge et al., 2002). However, deep peri-implant bony defects may negatively influence the implant prognosis. The main problem in the treatment is the optimal decontamination of the implant surface before augmentation of the defect. The aim of these case series was to show the long-term prognosis of failing implants, which were decontaminated with a laser immediately before augmentation.

Based on the previous experience using thermocouples, microbiological as well as morphological studies of implant surfaces after the laser decontamination we were able to successfully treat 27 peri-implant defects and to improve the implant prognosis without the changing of the implant restoration in cases of restored implants. Nineteen defects were decontaminated with the CO<sub>2</sub> laser and eight defects were treated with the diode (980 nm) laser using power settings of 2 Watt in a cont. wave and defocused beam for implant decontamination. The augmentation was performed with autogenous bone or BioOss<sup>®</sup> and a Biogide<sup>®</sup> collagen membrane coverage. Based on the radiological evaluation after at least 2 years post augmentation we showed a bone gain independent on the used laser or the implant surface. In some cases, with the use of autogenous bone grafts some resorption of the augmentation material was observed. The data presented in this report showed that these infrared lasers might be useful for the efficient decontamination of deep peri-implant bony defects.

54

### Histomorphometric study of bone density in augmented alveolar ridges

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**Objective:** Measurement of the bone density is an appropriate method for evaluation of the load bearing capacity of the bony bed prior to implant insertion. Sinus floor has an excellent bone forming capacity compared with the destructured alveolar crest.

**Methods:** Vertical augmentation (VA) of the alveolar ridge defect was surgically performed by Bio-Oss grafting combined with guided tissue regeneration in 20 cases. In 10 of them, sinus floor elevation (SFE) was simultaneously performed. After 6–8 months, tissue samples were taken from the grafted area and implants were inserted. Histomorphometric measurements were performed on decalcified, paraffin embedded sections by a computerized technique. Densities of the newly formed bone and of the bone substitute material were determined as a percentage of the total biopsy area.

**Results:** In the alveolar ridges new bone and graft densities were measured both in the group of patients with AV, and in the cases with combined AV and SFE surgery. The mean bone density of the 10 AV cases was lower ( $18.4 \pm 3.6\%$ ) compared with that of the AV + SE group ( $22.3 \pm 5.4\%$ ). Graft densities were similar in the two groups;  $28.8 \pm 10.1$  and  $29.6 \pm 13.2\%$ , respectively.

**Conclusions:** Bone density of the Bio-Oss grafted alveolar ridge can be enhanced by simultaneous sinus floor elevation.

55

### Regenerative therapy of peri-implantitis intrabony defects

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The aim of the present clinical study was to evaluate healing of peri-implantitis intrabony defects following application of a nanocrystalline hydroxyapatite (Ostim) or a bovine-derived xenograft combined with a collagen membrane (BDX + BG). Twenty-four patients suffering from advanced peri-implantitis ( $n = 24$  defects), were randomly treated with (i) access flap surgery (AFS) and the application of Ostim ( $n = 12$  implants – test), or with AFS and the application of BDX + BG ( $n = 12$  implants – control). The bone defects and implant surfaces were cleaned with plastic curets + cotton pellets soaked in saline. Clinical parameters (probing depth – PD, clinical attachment level – CAL) were recorded at baseline and after 6 months of non-submerged healing.

Postoperative wound healing revealed that Ostim seemed to compromise initial adhesion of the mucoperiosteal flaps in all patients. At 6 months after therapy, the test group showed a reduction in mean PD from  $7.2 \pm 0.8$  to  $5.0 \pm 0.8$  mm and a change in mean CAL from  $7.7 \pm 1.1$  to  $5.8 \pm 1.1$  mm. In the control group, the mean PD was reduced from  $7.1 \pm 0.8$  to  $4.6 \pm 0.7$  mm and the mean CAL changed from  $7.5 \pm 1.0$  to  $5.2 \pm 0.7$  mm. The differences between both groups were statistically non-significant. Within the limits of the present study, it can be concluded that at 6 months after surgery both therapies resulted in statistically significant PD reductions and CAL gains. However, postoperative wound healing seemed to be ameliorated following application of BDX + BG.

56

### Full arch immediate functional loading of maxilla implants with cemented prosthesis in post-extractive sites. A prospective clinical study

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Aim of this study is to present a protocol for complete rehabilitation of the upper jaw, using immediately loaded post-extractive implants and analyse the outcomes at 18 months. Passed the entry criteria, patients underwent a diagnostic process. Once diagnosis was established and decision to remove all natural teeth was taken, feasibility of full arch-rehabilitation with implant and cemented prosthesis was assessed. At the time of surgery measurements were recorded: number, length, diameter of implants; number of post-extractive implants, gap between bone crest and implant, torque, number of immediately loaded implants. Follow up measurements were registered: FMPS, FMBS, PD and BOP (six measures per implant), implant failure, implant suffering, aesthetic evaluation throughout time. The same operator treated eight patients; 67 implants were placed (Xive-Friadent<sup>®</sup>); 51 implants were immediately loaded with a provisional arch (torque  $\geq 35$  N/cm<sup>2</sup>) and 44 were placed in post-extractive sites. Six months later a metal-ceramic prosthesis was done. Average implant length was  $11.84 \pm 1.18$ ; average diameter was  $4.31 \pm 0.57$ . Eighteen months after surgery, patients were re-evaluated and showed an average PD of  $2.77 \pm 0.62$ ; BOP was 14.28%; survival rate was 100%; implants suffering rate was 1.49%. Papilla presence rate between post-extractive implants was 78.23%. On this data immediate functional loading is a reliable surgical-prosthetic procedure with a good predictability of aesthetic maintenance in multiple post-extractive implants.

57

### Analysis of early failures of two-piece non-submerged implants

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**Background:** Non-submerged placement of two-piece implants is well documented as a predictable and efficacious treatment, but its use in a daily practice is not very common.

**Aim:** The aim of the study was to evaluate the success rate of non-submerged two-piece implants prior to prosthetic loading.

**Materials and methods:** A total of 421 two-piece Biohorizons (Birmingham, Alabama, USA) implants were installed in 189 patients, 70 male and 119 female, partially dentate and edentulous. Immediately after implant placement-healing abutments were connected and peri-implant mucosa was sutured. In the maxilla 197 implants were placed, while mandible received 224 implants. Seventy-five implants were placed in anterior segment (from canine

to canine) and 346 in the posterior regions. After 3 months in the mandible and 5 months in the maxilla, osseointegration was evaluated clinically and radiographically for the possible early failures prior to prosthetic loading.

**Results:** From all 421 implants placed, 12 were lost as early failures comprising 2.85%. Implant failure rate in the mandible was 1.78% (four implants lost from 224) while in maxilla it was 4.06% (eight from 197). Anterior segments had 5.33 % of failures (three from 75), while posterior regions exhibited 2.6% implant loss (nine from 346).

**Discussion and conclusion:** With the limitations of this retrospective study it can be concluded, that high success rate of non-submerged two-piece implant placement can be achieved.

58

### Diabetes and dental implants: a comprehensive and critical review of the literature

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**Background:** A comprehensive review of implant placement in diabetic subjects has not been published.

**Aim:** The aim of the present study was to perform a comprehensive and critical review of experimental and clinical studies, regarding implant placement in diabetic subjects and to draw evidence-based conclusions on its effectiveness and predictability.

**Material and methods:** Search for articles up to and including March 2005 was performed by using The National Library of Medicine and Cochrane databases. The references of identified articles, workshops and hand-searched journals were also used. At the first phase of selection the titles and abstracts and at the second phase full papers were screened independently by three reviewers (S.K., I.K.K., I.F.). Any disagreement was resolved by discussion.

**Results:** The search provided 227 titles and abstracts. After the first phase of evaluation, 28 papers, including 11 experimental studies and 16 clinical studies (one clinical study corresponded to two papers), were selected. After the second phase, 11 experimental and eight clinical studies were accepted.

**Conclusion:** Experimental studies seem to reveal an impaired bone healing response to implant placement in diabetic animals compared to non-diabetic controls. Most clinical studies indicate that controlled diabetes is no contraindication for implant placement. However, guidelines with objective criteria (e.g. type, duration of diabetes, glucosylated haemoglobin levels) need to be established.

59

### Transtomography for implant placement guidance in non invasive surgical procedures: presentation of surgical protocol, interest and applications

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Transtomography use X-rays narrow beam and a digital detector to perform tomograms. Image appears instantly. So intraoperative tomograms may be done for implant placement guidance during non invasive surgical procedures. The aims of the report is to present this new surgical protocol, its interest and results of experimentation. Clinical cases illustrate applications of the technique.

**Materials and methods:** Cross-sectional and longitudinal transtomogram is done after a first pilot drill through the mucosa and the bone as planned preoperatively (first pilot drill is 2 mm section and shorter than the distance to critical zones). A radiographic reference guide (2 mm section) is then inserted in the

bone. This titanium guide closes all the drilled zone during radiographic control. Patients move a few meters from the surgery table to the panoramic machine installed in the surgery room. The guide is securely sutured to the adjacent tooth or to the surrounding mucosa.

**Results:** Position of the guide on the tomographic images allow to control the planned surgical drilling trajectory in the three dimensions. The axis of the drill can be corrected accordingly when needed. Full pilot drill and enlargement are then performed.

**Conclusion:** Intraoperative transtomograms during noninvasive surgeries may minimize the risk of damaging critical anatomic structures and bone wall perforation. This protocol enables to extend noninvasive techniques to patients with a narrow alveolar bone ridge.

60

### The retrograde peri-implantitis: about five clinical cases and review of the literature

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The use of dental implants as an integral part of rehabilitation therapy increased in recent years. Long-term studies have confirmed their predictability in restoring partially and completely edentulous patients. The failure of osseointegrated dental implants primarily occurs as a result of peri-implantitis and occlusal overload. Even if less frequently, reported failures had been attributed to retrograde peri-implantitis or implant periapical lesion. The aetiology and management of such lesion have been speculated throughout the literature. Five cases reports under diagnosis of retrograde peri-implantitis are presented with a successful treatment: apicoectomy of the implant. This communication discusses possible aetiologies and treatment for retrograde peri-implantitis based on the existing literature and five cases reports.

61

### Sinus floor elevation using osteotomes: a systematic review and meta-analysis

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**Background:** Various techniques of sinus floor elevation (SFE) are described. The elevation with osteotomes (OSFE) from a crestal approach is a relatively new technique. The aim of this systematic review and meta-analysis was to evaluate the clinical outcome of implants placed with an OSFE technique.

**Methods:** A systematic online and manual review of the literature identified articles dealing with OSFE. Applying rigid inclusion criteria, screening and data abstraction were performed independently by two reviewers. The follow-up of loaded implants had to be at least 6 months. The identified articles were analysed in regards to implant outcome and defined surgical aspects. Survival and success rates were estimated by Kaplan-Meier curves.

**Results:** Eight out of 44 articles dealing with OSFE met the inclusion criteria. Five of the eight selected studies met established success criteria. The survival and success rates were 95.7% and 96.0% after 24 and 36 months, respectively. The median and mean follow-up periods were 24 and 18.73 months for the survival rate and 24 and 19.7 months for the success rate. Due to a multivariate database, no statistical analysis could be performed on surgical parameters.

**Conclusions:** Short-term clinical success/survival (up to 3 years) of implants placed with an OSFE technique seems to be similar to

that of implants conventionally placed in the partially edentulous maxilla. Controlled prospective clinical long term studies are needed.

62

### Immediate provisionalization of maxillary single premolars: a 2-year prospective study

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**Purpose:** This 2-year prospective study evaluated the implant success rate and marginal bone changes of immediately provisionalized maxillary premolar single implants in edentulous ridges.

**Materials and Methods:** Ten patients (three men, seven women) with a mean age of 51 years (range 40–82) were included in this study. Ten threaded and tapered implants with a porous surface were placed and provisionalized immediately with a temporary screw-retained acrylic resin crown. The definitive restoration was placed 6 months later. The patients were evaluated clinically and radiographically at implant placement and at 6, 12, 18 and 24 months after implant placement.

**Results:** At 2 years, all implants remained osseointegrated. The mean marginal bone change from the time of implant placement to 24 months was  $-1.01$  mm and presented a statistically significant difference (ANOVA,  $p < 0.05$ ).

**Discussion:** Although marginal changes were statistically significant from implant placement to 2 years of follow-up, they were well within clinical expectations and appeared to be similar to findings of previous studies regarding the delayed loading protocol.

**Conclusion:** The results of this study suggest that favourable implant success rates and marginal bone changes can be achieved with immediately provisionalized maxillary premolar single implants in edentulous ridges.

63

### Long-term retrospective analysis of marginal bone loss and implant failure of MIS external HEX implants

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**Objectives:** The aim of the study was to retrospectively evaluate bone loss and success rate of external hex implants (MIS) and to construct a multivariate correlation model between implant variables (time, smoking habits, fixed/removable prosthesis, implant dimensions) and bone loss.

**Methods:** Patients that had completed at least 30 months of follow-up were recalled for clinical and radiographic examination. The radiographic bone loss was measured, and used to calculate the actual bone loss.

**Results:** Thirty-four out of 52 patients were available, with total of 74 implants. Follow-up time range was 30–84 months (mean 45.8). Two patients were smokers, and in seven patients the procedure involved bone augmentation. Two implants failed at the first healing phase (3% early loss), and none were lost through time of function, exhibiting an overall success rate of 97%. The average number of exposed threads was found to be  $1.4 \pm 0.93$  (0–3.5) and the average radiographic bone loss was  $2.35 \pm 0.87$  mm (0.7–4.4). No correlation was found between the time of service and crestal bone loss. From the all tested variables, only removable prosthesis was found to be positively correlate with bone loss ( $P = 0.002$ ).

**Conclusions:** The results of the present study confirm that MIS external hex implants exhibited an overall success rate of 97% in a long-term follow-up period. Except for the prosthesis type, none of the other suspected variables were found to influence crestal bone loss.

**Acknowledgement:** Supported by MIS Implants.

64

### Crest expander-the use of distraction osteogenesis as an alternative technique to bone augmentation

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**Aim:** To introduce a new device and technique to widen a narrow alveolar crest prior to implantation.

**Materials and methods:** A new distractor was used to widen a narrow alveolar crest. Under local anaesthesia, three mucoperiosteal incisions were performed. One along the crestal ridge and two vertical incisions at the anterior and posterior buccal aspects. Through these incisions, bone cuts were made without stripping the mucoperiosteum. The bone transport was fractured by an osteotome and the distractor was inserted into the crestal bone cut. Distraction started 7 days post-op and was stopped when adequate width was achieved. Consolidation period was 1 week and then removed under local anaesthesia. Implants inserted at the same session.

**Results:** Study group consisted of 19 patients who suffered from a narrow alveolar crest. Crest widening ranged from 4–6 mm. Distraction periods ranged from 10–16 days. Latency period was 7 days. Total of 57 implants were inserted. No complications were observed. No complications were observed 18–30 months after prosthetic treatment.

**Conclusions:** Crest widening by distraction may be a better alternative to bone augmentation in cases of narrow crest with sufficient height. The advantages of the new technique are: early implant placement, simple procedure, no painful donor site, no post-op bone resorption, the technique can be easily performed in the private office. A long-term and multi center trial is needed.

65

### Prospective evaluation of hard- and soft-tissue remodelling after ridge preservation with and without primary wound closure

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**Background:** A significant problem of ridge preservation procedures is the loss of attached keratinized tissue on the buccal side due to flap advancement when primary closure of the extraction socket is attempted. The present RCT investigates hard-, and soft-tissue changes after ridge preservation using PepGen, DFDBA and ADAPT with and without primary wound closure.

**Materials and methods:** Twenty patients are enrolled in this clinical prospective randomized controlled trial that compares the 'guided membrane exposure' (test) method with the 'flap advancement method' in a split mouth design. The changes of keratinized tissue, bone width and height and postoperative discomfort are evaluated over a period of 6 months.

**Results:** By the time of the presentation approximately 15 patients will have had bilateral ridge preservation. Several cases will be presented and the evidence and applicability of ridge preservation will be explained. The surgical method and the significant difference in postoperative discomfort and swelling will be presented for each site. The initial clinical results will be presented together with obtained histological sections.

**Discussion:** It seems that the 'guided membrane exposure' method has significant advantages in regards to preserving the keratinized tissue and decreasing postoperative discomfort and swelling without having a negative impact on the amount of preserved bone volume.

66

### Stabilizing of the extraction clot to promote alveolar ridge resorption

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The stabilization of the extraction clot is becoming more important to promote ridge preservation after tooth extraction. The aim of this study was to evaluate whether alveolar ridge resorption following tooth extraction could be reduced using absorbable collagen matrix impregnated with gentamicin and sealed with an autogenous soft tissue to stabilize the extraction clot, compared to the natural healing by using a classic extraction technique. 110 extraction sites were including. Three Groups were compared: classic technique (Group A,  $n = 50$ ), atraumatic extraction sealing the socket with autogenous soft tissue graft (Group B,  $n = 33$ ), and atraumatic extraction with gent-coll plug and soft tissue sealing (Group C,  $n = 27$ ). Casts were used to measure the width of the alveolar bone at the extraction area. The clinical measurement before and 3-month after the extraction revealed a loss of bone weight of  $2 \pm 1.48$  mm in Group A, a loss of  $0.34 \pm 0.18$  mm in the group B and a loss of  $0.16 \pm 0.066$  mm in the group C. The bone loss in group B and C was significantly less than that in Group A ( $P < 0.05$ ). After 10 days, all grafts from group C and 20 from group B were vital. Based on the present observations, connective tissue grafts sealing fresh extraction sites significantly reduced ridge resorption following tooth extraction and created an increased width of keratinized tissue and the local application of gentamicin stimulated more vascular in-growth in the granulation tissue beneath the graft.

67

### Preliminary clinical results with zirconium oxide dental implants in humans

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There is extensive literature in prosthetic dentistry showing the optimal biocompatibility, aesthetics and long term results with zirconium oxide. However there is little literature about zirconium oxide in implant dentistry. The aim of this communication is to present the preliminary clinical results of a zirconium oxide dental implant. Also to analyse the initial *in vitro* and animal investigations made with this implant. The zirconium oxide implants used in these *in vitro* and animal investigations, as well as the clinical tests, were specially designed and made for this purpose. The composition, design and surface characteristics will be presented. The preliminary results in the clinical test have shown an optimal gingival health without inflammation or bleeding on probing, no implant mobility, and no pain on percussion. Also the x-rays have shown no loss of bone around the implants. The aesthetic results have been considered very well. The *in vitro* investigations have shown a good biocompatibility of the material, and a good fatigue resistance of the implants. The animal investigations have proven a good osseointegration and no foreign body reaction or encapsulation of this zirconium oxide implant. From all the results of the *in vitro*, animal and clinical tests we can conclude that this zirconium oxide implants are a good alternative to conventional titanium implants, especially in patients with metal allergies or when there is an important aesthetic component.



68

### **Preliminary results on the syncrystallization technique for fixed provisionalization of immediately loaded implants: a twelve months follow-up**

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**Aim:** The objective of this article was to evaluate a prosthetic concept for an accelerated rigid splinting of multiple implants for same-day immediate loading with metal-reinforced provisional restorations using a novel technique of welding temporary implant abutments with a pre-fabricated titanium bar directly in the oral cavity (Syncrystallization).

**Materials and Methods:** Immediate loading of threaded implants with a metal-reinforced, acrylic resin provisional restoration at stage one surgery was evaluated in 40 consecutive patients. A total of 192 implants were placed in selected edentulous or partially edentulous patients using the Syncrystallization technique. Once the titanium bar was welded intraorally to the abutments, opaque was applied and the provisional restoration was relined and screw-retained the same day. In addition, a comparison of deformations and stress distributions in implant-supported, metal-reinforced and non-metal-reinforced resin provisional restorations were analysed in the edentulous mandible by a three-dimensional finite element model (FEM).

**Results:** All of the 192 rigidly temporized immediately loaded implants osseointegrated. An implant success rate of 100% was achieved over a period of 6 months post placement. Compared to mere acrylic superstructures, a significant reduction of deformation and strain within metal-reinforced provisional restorations was detected by FEM analysis.

69

### **Using a space maintaining biodegradable membrane for guided bone regeneration – a novel method for ridge augmentation**

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Reconstruction of the deficient alveolar ridge requires using a space-maintaining barrier to promote osseous regeneration. Guided bone regeneration using a space-maintaining E-PTFE membrane had been successfully incorporated into the dental practice with predictable long-term results. One of the disadvantages reported with using this membrane is premature exposure followed by infection and graft resorption. A new biodegradable PLGA/TMC copolymer membrane with a space maintaining physical properties and a lasting barrier function of 12 weeks is reported. Fifteen patients requiring lateral and vertical bone regeneration were treated with the new membrane. Exposure of the treated sites 3–6 months post operatively revealed new bone formation in the regenerated defects as shown in histological bone cores taken. One-year follow-up of the regenerated sites with dental implant placement showed no signs of crestal bone resorption. Within the limits of this study it can be concluded that using a biodegradable space maintaining membrane should be considered as an alternative treatment option to the nonresorbable barriers.

70

### **A 5-year life table analysis from a prospective study on the treatment of edentulism in the posterior region by short (6–8 mm) SLA ITI implants**

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*Private Practice*

The aim of this prospective study is to report a private practice experience on the use, as an alternative to advanced surgical

techniques, of short (6–8 mm) SLA ITI implants placed in the posterior region and loaded for at least 1 year. Starting from January 2000, 36.6 mm, and 76.8 mm implants were placed in the molar and premolar area in 68 patients, following a standard surgical procedure. The purpose was to replace single or multiple missing teeth. The prosthetic procedures started after 6 weeks and the functional load with acrylic prosthesis was applied after about 8 weeks. After at least 3 months of progressive load, metal-ceramic crowns or bridges were placed on implants. Six millimetre implants were never used for single replacement and were always connected to longer implants (8 mm or more). When two or three implants were placed in the same area, a rigid prosthetic connection was always used, as well during the period of progressive load. Implant-tooth connection and cantilevers were avoided. Bone level was recorded, through peri-apical radiographs, at the time of surgery, before prosthetic load and after every year. At the present no implants were lost; two implants (4.1 × 8 mm) don't satisfy Albrektsson criteria, with a cumulative success rate of 98.2% (100% for 6 mm and 94.7% for 8 mm). The present study shows that, within the condition described, the use of short SLA ITI implants in the treatment of posterior edentulism is successful in a medium-term period.

71

### **Histomorphometric analysis of bone formation after maxillary sinus floor augmentation using a ground cortical bone allograft**

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**Background:** Grafting of the maxillary sinus floor has become a common procedure for increasing alveolar bone height. Demineralized freeze-dried bone allograft (DFDBA) has limited effectiveness for such procedures. (AO sinus graft consensus conference 1996).

**Aims:** To evaluate clinically, histologically and histomorphometrically the efficiency of a Freeze Dried Bone Allograft (FDBA) in sinus lift procedures.

**Materials and methods:** The lateral antrostomy technique with FDBA (Oragraft® Life-Net) allograft and collagen membrane was used. A double (internal-external) collagen membrane was used in 16 cases. Core biopsies were harvested 9 months after sinus floor augmentation. Biopsies were stained with H&E and Mallory. Histomorphometrical measurements were made using the point-counting procedure. Each section was examined using a projection microscope (Visopan). The percentage fraction of each of the following tissues: newly formed bone, residual graft material, bone marrow and connective tissue) was calculated for each section.

**Results:** Graft particles were observed in all specimens surrounded by newly formed bone in direct connection or by soft tissue marrow. The histometrical analysis of the sections showed an average new bone formation of 37%, residual graft particles of 18% and connective tissue of 45%.

**Conclusion:** FDBA (Oragraft®) is comparable to DFDBA Allografts or Deproteinized Bovine Bone Mineral Xenograft for sinus lift augmentation procedure.

72

### **Papillon-lefèvre syndrome: a case report of the 12-year boy treated by tissue engineering method**

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A case is reported of periodontal 12-year-old patient diagnosed with Papillon-Lefèvre Syndrome. Papillon-Lefèvre syndrome is a very rare autosomal recessive condition characterised by pronounced palmoplantar hyperkeratosis and severe early onset periodontitis,

leading to early loss of teeth. Our patient presented with palmo-plantar hyperkeratosis, intraoral examination revealed parodontitis with generalized recession, periodontal pocketing and severe mobility affecting most of the teeth. Panoramic radiograph confirmed the presence of generalized destruction of the alveolar bone around the permanent dentition with maximal manifestation in regions of incisors and 1st molars. Among the possible treatment modalities, we decided for surgical approach by tissue engineering with concomitant systemic antibiotic therapy. For reconstruction of alveolar bone and the periodontium we used an organic bovine bone (Bio-Oss) mixed with autologous platelet rich plasma (PRP), which was activated by tissucol. Today, PRP remains the sole growth factor preparation for tissue engineering available for clinical practice. Periodontal tissue engineering by means of an organic bovine bone and PRP is one of the best alternatives in the treatment of periodontal destruction associated with Papillon-Lefèvre syndrome.

73

### A 2–10 years study of 527 implants placed directly into extraction sockets

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**Aim:** To determine the long-term survival rate of 527 dental implants placed into extraction sockets.

**Methods:** Between 01/94 and 11/02, 309 immediate implants and 218 delayed implants were placed in 247 patients. Three different Implant System were used (42 Branemark Implants; 207 Straumann Implants and 278 Ankylos Implants). Bone augmentation procedures were combined with implant placement. After conventional healing period all implants were osseointegrated from clinical and radiographic point of view and fixed restorations were inserted. In different time intervals mPFI, mSBI, standardized peri-apical radiographs, technical complications and patients' satisfaction have been registered.

**Results:** Twelve patients with a total of 19 implants were not present to recall. During a total observation period of 4.7 years (range 2–10 years), 20 implants were lost and the cumulative survival rate was 96%. The majority part of implants presented healthy peri-implant soft tissue conditions (mPFI > 1, mSBI < 1) and stable peri-implant bone level. Twenty-one patients reported technical complications. Fourteen patients were not satisfied with the aesthetic result.

**Conclusion:** Immediate implants positioning is a predictable method to restore missing teeth with high survival rate. Technical complications can have a negative influence on the patient's comfort. The internal-tapered implant-abutment connection can have a positive influence on the healing and long-term stability of peri-implant tissues.

## Posters: Epidemiology and delivery of care

74

### An investigation of localized aggressive periodontitis prevalence among 11–19 years. students in mashhad city, Iran

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**Background:** The aim of this study was to evaluate the prevalence of localized aggressive periodontitis (LAP) among an Iranian population.

**Materials and methods:** From a total of 15 237 junior high-school, and high school of six educational districts of Mashhad City, cluster sample comprising of 3909 11–19 years. Subjects including 1726 female and 2183 male students were clinically examined. Full-mouth periapical radiographs were taken for putative LAP subjects before a definitive diagnosis of LAP was established.

**Results:** Nine subjects were diagnosed as LAP. The overall prevalence of LAP was 0.23%. The female to male ratio was 3.5 : 1.

**Conclusion:** The prevalence of LAP in Iran population corroborates with previous reports on other ethnic/racial groups.

patients were given oral hygiene instruction. Their toothbrushing and interdental cleaning attitudes were observed 6 months after therapy. Patients answered to structured questionnaire including information about social-demographics, oral hygiene knowledge and attitudes, and dental visiting habits. Chi square or Fisher exact test was used to analyse the relation between questionnaire data and oral hygiene attitudes.

**Results:** Majority of patients (94%) never visited a dentist unless they have dental problem. All patients reported to brush their teeth, but only 76% were brushing with proper technique twice a day. Seventy-two percent of patients reported to use interdental hygiene aids, however 57% were using them properly. Patients who were performing adequate interdental cleaning were also brushing their teeth properly.

**Conclusion:** It appears that classical intervention based on information and training about oral hygiene techniques is not enough for the long-term effectiveness of this intervention. Other than knowledge and training could play a role in oral hygiene attitudes. We suggest that educational intervention needs to be improved perfectly in periodontal patients.

75

### Changes of the oral hygiene habits, knowledge and attitudes of the patients after active periodontal therapy

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**Aim:** The aim was to assess the changes about oral hygiene habits, knowledge and attitudes of patients after active periodontal therapy.

**Methods:** One hundred patients from middle/lower socio-economic class, 14–72 years old, were recruited from patient pool of Ege University Dental School. During periodontal therapy, all

76

### Prevalence of osseous defects on a population of patients undergoing periodontal surgery

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**Introduction:** Periodontitis causes osseous destruction resulting in architectural changes in the alveolar supporting bone, reducing stability and dental support. Those osseous changes or defects can be found in very different ways, as their morphology and aggressiveness can vary from one tooth to another, or between surfaces of the same tooth.

**Aim:** The aim of the study is to know the prevalence and distribution of osseous defects, in patients from the periodontics area of the universidad internacional de cataluña dental clinic, who undergo periodontal surgery.

**Materials and methods:** The study includes 130 patients above 18 years, with periodontal surgery done, excluding those with systemic illness that could affect alveolar bone such as osteoporosis. Probing depth is registered previously and during surgery, and defects are classified by direct vision using various classifications.

**Results:** In 130 surgeries, 248 defects have been found. Most prevalent defects are intraosseous, and from these, the crater or two wall defects are the most prevalent (88). Maxillary defects are more frequent than mandibular and most of the patients with osseous defect were smokers.

**Discussion and conclusion:** We observed a great prevalence of two wall-defects, which means that most of these defects wouldn't have been detected unless a surgical therapy was performed.

77

### The evaluation of patient profile and satisfaction (a pilot study)

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The satisfaction of the patient and the quality of the treatment are accepted to be related. A detailed assessment of patient satisfaction plays a major role in revealing the quality of the treatment. In this study, the relation between the patient satisfaction and the social and medical profile a group of Turkish patients are evaluated. To assess the patient profile and patient satisfaction two separate questionnaires were used. The first one used for evaluating the profile of the patient and it is divided into two sections: social profile and medical profile. The second questionnaire used for evaluating the patient satisfaction. Subjects were chosen from Istanbul University School of Dentistry Periodontology Department (From Periodontal Surgery Clinic 31, student Clinic 70 patients). Sixty-seven percent of the patients were chronic periodontitis, 25% gingivitis, 3% aggressive periodontitis and 5% have mucogingival problems. As a result, 56% of the patients mentioned that they were very satisfied with the attitude of the dentist. In this group, the graduation from high school and university was 56%. Six percent of the patients mentioned that they had too much pain, also it is found that sex is not related with pain. In this study population, it seems that a general satisfaction of the patient group have obtained from the treatment that they received.

78

### Knowledge, motivation and habits in oral hygiene in relation to oral health of young people in Croatia

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**Aims:** The main condition for establishment and maintenance of oral health is patient who is educated and motivated in oral hygiene. The aim of this study was to assess the knowledge, motivation and habits in oral hygiene of young people in relation to dental status.

**Materials and methods:** The assessment was conducted in the sample of 750 individuals aged 18–28 in Croatia using the questionnaire and clinical examination according to the recommendation of the WHO.

**Results:** Oral health habits of Croatian young men do not satisfy because only 57% of them brushed their teeth twice a day, only 10% used auxiliary devices and less than half went to dental check-ups. Oral health knowledge is limited especially in periodontology.

Perception of oral health is at a higher level because more of 80% of them think oral hygiene is necessary and believe they practice it properly. Motivation to keep the natural teeth is also at higher level but although 82% would seek conservative treatment instead of extraction when toothache occurs, still the toothache is the main reason for dental visits (53%) instead of check-ups. Knowledge, habits and motivation positively correlate with clinical status of oral health. Only 20% of population did not need any dental treatment.

**Conclusions:** Although perception of oral health and motivation are at higher level, low-level perception of oral diseases must be improved by educational public dental program.

79

### Survey of members of the French society of periodontology on bone grafts and implant materials

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**Objective:** The aim of the present survey through questionnaires to members of the French Society of Periodontology (SFPIO) was to determine the use of bone grafts and implant materials in periodontal and implant indications.

**Materials and methods:** An anonymous questionnaire has been sent to the 800 members of the SFPIO registered in 2003. The data were collected between May and October 2004.

**Results and Discussion:** A total of 307 exploitable questionnaires were returned to give a 38% response rate. The results show a great implication of university institution in periodontal post-graduate training, which contrast with a lack of university-based implantology training. Autogenous bone graft is still the gold standard both in periodontology and implantology. Of the wide range of implant materials, Perioglas® (in periodontal indications) and Bio-Oss® (both in periodontal and implant indications) are by far the most frequently used. On the other hand, in contrast with some Anglo-Saxon countries, the use of allogenic grafts is insignificant in France. The most frequent indications of bone grafts and implant materials use are three-wall periodontal lesions and pre- or per-implant placement. It is comforting to notice that the choice of the clinicians is mainly influenced by research publications and very few by the effects of marketing by companies. The use of bone grafts and implant materials is fully part of the treatment facilities offered by a large majority of members of the SFPIO.

80

### Compliance with supportive periodontal therapy in patients with periodontal disease at university of Valencia

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**Introduction:** Professional maintenance care is important in preventing further periodontal breakdown. In these visits the clinical parameters are controlled, as well as risk factors. The knowledge of the profile of the patient and degree of compliance of supportive periodontal visits will help us in the daily practice.

**Aim:** To know the variables that influence the compliance of patients treated at Department of Periodontics at University of Valencia.

**Materials and methods:** Data were obtained from 400 records of patients seen over 12-year period. All of the patients had received hygienic treatment and had been informed about the importance of realizing periodic supportive periodontal visits. Analysis was made to correlate the degree of compliance with gender, age, level of

studies, distance university-residence, origin of the patient, care seeking, periodontal disease diagnosed, severity of periodontal disease, type of treatment received, systemic diseases and smoking habits. Descriptive and statistical analysis of the population is realized.

**Results:** 25.4% were found to be complete compliers, 11.1% were erratic compliers, 33.3% never initiated maintenance visits and 30.2% have initiated it but later they dropped out. The statistically significant variable with compliance of maintenance periodontal is severity of periodontal disease.

**Conclusions:** We will have to be aware in insisting the importance of supportive periodontal treatment in those patients with early periodontal disease.

81

### Oral hygiene aids: which are used by persons before a group prophylaxis? – results of a questionnaire

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In preventive dentistry oral health education increasingly gains in importance. One aspect is giving competent and customized information on oral health aids. As a stock-taking we investigated which aids are used by persons before receiving corresponding advice. Oral health behaviour was assessed with a standardized questionnaire before starting a group prophylaxis. A total of 196 persons aged 11–57 years (Ø25.9 years) participated: 65 pupils, students, trainees; 71 university students; 60 employees. 92 were males and 104 females. Tooth brush: 54% of the students and trainees used a standard head, a short head was used by half of the university students and to the employees this item did not matter (55%). The results were significant ( $P < 0.001$ ). The females as a whole preferred using a short head brush. Relating to the brushing profile this item did not matter to the students and trainees (53%), half of the university students stated using a brush with a jagged profile. The employees used to 43% flat and to 45% jagged profiles. Some of the differences were significant ( $P < 0.05$ ). Dental floss: 74% of the students and trainees, 42% of the university students and 73% of the employees never ever performed flossing, whereas 35% of the university students used dental floss, mainly unwaxed floss. The results show that competent information on oral health aids should mainly focus on the use of dental floss and further aids for interdental cleaning.

82

### Oral hygiene aids: which are preferred by dental professionals? – results of a questionnaire

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In preventive dentistry oral health education increasingly gains in importance. One aspect is giving information on oral health aids. This responsibility applies to the dental professionals. Therefore we investigated which aids they use and if there are differences between the occupational categories. Oral health behaviour was assessed with a standardized questionnaire during a dental meeting on prevention. A total of 160 dental professionals including 55 advanced dental students participated (79 dentists, 26 dentist's assistants). Tooth brush: The size of the head was important for the majority of the interviewees (88%), whereas most of them preferred a short head (78%) and 10% a standard head. The differences between the occupational categories showed a slight significant tendency ( $P = 0.055$ ). Relating to the brushing profile one third preferred a flat profile, another third a jagged and for the last third this item did not matter. The differences between the occupational categories were not significant ( $P = 0.55$ ). Dental floss: 84% of the

dental professionals performed flossing, mainly with unwaxed floss (52%). The dentist's assistants used significant more waxed floss than dentists ( $P = 0.03$ ) and students ( $P = 0.01$ ). Tongue cleaner: 13% of the participants stated using this aid, half of them were dentists. The results show that most of the interviewed dental professionals choose their own oral hygiene aids carefully. This result meets good requirements for reliability in oral health education.

83

### Oral malodor in children

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**Objective:** Oral malodor is a problem both for adults and children. The purpose of this study is to determine the relationship between oral malodor, tongue coating and periodontal parameters in children.

**Materials and Methods:** The study included 620 children (306 girls and 314 boys) aged between 8 and 12 years. Malodor related parameters included volatile sulphur compounds (VSC) measured with a portable sulphide monitor (Halimeter®). Periodontal related parameters included periodontal screening and recording (PSR) and tongue coating index. (TCI). The relation between VSC, PSR and TCI were tested with Spearman's Correlation Analysis.

**Results:** The VSC scores for girls was  $203.54 \pm 91.45$  ppb and  $203.56 \pm 93.84$  ppb for the boys. The average PSR values for girls and boys were  $0.66 \pm 0.75$  and  $0.69 \pm 0.74$  respectively. For all evaluated parameters there was no significant difference between the genders. The tongue coating index was  $1.73 \pm 0.84$  and  $1.67 \pm 0.89$ . According to the results of the correlation analysis, there was a significant relation between VSC, PSR ( $r = 0.329$ ,  $P \leq 0.001$ ) and tongue coating ( $r = 0.606$ ,  $P \leq 0.001$ ).

**Conclusion:** Our data suggested that as in adults, oral malodor in children is associated with tongue coating. Particular emphasis must be placed on cleaning the tongue surface for the treatment of malodor in children.

84

### Prevalence of periodontal diseases in the Belgian army

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The aim of the study was to assess the periodontal status of the Belgian military population. The survey was conducted using the Community Periodontal Index of Treatment Needs (CPITN). A total of 484 military personnel belonging to the 10th Tactical Wing, on recall for a routine Annual Dental Inspection, participated in the study. Lifetime of the participants ranged between 21 and 56 years. 14.5% of the population presented pockets at least 6 mm deep (CPITN 4); 40.2% showed pockets ranging between 4 and 5 mm (CPITN 3); 45.2% showed no pockets exceeding 4 mm (CPITN 0, 1 and 2). Calculus or plaque retaining factors with no pockets exceeding 4mm (CPITN 2) could be detected among 41.5% of the population. When analysing CPITN scores according to age, CPITN 4 could be detected in 1.8% of the people 21–29 years old, in 10.6% of the people 30–39 years old and in 22.9% of the people 40–56 years old. These results are at variance with an epidemiological survey conducted in Belgium among hospital staff members (Bercy et al., 2002) where a CPITN 4 was detected among 29.7% of the population 35–44 years old, among 39.7% of the population 45–55 years old and among 55.9% of the population aged 55 years or more. The lower prevalence of CPITN 4 cases in the Belgian Army compared to a non-military Belgian population could be explained by the regular dental recalls to which Belgian

military patients are submitted, resulting in a better on time treatment of periodontal diseases.

85

### Oral health status in elderly institutionalized adults in arcos de valdevez district, Portugal

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The main purpose of this study was to investigate the periodontal status and oral hygiene, in 503 old adults (76.6% was women), institutionalized in 10 community hospitals in Arcos de Valdevez district in Portugal. All examinations were carried out within the 'PASOP' (Ambulatory Project of Oral & Public Health) of the University Fernando Pessoa (UFP) and were conducted according to WHO criteria for oral health surveys. The Periodontal status was assessed by the CPI index and the oral hygiene habits through a questionnaire. All data was analysed by SPSS 12.0. The results indicate that 39.4% of the sample was edentulous and the tooth mortality rate was 70.2%; 71.8% of the individuals have an inappropriate method of teeth and prostheses hygiene. In 95.6% of the individuals has gingival bleeding; 82.1% had presence of calculus, in 68.2% had shallow periodontal pockets and 40.2% had deep periodontal pockets. The daily oral hygiene habits occur in only 73.4% of the individuals. The OCDE perspectives about the demographic evolution of the elderly people (>60 years) in Portugal for the next 50 years, with an estimated increase of 37%, and take attention that the oral hygiene habits and the assess to oral health consultation in Portugal are desadequated to adults needs and indicates that it is necessary a change of strategies in planning and organising the dental services attending the particular attention need for the elderly persons.

86

### Significance of dentist-patient-communication in dentist's education and work

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**Aims:** The aim of our investigation was to survey the significance of dentist-patient-communication particularly with regard to integrating communicative topics into dental education.

**Materials and methods:** Patients, dentists and students were questioned on communicative issues using questionnaires. After the students had been given a 45-min lecture on a didactic training program in patient management, they were again asked to fill in a second questionnaire.

**Results:** A total of 730 questionnaires were available for evaluation (233 dentists, 185 patients, 312 students). Eighty-six percent of the patients and 87% of the dentists supported an integration of communicative issues into dental university education. Following the lecture 85% of the students judged an extension of their education to include communicative topics as important or very important. Only 15% had no preference or considered it as of low or very low importance. On a licert-scale (scaled from 1 to 5) dentists found the friendliness of the office staff (1.5) as important as the professional competence (1.5), followed by detailed informative talk (1.7) and modern instruments (2.6). Eighty percent of the dentists and 51% of the patients attached vital importance to the dentist-patient-relationship concerning therapy outcome.

**Conclusions:** Since patients, dentists and students of dentistry all agreed on the importance of the dentist-patient-communication, this topic should become part of the dental curriculum.

87

### The prevalence and severity of dental fluorosis and periodontal disease in an above optimal and optimal fluoride area of Sudan

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**Objectives:** Dental fluorosis is a public health problem in many part of the Sudan. A cross sectional study to determine the prevalence of dental fluorosis and to study the effect of fluoride in potable water on periodontal disease in an above optimal (Um Duwanban) and optimal fluoride area (Tiraat El-Bijah).

**Materials and methods:** A sample of 400 adults aged 15–65 years were randomly selected from life long residents of Um Duwanban and Tiraat El-Bijah. The Modified Dean's index was used to record the dental fluorosis on the two most affected teeth. The periodontal status of the participants was determined using the gingival and plaque indices. The probing pocket depth and loss attachment were also measured.

**Results:** The mean fluoride concentration in Um Duwanban was 1.36 mg/L and in Tiraat El-Bijah was 0.45 mg/L. The prevalence of dental fluorosis was 70% in Um Duwanban and in Tiraat El-Bijah was 42.5%. All the periodontal parameters increased with age in both communities. Except for the attachment level all the periodontal parameters were statistical significance ( $P < 0.05$ ).

**Conclusions:** Findings suggest that there is a need for continued monitoring of dental fluorosis and further investigation into the fluoride intake from all sources in Sudan. There is a need to change the drinking water supplies in the Um Duwanban area. The fluoride level in the potable water has no influence of clinical significance on the periodontal parameters.

88

### Baseline periodontal status, oral hygiene and smoking habits in head and neck cancer patients

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Periodontally diseased sites are a high risk for osteoradio-necrosis following cancer therapy, especially furcation involved sites.

**Aim:** To assess the periodontal status and smoking habits of head and neck cancer patients scheduled for radiotherapy.

**Material and methods:** Initial records for 287 patients were reviewed.

**Results:** There were 64 females and 223 males with 61% in the 46–65 age group. The cancer diagnosis was squamous cell carcinoma in 80% of cases. Forty-nine percent had never smoked cigarettes or had quit long-term, 51% had stopped in the previous 12 months or currently smoked. Sixty-nine percent of subjects had more than 16 teeth, 44% had a plaque score of >81% and 4% had a bleeding score >81%. The highest CPITN score was '0' in 1% of subjects, '1' in 5%, '2' in 23%, '3' in 39% and '4' in 31%. Associated periodontal factors were gingival recession (39% of subjects), furcation involvement (32%), mobility (2%) and gingival hyperplasia (0.7%). Pre-radiation dental extractions were advised for periodontal reasons in 43% of cases.

**Discussion:** A highest CPITN score of 4 was recorded in 31% of individuals and 32% had furcation involvement. These clinical findings will have an impact on decisions regarding pre-radiation extraction if affected teeth are in the field of radiation.

**Conclusion:** Dental consultation and treatment prior to radiation therapy to the head and neck are an important part of care of the head and neck cancer patient.

89

### Epidemiological study of periodontal diseases – holistic approach

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**The aim:** The research establishes the interrelations between periodontal and systemic diseases (as possible risk factors).

**Materials and methods:** The epidemiological study was realized on 18 932 individuals, clinically investigated in collaboration with WHO Collaborating Center for Oral Health Systems in Transition, Iasi, as a national research for Romania, in 2002–2005 period.

**Results:** The values showed chronic periodontal diseases in 39.41% cases, calculus index >1 in 14.41%, gingival hyper-growth in 9.98%, gingival recession in 21.49% associated with a cardiac, metabolic and other chronic systemic diseases.

**Conclusion:** Systemic diseases have a direct influence on periodontal health status.

90

### Oral health status and dental treatment needs among non-institutionalized psychiatric patients

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**Objective:** To describe oral health status among non-institutionalized psychiatric patients receiving long-term antipsychotic and anti-depressant drugs.

**Method:** Forty psychiatric outpatients were selected as a study group on the basis of having chronic psychotic illness and on neuroleptic medications for at least 2 years. The control group consisted of 40 healthy patients who were selected to match the study group by age and gender. Demographic characteristics, smoking and brushing habits in addition to dental examination including DMF-T, plaque index and CPITN were recorded for each patient in both groups.

**Results:** Psychiatric diagnosis revealed that 57% were having schizophrenia and 43% mood disorders; the mean duration of illness was 11 years. Dry mouth was the chief complaint among 40% of the psychiatric patients. The mean indices for the study vs. control groups were as follows, (9.07 vs. 8.65) for the DMF-T, (1.96 vs. 1.55) for the plaque index and (2.3 vs. 1.97) for the periodontal treatment needs. Filled teeth and plaque index score-0 were significantly greater in the control group, while plaque index score-3 and periodontal treatment need score-1 were significantly greater in the study group.

**Conclusion:** Oral health status of chronic psychiatric outpatients seems to be worse than that of the mentally healthy population. Mental health professionals should pay more attention to dental care of outpatients.

91

### Evaluation of the relationship between cholesterol and triglyceride blood values and periodontal parameters in patients of mashhad health center

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Although the causal link between periodontitis and cardiovascular disease is supported by some research and seems biologically plausible, it remains a controversial topic. Now the evidence suggests that periodontitis once established, possibly provides a biological burden of endotoxin and inflammatory cytokines, which serve to initiate and exacerbate atherogenesis and thromboembolic events. The aim of this study was to evaluate the relationship between periodontitis and blood level cholesterol and triglyceride

changes. Seventy-one patients took part in this study. For each patient, a blood sample was taken and the level of the blood total cholesterol, triglyceride and glucose was measured and a periodontal examination including of probing depth and bleeding on probing was carried out. Statistical analysis of results suggested that with increasing the level of total blood cholesterol, average probing depth and percent of pockets more than 4 mm increased. Although there were direct correlation between periodontal and the measured biochemical parameters, none of these correlations turned out to be a significant. More research is needed to draw any conclusion on the relationship between periodontitis and hypercholesterolemia and hypertriglyceridemia.

92

### Dental anxiety among patients referred for periodontal treatments in hospital Rancagua, Chile

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**Background:** Dental anxiety is an important component of distress to patients in the dental treatments. Avoidance of dental treatment due to anxiety is very common and may be associated with deterioration of dental health.

**Objective:** To register specific anxieties by patients referred for specialist periodontal treatment.

**Materials and methods:** The study was carried out among 176 new patients referred for periodontal therapy at dental service, Hospital Rancagua, during the month of July and August 2005. Prior to periodontal procedures by specialist completed an anxiety questionnaire. All patients were included in the study, and their levels of anxiety were assessed. Dental anxiety was evaluated by the administration of a questionnaire based on the Corah's Dental Anxiety Scale (DAS). Dentally anxious individuals were defined as those with a DAS score 13 or more.

**Results:** Females recorded higher total DAS scores than males (9.49 and 9.16 respectively). Patients in the 24–34 years age group showed the highest total DAS scores (9.25) followed by <24, 35–49, and >50 year age groups in descending order. The total DAS scores for age groups 35–39 and >50 years differ significantly from those of age groups <24 and 24–34 years, but the 24 to 34-year and <24 year age groups did not differ significantly.

**Conclusion:** The periodontal patient's referrals had moderate levels of anxiety. In Chile a large percentage of the population avoids dental treatment except when they feel severe pain.

93

### An assessment of oral self-care among Romanian dental students using the Hiroshima university – dental behavioural inventory

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**Objective:** To determine the differences in oral self-care levels between 322 Romanian dental students.

**Design:** Cross-sectional survey of dental students. Setting: Romania.

**Participants and methods:** The examination was based upon responses to a questionnaire titled 'Hiroshima University – Dental Behavioural Inventory (HU-DBI)' and three additional questions.

**Results:** Statistical significant differences of the mean HU-DBI scores were observed between Years 1 & 2 (level of basic science course) and Years 5 & 6 (level of clinical course) and between genders ( $P < 0.001$ ). Statistical significant differences were notable in 10 items out of 20 between three levels of dental education (basic, preclinical and clinical levels). The most striking results were

that Years 5 & 6 students were less likely to use a toothbrush which has hard bristles ( $P < 0.001$ ), and less likely to worry about the colour of their teeth ( $P < 0.01$ ). While 20% of Years 1 & 2 students reported daily flossing, almost 46% was reported in Year 5 & 6 students ( $P < 0.001$ ). A significant difference was also observed on flossing behaviour between genders ( $P < 0.001$ ). A total of 176 of 205 Years 1–4 students (85.9%) and 177 of 193 female students (91.7%) were correctly predicted by the models.

**Conclusions:** There were considerable differences in dental health attitudes/behaviour between the three levels of dental education and between genders.

94

### Periodontal destruction and oral health-related quality of life among adolescents

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**Background:** The need for appraisal of oral health related quality of life (OH QoL) has been increasingly recognized over the last decades. Our aim was to assess the association between periodontal outcomes and the OH QoL in adolescents considering selected socio-demographic correlates.

**Methods:** The data originated in a study conducted among high school students from Santiago, Chile. The participants were sampled using a multistage random cluster procedure yielding 9203 students. Clinical examinations comprised direct recordings of clinical attachment levels and the presence of necrotizing ulcerative gingival lesions. The students answered a Spanish version of the Oral Health Impact Profile (OHIP-Sp), and the headmasters of the schools provided information on monthly tuitions and annuals fees for each school. The dichotomous outcomes 'presence of attachment lost  $\geq 3$  mm'; and 'presence of necrotizing gingival lesions' were computed and two multivariable linear regression models for the OHIP-Sp total score were obtained using the covariates age; gender; and annual education expenses.

**Results:** A total of 9155 students filled the OHIP-Sp. The coefficients of the linear regression models revealed that students with attachment lost  $\geq 3$  mm (3.5) or necrotizing gingival lesions (2.4); as well as older students (2.1); girls (2.0); and students from schools with lower tuitions and fees (3.5) were more likely to have higher scores for OHIP-Sp and consequently poorer OHQoL.

95

### The new European credit system (ECTS): determinants in dental students to acquire the periodontics competencies to achieve the degree in dentistry

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The purpose of this innovative educational research project has been to determine the total workload of students (TWS) needed to acquire the competencies in the subject of Periodontics to obtain the DMD degree. Eleven students developed an agenda o schedule to establish the work time need to study the Periodontic subject. Also the students classified and quantified the work performed for the Periodontic subject course.

**Results:** The mean VTT of the student to acquire the competencies was 19 377 (mean of presence hours 78 and 115.7 h not present at class). The student employed a mean of 18.15 h for each week. The student profile was studying alone, use of notes, highlighting, performing schemes, no summaries, no use of books, and performed reviews.

**Conclusion:** The equivalent number of European credits for the Periodontic subject was 28.1 h of work for student by credit. The mean grades about knowledge were not related to number of hours dedicated to self-study neither to total workload of students. The present study techniques used by dentals students need a change in order to enhance performance in the new European credit academic system (ECTS).

**Acknowledgement:** Supported by Educational Science Institution (ICE).

## Posters: Periodontal systemic interactions

96

### Inflammatory cytokine production propensity of the main inflammatory cells before and after periodontal treatment

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In the case of a chronic periodontal destruction, the amount of cytokines produced in the local tissues could be little worth to elicit systemic damage. But it seems to be worth to determine the interleukin production propensity of the main inflammatory cell lines, rather than the current sera interleukin level.

**Aim of the study:** Detection of IL1-b, IL6, IL8, IL-10, IL-12p70 and TNF- $\alpha$  production of Monocytes and Lymphocytes by BCA method at patients with periodontitis gravis. Two assays were carried out: first before the beginning of the periodontal treatment and then 6 weeks after the completion of treatment. The further periodontal indexes were recorded: bleeding on probing (BOP), periodontal pocket depth (PPD), gum recession (R), tooth mobility (M), and furcation involvement (F).

**Results:** A slight but not remarkable increase of cytokine production was detected with BCA method in the case of periodontal patients. IL-6 production propensity showed the most remarkable decrease 6 weeks after the periodontal treatment completion. The production of other cytokines decreased almost in all cases after the therapy but showed not a coherent rather a diverse pattern.

**Conclusions:** Some consequent elevation in the macrophage cytokine production at periodontitis patients was demonstrated by the present study. This increased cytokine production propensity could be present through the whole disease process.

97

### Periodontal changes during pregnancy and post-partum

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**Aim:** It is unclear whether pregnancy gingivitis exposes an individual to periodontitis. Our aim was to examine longitudinally the severity of periodontal changes during pregnancy and post-partum.

## Posters: Periodontal systemic interactions

**Methods:** Twenty-eight healthy women (age 24–35 years) with three visits during pregnancy (I: at 12–14 weeks, II: at 25–27 weeks, and III: at 34–38 weeks), and two visits post-partum (IV: after delivery, and V: after lactation) were included in this study. At each visit, visible plaque index (VPI), bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment level (CAL) were measured from six sites/tooth.

**Results:** VPI scores (mean  $\pm$  SD) were  $25.3 \pm 12.76\%$  at visit I,  $20.8 \pm 11.38\%$  at visit II,  $18.2 \pm 10.2\%$  at visit III,  $17.96 \pm 11.6\%$  at visit IV, and  $14.1 \pm 7.8\%$  at visit V. The corresponding percentage for BOP were  $24.97 \pm 16.36\%$ ,  $34.7 \pm 13.2\%$ ,  $28.0 \pm 13.38\%$ ,  $17.6 \pm 12.26\%$ , and  $7.9 \pm 3.53\%$ , all changes in BOP being statistically significant. The increase in BOP between the 1st and 2nd trimester ( $P = 0.0009$ ) was not associated with plaque. The mean number of pockets  $>3$  mm increased from  $0.18 \pm 0.78$  at visit I to  $12.1 \pm 10.99$  at visit II ( $P < 0.0001$ ) but decreased to  $8.24 \pm 9.54$  at visit III ( $P < 0.05$ ) and  $2.3 \pm 4.42$  at visit IV ( $P < 0.05$ ) and, further to  $0.14 \pm 0.47$  at visit V ( $P < 0.05$ ). No PPD  $>4$  mm nor changes in CAL were detected.

**Conclusion:** Major changes in clinical parameters occur during the 2nd trimester. Pregnancy gingivitis develops regardless of the amount of plaque, and pocket formation occurs transiently due to gingival swelling.

98

### Non-surgical periodontal therapy and lipid levels in a population of Singaporean diabetics

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Diabetes mellitus is a known risk factor for periodontal disease. Poor metabolic control of diabetes is associated with increased risk for coronary heart disease (CHD). Periodontitis has also been associated with CHD and a significant association between periodontitis and low HDL-C levels has recently been shown.

**Aim:** To determine the effects of non-surgical periodontal treatment on serum lipid levels, as measured by HDL-C levels, in diabetic patients.

**Methods:** In a randomized, clinical controlled trial, 84 diabetic patients were examined at baseline and randomly subdivided into three different groups of intervention [Group 1 – scaling & root planning plus oral hygiene instructions (OHI); Group 2 – OHI alone; Group 3 – a control group without treatment or any OHI] and then re-examined at 3 & 9 months later. Full mouth plaque and gingival bleeding scores, serum lipid panel, serum HbA1c and CRP levels were taken at all three visits. The total cholesterol: HDL-C ratio and HDL-C levels were compared to baseline.

**Results:** A significant and sustained improvement in periodontal parameters was seen in groups 1 & 2 while group 3 only showed minor improvement in percentage plaque. Group 1 also showed some increase in HDL-C levels as compared to Groups 2 & 3.

**Conclusion:** Within the limits of this study, there were no marked changes in the HDL-C levels for all the three groups at the review visits. The improvement of oral health status is attributed mainly to the modality of treatment rendered.

99

### Duration of diabetes on periodontal status and serum markers

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**Background:** Diabetes has been recognized as a risk factor in periodontal disease. While periodontal disease severity has been found to be related to glycaemic control, few studies have

evaluated the impact of the duration of diabetes on periodontal health and related systemic factors.

**Aim:** The aim of this paper is to assess the periodontal status of a cohort of patients with diabetes of different duration.

**Materials & Method:** A total of 191 patients with diabetes were recruited from two Diabetic centres. Subjects were examined for plaque, bleeding, calculus and probing depths. Blood serum was collected for analysis of HbA1c, Cholesterol and HsCRP (High sensitivity C Reactive Protein). Subjects were categorised into two groups ( $<10$  years, 10 years and more) based upon the duration of diabetes since diagnosis.

**Results:** A total of 117 subjects had diabetes for 10 years or less. For these patients, the HbA1c values were lower and the number of teeth present were higher than those who had the condition for more than 10 years ( $P < 0.05$ ). Only marginal differences were found for the other periodontal parameters. Similarly, there was no marked difference for the HsCRP and cholesterol levels.

**Conclusion:** The results of the study showed that although the duration of diabetes have no direct impact on the periodontal status, the importance of glycaemic control remains a major target in the management of patients with diabetes and periodontal disease.

**Acknowledgement:** Supported by NUS FRC Grant 222-000-017-112.

100

### Bernard soulier syndrome with prolonged gingival bleeding

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Bernard Soulier Syndrome (BSS) is a rare autosomal recessive disease, which is characterized by prolonged bleeding time, thrombocytopenia, and extremely large platelets. A 21-year-old female presented with a complaint of prolonged gingival bleeding for 3 months. The case was diagnosed as BSS in Department of Paediatric Haematology at the age of three. She was the second child of the consanguineous healthy parents. Her younger female sibling has also BSS and achondroplasia. In order to prevent prolonged menstrual and/or spontaneous bleeding, hormonal therapy, coagulant agents were prescribed. In intra-oral examination, the gingiva was oedematous and spontaneous gingival bleeding was noted around the prosthesis in both maxillary and mandibular posterior regions. The amount of plaque was not consistent with the severity of gingival inflammation. Haemoglobin, haematocrit level and the number of platelets were significantly low (Hgb: 6.1 g/dl Hct: 19.9% PLT: 76.000) when compared to normal range values. Since the systemic condition of case was not stable, thrombocyte transfusion and iron supplementation was performed and concomitantly professional dental care including scaling and polishing and daily irrigation with Chlorohexidine gluconate was carried out for 1 week. To maintain gingival health, monthly recall and additional oral irrigation device was suggested to control plaque accumulation and gingival bleeding. Any spontaneous bleeding was not observed at third month visit.

101

### Comparison of gingival crevicular fluid interleukin-8 levels in chronic periodontitis patients with or without type 2 diabetes mellitus

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Chronic periodontitis (CP) is accepted as a complication of diabetes mellitus (DM). Defects that can be seen in functions and chemotaxis of neutrophils are reported to be related with the



severity of periodontal disease in diabetic patients. IL-8 has an important role in restricting the destructive effects of plaque bacteria by its chemotaxis effect on neutrophils. This study aimed to determine IL-8 levels in gingival crevicular fluid (GCF) of CP patients ( $n = 15$ ) which had been shown to have type 2 DM, CP patients ( $n = 15$ ) which were systematically healthy and patients which were periodontally and systematically healthy (control  $n = 24$ ). Full mouth periodontal clinical parameters and GCF sampling was done before and after Phase I therapy. ELISA was used to determine the levels of IL-8. No significant differences were seen between CP and DM groups regarding to GCF parameters at baseline and after therapy. However, mean GCF volume was found to be significantly lower, and mean IL-8 concentration was found to be significantly higher in control group. A general reduction in GCF parameters of CP and DM groups were seen after therapy. The marked reduction of cytokines in GCF following treatment observed in the present study suggests a relationship between disease and cytokine production.

102

### Periodontitis and markers of platelet activation

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**Background:** Chronic inflammation may result in increased platelet activation, an important factor in atherosclerosis. Periodontitis is epidemiologically associated with atherosclerosis.

**Objective:** To explore whether two markers of platelet activation are increased in periodontitis.

**Materials and methods:** Plasma levels of sCD40 ligand (sCD154) and sP-selectin (sCD62) from 62 moderate and 43 severe periodontitis patients and 46 controls were determined. Age, gender, race, smoking, cholesterol, BMI and education were recorded.

**Results:** No significant association between plasma levels of sCD154 and periodontitis was found ( $P = 0.452$ ). sCD62 was modestly elevated in periodontitis; controls, moderate and severe periodontitis patients showed median values of 52, 67 and 82 ng/ml respectively ( $P = 0.006$ ). Patients more frequently had plasma levels of sCD62 above the 75th percentile value (84 ng/ml) of the controls (34% and 49% for moderate and severe periodontitis respectively,  $P = 0.014$ ). In a regression model, cholesterol ( $P = 0.002$ ), periodontitis ( $P = 0.024$ ) and education ( $P = 0.030$ ) were explanatory variables for sCD62 levels.

**Conclusions:** sCD62 levels were related to the presence and severity of periodontitis, therefore platelet activation may occur in patients. However, the association found was not independent. Furthermore, it cannot be excluded that the observed raise in sCD62 is partially originating from endothelial cells, since sCD154 was not elevated in periodontitis.

103

### Relation between periodontitis and rheumatoid arthritis

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Rheumatoid arthritis (RA) is a chronic destructive inflammatory disease characterized by the accumulation and persistence of an inflammatory infiltrate in the synovial membrane that leads to synovitis and the destruction of the joint architecture. Periodontitis has a remarkably similar pathobiology to RA. The aim of present study was to investigate a relationship between RA and periodontitis. Two groups, RA and control, were compared with regard to medical and dental status. The study population was comprised of 73 individuals. RA group was comprised of 33 patients with proven RA (nine males and 24 females) and control group was comprised of 40 random patients, matched for sex and age (11 male and 29

females). All individuals had at least 12 teeth. The study subjects were clinically examined by full-mouth probing depth, gingival index, clinical attachment loss and plaque index. Also the number of remaining teeth was recorded. The amount of alveolar bone loss was measured by means of Shei ruler on panoramic radiographs and defined as percentage. Statistical analysis of data revealed all of the clinical parameters were significantly higher in RA than control group ( $P < 0.05$ ). However the number of remaining teeth was significantly lower in RA group ( $P < 0.05$ ). In conclusion, this study highlights a potential relationship between two of the most common and debilitating chronic inflammatory conditions affecting humans. However, further analyses were needed to confirm this relationship.

104

### Study of the dental and periodontal health of children suffering from leukaemia in northern Turkey

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**Background:** The relation between leukaemia and oral manifestations has been the topic of many investigations.

**Aim:** The aim of this study was to evaluate the dental and periodontal health status of 39 children suffering from acute lymphoblastic leukaemia (ALL) in Northern Turkey.

**Materials and Methods:** The female to male ratio in the study population was 22/17 and mean age was 7.7, ranging between 4 months-2 years old. All the patients were under remission-induction therapy. Periodontal examination was performed using plaque and CPITN indices. Dental status was evaluated by using dmf-t and DMF-T indices. Presence of oral lesions was also noted in the routine intraoral examinations.

**Results:** Three patients had mucositis and another three had localized gingival hyperplasia. The mean plaque index score was 1.42. CPITN scores revealed that 29% of the patients had healthy gingiva; 63% of the patients had plaque who needed only oral hygiene instruction and 7.9% of the patients had calculus who needed scaling and oral hygiene instruction. The results of dmf-t and DMF-T indices showed that 32 patients had decayed teeth ( $n : 119$ ), 13 had missing teeth ( $n : 28$ ) and only four had filled teeth ( $n : 5$ ).

**Conclusion:** It may be concluded that, most of these hospitalized leukaemic children had poor oral hygiene and dental status. They needed proper oral hygiene instructions as well as decay treatment. Since, presence of bacterial plaque and caries lesions might be life-threatening.

105

### Periodontal disease, preterm birth and low weight children: a cross-sectional study

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**Introduction:** Several studies in medical literature report risk factors related to preterm birth and low weight birth. Also several reports and studies have been done in order to relate periodontitis as a possible risk factor in preterm birth and low birth weight.

**Aims:** The purpose of this trial is to establish a possible relationship between periodontal status in 88 pregnant women and the results of their pregnancy.

**Material and methods:** The sample was 88 pregnant women aged between 16 and 38. Measurements recorded were: Age, race, height (cm), weight (kg), number of children, number of pregnancies, number of medical controls during pregnancy, and the mother's periodontal status. Ramfjord's Index Teeth (#16, #21, #24, #36,

#41, #44) were taken to record the grade of bleeding on probing, mobility, probing depth and clinical attachment loss, in six different points at each tooth. Once the child was born, gestational time (weeks), child weight (g), length (cm) and type of birth were recorded.

**Results:** Means of probing depth, clinical attachment loss, mobility and grade of bleeding on probing, are evaluated in order to relate them to preterm birth and low weight birth and analysed with the analysis of variance ANOVA.

**Conclusions:** It seems to be that low weight birth and low weight infants are related to periodontal disease, but further investigation would be necessary in order to relate the mother's periodontal status with the pregnancy results.

106

# **Relations between periodontal disease, cytokine gene polymorphisms, preterm birth and the intra-uterine growth restriction in postpartum women**

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**Aim:** The aim of this study was to evaluate the relations between periodontal disease, cytokine gene polymorphisms; preterm birth (PB) and intra-uterine growth restriction (IUGR) in postpartum woman.

**Materials and Methods:** Out of 156 subjects, 64 subjects with normal birth (NB), 45 subjects with PB and 47 subjects with IUGR ranging in age from 18 to 34 years were included in this study. Dental plaque, probing depth, bleeding on probing and clinical attachment level was recorded and three groups were made: healthy group ( $n = 46$ ), mild group ( $n = 7$ ) and periodontitis group ( $n = 103$ ). Polymorphisms of IL-1A (IL-1 $\alpha$ <sup>+4845</sup>), IL-1B (IL-1 $\beta$ <sup>+3954</sup>) and IL-1RN (IL-1ra) genes were determined with polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis.

**Results:** As regards to the genotype frequencies of IL-1A and IL-1B, no differences were found between NB, PB and IUGR groups ( $P > 0.05$ ). In this study, we found that IL-1RN 'allele' 2+ property was closely related with PB and IUGR ( $P < 0.05$ ). There was no relationship between IL-1A, IL-1B, IL-1RN polymorphisms and periodontal disease.

**Conclusions:** This data provide evidence that IL-1RN 'allele' 2+ property and periodontal disease characterized with CAL are associated with PB and IUGR.

107

# **Initial periodontal therapy on IL-1b levels in patients with type II diabetes mellitus**

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The aim of this study is to investigate the effect of initial periodontal therapy (IPT) plus systemic doxycycline (DOC) on clinical parameters and gingival crevicular fluid (GCF) interleukin-1 beta (IL-1 $\beta$ ) levels in chronic periodontitis (CP) patients with diabetes mellitus (DM) type II. Twenty patients and 10 medically and periodontally healthy subjects were included. DM patients were divided into two groups: Group 1 received IPT and DOC; Group 2 received IPT only. At baseline and 7 weeks post-therapy, plaque index, probing depth (PD), bleeding on probing and relative attachment level were recorded, GCF samples were collected from sites with initial PD  $\geq 5$  mm and IL-1 $\beta$  levels were further determined by enzyme-linked immunosorbent assay. Both treatments provided significant clinical improvements 7 weeks after therapy ( $P < 0.01$ ). However, no significant differences were observed between G1 and G2. Although levels of HbA<sub>1c</sub> decreased

markedly in both groups, intra- and inter-group comparisons revealed no statistically significant differences. While total amounts of IL-1 $\beta$  in GCF were reduced in both G1 and G2 ( $P < 0.05$ ), the difference between the groups was not significant. IPT provided clinical improvement together with reduction in IL-1 $\beta$  levels. Although not significant, periodontal therapy improved glycaemic control in these patients. However, within the limits of this study, the use of systemic DOC did not provide additional effect to IPT alone.

108

# **Effect of periodontal therapy on glycaemic control and gingival tissue lipid peroxidation and glutathione levels in type 2 diabetes mellitus**

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The effects of initial periodontal therapy (IPT) and plus systemic doxycycline (DOC) administration on clinical parameters, glycaemic control and gingival tissue lipid peroxidation (LPO) and glutathione (GSH) levels were investigated in chronic periodontitis (CP) patients with type II diabetes mellitus (DM). Twenty patients and 10 medically and periodontal healthy subjects were included. DM patients were divided into 2 groups: Group 1 received IPT and DOC; Group 2 received IPT only. Plaque index, bleeding on probing, probing depth (PD), relative attachment level (RAL) and HbA<sub>1c</sub> were measured at 0 and 49 days. Gingival tissues were collected from the selected sites. Levels of malondialdehyde and GSH were measured by biochemical methods. Clinical evaluation revealed statistically significant improvements in all clinical parameters of both treatment groups ( $P < 0.01$ ), however inter-group differences were not significant ( $P > 0.05$ ). No significant differences were observed at in the HbA<sub>1c</sub> levels. Gingival tissue LPO and GSH levels showed significant changes in both treatment groups after therapy ( $P < 0.01$ ). There was no significant difference between the groups ( $P > 0.05$ ). Clinical and laboratory results of this study demonstrated no superior effect of systemic DOC usage combined with IPT in comparison with IPT alone in CP + DM patients. Clinical and laboratory parameters of study groups after periodontal therapy were comparable with those of healthy group.

109

# **Anti-TNF $\alpha$ biological agents and periodontal conditions: a pilot study**

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**Background:** Periodontitis and rheumatoid arthritis share a number of similarities concerning the pathogenic mechanisms of disease initiation and progression. Proinflammatory cytokines and matrix metalloproteinases are common features of both diseases. Modulation of host inflammatory activity with anti-TNF $\alpha$  agents is a new approach in the treatment of rheumatoid arthritis and has shown promising results in experimental periodontitis.

**Aim:** Aim of the present study is to investigate the effects of anti-TNF $\alpha$  agents, on clinical and radiographic parameters of periodontal conditions.

**Materials and methods:** Ten patients with severe RA (Disease Activity Score DAS28  $> 5.1$ ), were scheduled to initiate anti-TNF $\alpha$  treatment. On entry and after 6 months of treatment, patients were evaluated concerning Probing Depth, Probing Attachment Level, Bleeding on Probing and Presence of Plaque. Full-mouth radiographs were taken. Changes in bone-crest height

were assessed with the Emago digital radiographs analysis programme vs. 3.4.

**Results:** Non statistically significant changes were observed in clinical or radiographic parameters (Wilcoxon test,  $P > 0.05$ ) with the patient as observational statistical unit.

**Conclusions:** Despite poor oral hygiene of these patients, anti-TNF $\alpha$  treatment did not cause deterioration of periodontal conditions. Findings from this pilot study suggest that the possible anti-inflammatory effects of anti-TNF $\alpha$  agents on periodontal conditions require further investigation.

110

### The effects of menopause on periodontal condition by means of clinical and hormonal parameters

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Menopause should not be accepted as a negative experience in women life. Post-menopausal women suffer from hot flushes, atrophic changes, osteoporosis, tooth loss, etc. Periodontitis is also a complex disease, which its progression is influenced by modifying factors such as systemic diseases and age. Some of the risk factors are the same in menopause and periodontitis. There is limited data trying to correlate hormone therapy, hormonal parameters and periodontal condition. The aim of this study is evaluating the effects of menopause on periodontal condition by correlating clinical and hormonal parameters and. A total of 24 women were included in the study. Eight of them are menopausal and receiving hormone therapy, eight of them are menopausal and not receiving any therapy and eight of them are not menopausal. Clinical parameters were; Plaque index (PI), sulcus bleeding index (SBI), relative clinical attachment levels (RCAL) and probing depth (PD). Oestrogen levels were collected from each patient. The clinical and hormonal correlation between the groups was performed by the Mann Whitney U test. Non-menopausal group had statistically higher oestrogen levels and statistically lower RCAL. As conclusion, in the post-menopausal period, women have a greater risk of RCAL loss. A adequate periodontal recall programme might reduce this risk.

111

### The effect of behçet's disease on the clinical periodontal status and amount of gingival crevicular fluid

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The aim of this study was to evaluate the effects of BD on periodontal clinical parameters and volume of the gingival crevicular fluid (GCF). Test group of this study is consisted of 16 volunteers (female: 12 and male: 4, aged 20–65 years, mean age:  $35.06 \pm 10.66$ ), patients with Behçet's disease and who referred to Dermatology department of University of Selcuk, Meram Medicine Faculty and control group is consisted of randomly selected 16 volunteer (female: 12 and male: 4) patients with aged 23–55 years (mean age:  $39.81 \pm 7.36$ ), and who referred to the Periodontology department of University of Selcuk, School of Dentistry. In all patients, Probing Depth, Clinical Attachment Level, Gingival Index and Plaque Index were recorded and GCF sampling was performed. No statistically significant differences were observed for all clinical parameters and amount of GCF between groups ( $P > 0.05$ ). Data obtained from this study suggest that Behçet's disease is not directly a risk factor for periodontal diseases.

112

### Serum antibodies in *Porphyromonas gingivalis* induced bacteraemia after scaling and root planning in periodontitis patients

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**Background:** It is little known the role, kinetic and protective value of anti-*Porphyromonas gingivalis* antibodies during bacteraemia induced by scaling and root planning in periodontitis patients.

**Materials and methods:** Thirty-nine patients diagnosed with periodontitis participated in the study: 15 with Generalized Aggressive Periodontitis (GAP) and 24 with Generalized Severe Chronic Periodontitis (GSCP). *P. g* was isolated in each one of the patients in subgingival plaque before scaling and root planning. Four blood samples from the ante-cubital vein were taken at different times: (i) Before the scaling and root planning; (ii) Immediately after treatment; (iii) 15 min after treatment and (iv) 30 min after treatment, in order to identify by culture *P. g* in blood. Samples of serum pre- and 1 h after-treatment were processed to evaluate IgG and IgM antibodies specific to *P. g* and its LPS using an ELISA.

**Results:** Bacteraemia associated to *P. g* was detected in 28.2% patients (11/39), no significant differences were found in bacteraemia between GAP and GSCP patients ( $P > 0.05$ ). There was no difference between IgG and IgM pretreatment levels comparing patients with or without *P. g* in blood induced by scaling ( $P > 0.05$ ). The sample taken one hour after the procedure showed a significant increase of anti-*P. g* and anti-LPS specific IgG in GSCP patients ( $P < 0.05$ ). These results show evidence of immune activation during bacteraemia, but do not evidence a protective value in its early phase.

113

### Evidence-based dentistry: a systematic review to evaluate antibiotic therapy for gingival overgrowth induced by assumption of cyclosporine A

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**Background:** Gingival overgrowth (GO) is a common finding in periodontal patients under pharmacological treatment with Cyclosporine A (CsA). A systematic review was conducted to evaluate the efficacy of azithromycin (AZM) in patients with GO induced by assumption of CsA.

**Materials and methods:** Using an ideal key-word to search the online database MEDLINE, EMBASE and Cochrane Central of Register Controlled Trials (CENTRAL), we found 21 articles, only four of which were Randomized Controlled Trials (RCTs) published in English, thus meeting the inclusion criteria. The great heterogeneity between proposed treatments and final data did not allow to conduct a meta-analysis.

**Results:** A systemic antibiotic therapy without plaque and calculus removal does not reduce GO. A 5-day course of AZM with Scaling and Root Planning reduces the degree of GO, while a 7-day course of metronidazole is only effective on concomitant bacterial over-infection.

**Conclusions:** The great heterogeneity of diagnostic data and results found is due to the lack of precise diagnostic methods and protocols about GO. The pathogenesis of GO as well as the mechanism of action of AZM still remains unclear. In literature there are few RCTs on the efficacy of systemic antibiotic therapy in case of GO; that is mainly due to the absence of precise diagnostic methods and tools and of an adequate classification aimed to determine a correct prognosis and an appropriate therapy for GO.

114

### Periodontal disease and angiographic demonstrated coronary heart disease

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**Background:** Many epidemiological studies reported an association between periodontal (PD) and cardiovascular disease, another ones found no association. In most of these studies, Coronary heart disease (CHD) diagnostic was clinical instead of angiography. The aim of the study was to investigate the association between periodontal health and angiographic demonstrated CHD.

**Materials and methods:** Forty patients with coronary catheterization were included in this study. Twenty-three with coronary disease (A group) and 17 without disease (B group). Conventional cardiovascular risk factors were not statistically significant between groups. Missing teeth, plaque index (PI), probing depth (PD) and gingival index (GI) were recorded. Blood samples were taken for measurements of serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-CL) low density lipoprotein cholesterol (LDL-CL), and (PCR).

**Results:** The mean PD between A ( $2.52 \pm 0.70$  mm) and B ( $2.06 \pm 0.74$  mm) groups was nearly significant ( $P = 0.06$ ), percentage of sites with probing depth  $> 3$  mm, and GI were not statistically significant. We also found blood PCR higher levels in group A ( $15.43 \pm 29$ ) vs. B ( $4.81 \pm 4.90$ ) ( $P > 0.02$ ).

**Conclusions:** The results indicate that PD may be associated with angiographic demonstrated CHD. We are carrying out a prospective randomized study to determine whether PD is a risk factor of CHD and a possible clinically meaningful reduction in heart disease from the prevention or treatment of PD.

115

### Investigation of periodontal status in acromegalic patients

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Acromegaly is a chronic metabolic disorder caused by the presence of an increased growth hormone secretion due to an adenoma of the pituitary gland in nearly every case. It occurs after normal growth of the skeleton and other organs is complete. Acromegaly has an estimated prevalence of 40–60 cases per million. Patients with acromegaly may exhibit insulin resistance. The greatest anomaly is seen in the mandible, with greater enlargement of the ascending ramus. The most common oral features of acromegaly are a prognathic mandible, interdental spaces, macroglossy, and everted swollen lips. Periodontal status is not described in the literature. We investigated sixteen individuals, eight males and eight females, with mean age of  $41.5 \pm 10.5$  years. Periodontal probing was performed and gingival bleeding index (GBI) was calculated, followed by an assessment of gingival overgrowth, tooth mobility and gingival recession. All patients exhibited tooth mobility, mainly in the lower anterior teeth, seven patients presented with recession areas and no gingival growth was observed. No site with probing depth over 4 mm was found and mean GBI was  $0.35 \pm 0.2$ . The most interesting result was the complete absence of periodontal pockets. It is suggested that the presence of mobility and recession is caused by dental trauma resulting from the malocclusion caused by mandibular prognathism. The absence of periodontal pockets was not determined although growth hormone is anabolic for bone tissue.

116

### Comparison of the periodontal condition in Down syndrome and other mental retarded children

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**Objectives:** The aim of this case-control analytic study was the evaluation of periodontal condition in Down Syndrome (DS) children in compare with other mental retarded (MR) children.

**Patients and Methods:** Twenty-five DS (6–11 years) children as case group and 25 MR age-matched children as control group were studied. Plaque Index, Gingival Index, Probing Pocket Depth and Periodontal Disease Index were measured in both groups and the data were analysed by paired  $t$  test.

**Results:** GI in DS was  $2.51 \pm 0.63$  compared with  $2.43 \pm 0.55$  in MR, PI in DS was  $2.35 \pm 0.15$  compared with  $2.31 \pm 0.31$  in MR, PDI in DS was  $4.4 \pm 0.51$  compared with MR  $4.38 \pm 0.45$ , PPD in DS was  $4.90 \pm 0.54$  compared with  $5.8 \pm 0.71$  in MR. Clinical indices of periodontal condition were higher in DS but were not statistically significant ( $P > 0.05$ ).

**Conclusion:** Down syndrome children don't display different periodontal condition compared with age-matched other mental retarded children.

117

### Relationship of HbA1c level and smoking to periodontal status in a cohort of Croatian patients with insulin-dependent diabetes mellitus

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**Background:** There is firmly established correlation between periodontitis and diabetes mellitus (DM), but it is still unknown how does the duration of DM influence the periodontal status of individuals.

**Aim:** The aim of the study was to investigate the effect of DM duration and HbA1c level on periodontal status of patients with insulin-dependent DM, as well as the combined effect of smoking and HbA1c level on periodontal status.

**Materials and methods:** Subjects were randomly selected from the patients with insulin-dependent DM that are treated at the Department of Endocrinology in the hospital «Sveti Duh». Data was gathered by means of an interview, clinical periodontal investigation and patients' charts from the department. Sites with probing depth of 5 mm and greater were selected as sites with periodontitis. By means of Poisson regression model a relative risk with 95% confidence interval was computed, corrected for age of the subjects.

**Results:** Relative risk for attachment loss in smokers was 12.805 and for probing depth in smokers 7.99. Relative risk of the HbA1c level  $> 8.5$  in smokers were 10.681 ( $P = 0.002$ ), and 8.214 ( $P = 0.006$ ) for HbA1c level  $\leq 8.5$ . Relative risk for attachment loss in nonsmokers was 4.891 ( $P = 0.031$ ).

**Conclusion:** It was concluded that insulin-dependent smokers with poor glycaemic control have the greatest risk for development and progression of periodontal disease.

118

### The relationship between periodontitis and hyperlipidaemia

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**Objectives:** The aim of this study is to evaluate the relationship between chronic periodontitis and hyperlipidaemia.

**Material and methods:** In this case-control study 50 patients with chronic periodontitis (case) and 50 patients with normal periodontium (control) were examined through Probing Pocket Depth (PPD) and periodontal indices including: Plaque Index (PI), Bleeding Index (BI) and Periodontal Disease Index (PDI). Also Triglyceride (TG), Cholesterol (Chol), LDL-C and HDL-C levels were measured for all patients.

**Results:** The mean serum levels of TG ( $169.18 \pm 74$  mg/dL), Chol ( $206.06 \pm 56$  mg/dL) and LDL-C ( $143.19 \pm 58$  mg/dL) were significantly higher and HDL-C ( $46.70 \pm 11$  mg/dL) was lower in case group in compare with control which their TG levels were  $129.36 \pm 69$  mg/dL, and the levels of Chol, HDL-C and LDL-C were respectively  $180.94 \pm 51$ ,  $52.36 \pm 13$  and  $105.30 \pm 46$  mg/dL ( $P < 0.05$ ). There were a significant relationship between PPD and increase of TG ( $P = 0.00$ ), Chol ( $P = 0.00$ ) and LDL-C ( $P = 0.03$ ). Also hyperlipidaemia showed a significant relationship with BI and PDI ( $P = 0.00$ ).

**Conclusion:** There is a significant correlation between periodontitis and hyperlipidaemia.

119

### Long-term clinical outcomes of S/RP with SPT of HIV-seropositive patients taking highly active antiretroviral therapy

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The aim of this controlled study was to assess the treatment outcomes of S/RP with subsequent SPT in generalized, chronic periodontitis of HIV-seropositive patients undergoing highly active antiretroviral therapy (HAART). Twenty-two patients divided into test and control group, who were similar in age, sex and smoking behaviour, were treated by S/RP followed by a 15-month lasting SPT. The data collected in both groups were recorded before therapy as well as at the end of the study. The results were evaluated between the two dates of the survey and among the groups of trial subjects. The haemato-immunological marker values of the test group showed a stable phase of immunity reconstitution over the whole period of survey. The index BOP improved with all patients ( $P < 0.002$ ). The average PPD was reduced in the test group by 0.9 mm ( $P < 0.001$ ) and in the control group by 1.0 mm ( $P < 0.001$ ). The number of teeth classified as exhibiting from periodontal disease to teeth without clinical findings was reduced in both study groups ( $P = 0.001$ ). A so-called healing rate showed for the test group 84%, for the control group 87%; the comparison of the groups was not significantly different in terms of statistics ( $P = 0.4$ ). In conclusion there was no statistically significant difference concerning the clinical outcomes between both study groups. The conservative periodontal treatment by S/RP and strictly organized SPT in HIV-seropositive patients undergoing HAART is an effective and safe treatment concept.

120

### The effects of anabolic androgenic steroid abuse on gingival

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**Background:** Anabolic androgenic steroid (AAS) is the familiar name for synthetic derivatives of the male sex hormone, testosterone. A large number of young adults abuse AAS to enhance performance and physical appearance. The aim of this study was to evaluate the effects of AAS abuse on the gingival tissues in a group of bodybuilders and weight lifters.

**Methods:** The test group was composed of 24 athletes who have been using AAS for more than 1 year. All subjects were clinically examined for plaque levels (plaque index), gingival inflammation (gingival index), and gingival hyperplasia. The results were compared with a control group of 20 bodybuilders who had never used AAS drugs.

**Results:** Although there were no statistical differences between the plaque index ( $P > 0.05$ ) and gingival index ( $P > 0.05$ ) scores of the study group and the control group, the AAS abusers had statistically higher scores of gingival thickness, extent of gingival encroachment and total gingival overgrowth scores ( $P < 0.001$  each) when compared to non-users.

**Conclusions:** The results of this study have revealed that the prolonged use of AAS is closely associated with significant levels of gingival hyperplasia. Since recreational abuse and abuse in non-competitive sports are out of control and seems to increase despite legislation, dentists and periodontists should be familiar with the adverse effects of these synthetic derivatives of testosterone on the gingival tissues.

121

### Presence of respiratory pathogens in the oral biofilm of patients with nosocomial pneumonia

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Hospitalized patients receiving treatment at Intensive Care Units may have the oral cavity colonized by respiratory pathogens, increasing the risk for nosocomial pneumonia.

**Aim:** The aim of this study was to investigate the presence of respiratory pathogens in the oral cavity of hospitalized patients at Intensive Care Units.

**Material and methods:** Thirty patients from Hospital R. Sertã, NF, Brazil, with nosocomial pneumonia, were included in the study, and tracheal aspirate samples were cultured to identify the causing microorganisms. Microbiological samples from supragingival dental plaque, tongue and respiratory tube were cultured for a panel of respiratory pathogens.

**Results:** The most frequently found bacteria in the tracheal aspirate were *S. pneumoniae* 23.3% (7), *P. aeruginosa* 20% (6), *S. aureus* 13.3% (4), *K. pneumoniae* 13.3% (4), *C. albicans* 6.6% (2), *α-haemolytic streptococcus* 6.6% (2), *Staphylococcus sp.* 6.6% (2), *A. calcoaceticus* 3.3% (1), *E. coli* 3.3% (1) and *E. cloacae* 3.3% (1). 70.0% (21) of these microorganisms were found in the dental biofilm, 63.33% (19) in tongue samples; 73.33% (22) in the respiratory tube and 43.33% (13) in all sampling sites simultaneously. No differences in proportions could be observed between the sampling sites ( $P > 0.05$ ).

**Conclusion:** The results of this study show that respiratory pathogens associated with nosocomial pneumonia are present in the oral biofilm of hospitalized patients, which may serve as a reservoir for these microorganisms.

122

### Periodontal status of asthma patients

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**Aims:** Periodontal status investigated in 59 asthmatic patients and 83 healthy controls (mean age 48.9 years).

**Materials and methods:** We are using API, PBI and probing depth and results were compared according to age, gender, smoking and asthma intensity in both groups.

**Results:** API was significantly higher in patients with asthma than in controls ( $P < 0.001$ ). PBI was also significantly different compared to the controls (59% : 41%). All patients with asthma

had all four stages of gingival inflammation, while only four controls had inflammation stage III and none had stage IV. Analysis of probing depth showed a significant difference between patients with asthma and the controls in three teeth in maxillary (third sextant) and nine teeth (fourth and the sixth sextant) in mandibular jaw.

**Conclusions:** Results showed poorer periodontal status in asthmatic patients compared to healthy controls. Mean probing depth was 0.2 mm deeper in asthmatic patients than in controls ( $P = 0.022$ ). The percentage of API increased in patients with moderate and severe asthma. This trend of enhanced frequency was also visible in PBI in all three stages of persistent asthma compared to intermittent asthma. Tendency of increased probing depth almost reached statistical significance ( $P = 0.052$ ) in patients with persistent asthma, as compared with controls. API and PBI indices, probing depth and the number of extracted teeth showed no difference in both groups according to gender, age and smoking habits.

123

### Relationship between periodontitis and preterm birth: case-control study

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The introduction of Periodontal Medicine took doctors and dentists to face with a big heterogeneity of data, which suggest that periodontitis can contribute to the morbidity and mortality of individuals with some systemic conditions and to the preterm birth. The aim of this study was to determine whether maternal periodontitis could be associated with preterm birth.

**Materials and methods:** The case group ( $n = 22$ ) was defined as postpartum women with spontaneous preterm labour (gestational age  $< 37$  weeks) as a result of premature labour or premature rupture of membranes. The control group ( $n = 24$ ) included postpartum women with term birth (gestational age  $\geq 37$  weeks). Known risk factors for preterm birth and the periodontal status were recorded. Only postpartum women without any systemic disease were included. The patients were only enrolled into the study after giving informed written consent.

**Results:** The mean birth weight and the gestational age were both lower within postpartum women with periodontitis comparing to the group with gingivitis. The difference is significant ( $P = 0.002$  and  $P = 0.008$ , respectively). A significant association was found between periodontitis and preterm birth. The odds ratio was 2.9 at the 95% confidence interval.

**Conclusions:** The obtained data in this population revealed an association between the presence of maternal periodontitis and preterm birth. Further studies are needed to clarify this association and to determine whether this relation is causal.

124

### Periodontal and metabolic status after conversion to wholesome nutrition in adults

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The aim of the study was to examine the effect of the conversion from western style nutrition to wholesome nutrition on periodontal and metabolic health in adults. Twenty-three female subjects, mean age 51.9 years, were followed-up over 12 months during nutritional conversion. Attachment level (AL), probing depth (PD), bleeding on probing (BOP), gingival index (GI), interproximal plaque index (API) and serum variables (cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, apolipoproteins A-I and B, C-reactive protein, leukocytes, glucose, uric acid, creatinine,

homocysteine, folic acid, vitamin B12, vitamin E) were determined at baseline (0), after 2 weeks as well as after 3, 6 and 12 months. Friedman/Wilcoxon tests were used for statistical analysis. The results of the periodontal variables were as follows (12 months vs. 0): AL 2.7 vs. 2.9 mm ( $P = 0.024$ ), PD 2.2/2.4 mm ( $P = 0.001$ ), BOP 40/50% (n.s.), GI 0.9/1.1 ( $P = 0.014$ ), API 77/74% (n.s.). No significant changes were found for variables of lipoprotein metabolism (lipids, lipoproteins, apolipoproteins). CRP and leukocytes were unchanged. There was no significant change of folic acid (16.0/14.9 nmol/L) and vitamin B 12 276/259 pmol/L. Interestingly, for vitamin E a significant increase of serum concentration was observed (51.7/36.9 mmol/L,  $P = 0.001$ ). The results demonstrate a beneficial effect of the conversion to wholesome nutrition on periodontal variables after 1 year, especially on attachment level, probing depth and gingivitis.

125

### Effects of periodontal treatment and antioxidant usage in type II diabetes mellitus patients with chronic periodontitis

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The aim of this study is to assess effects of initial periodontal therapy with and without antioxidants on metabolic control and gingival glutathione (GSH) and malonaldehyde (MDA) levels in type II diabetes mellitus (DM) patients with chronic periodontitis (CP). Ten DM + CP patients received scaling and root planning (SRP) combined with systemic antioxidants (Vitamin C 1 g/day, Vitamin E 400 IU/day) for 2 months (SRP + A). Ten DM + CP patients received SRP. Ten systemically and periodontally healthy subjects undergoing crown-lengthening operation served as the healthy control group (H). HbA<sub>1c</sub> levels and plaque, sulcus bleeding indices (SBI), probing depths (PD) and relative attachment levels were assessed at baseline and gingival tissue samples from periodontal sites of DM patients with a PD  $\geq 5$  mm. All assessments were repeated 2 months after therapy. Gingival GSH and MDA levels were determined by Ellman and Thiobarbituric acid methods respectively. There were significant reductions in clinical parameters and HbA<sub>1c</sub> levels in SRP + A and SRP groups ( $P < 0.01$ ), whereas GSH levels increased significantly ( $P < 0.01$ ). MDA level reduced significantly only in the SRP + A group ( $P < 0.05$ ), there were no significant differences between the two groups. Biochemical parameters of both DM groups after periodontal therapy were comparable with the H group. Within the limits of this study the efficacy of antioxidants in conjunction with initial therapy on MDA levels warrants further detailed studies.

126

### Effect of diabetes on salivary antioxidant activity

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**Aims:** Exact mechanisms of interactions between diabetes and periodontal disease remain unclear. This study was conducted to compare clinical periodontal measurements and salivary glutathione, Vitamin C, and total antioxidant capacity between diabetic and non-diabetic patients.

**Methods:** Forty-one diabetic patients and 24 otherwise healthy control subjects matched with the diabetic group according to periodontal health status were included in the study. Whole saliva samples were obtained and full mouth clinical periodontal measurements were recorded at six sites/tooth. Saliva flow rate, salivary levels of glutathione, Vitamin C and total antioxidant

capacity were determined. Data were tested statistically by non-parametric tests.

**Results:** Diabetic group exhibited significantly less number of teeth present ( $P = 0.01$ ), reduced saliva flow rate ( $P = 0.001$ ). Clinical periodontal measurements and salivary glutathione, Vitamin C, and total antioxidant capacity values did not show significant differences between the groups ( $P > 0.05$ ). In diabetic group salivary glutathione level was significantly correlated with probing depths ( $P = 0.001$ ), and total antioxidant capacity correlated with saliva flow rate and Vitamin C level ( $P < 0.01$ ).

**Conclusion:** Within the limits of the present study, salivary glutathione, Vitamin C and total antioxidant capacity values do not seem to play a role among mechanisms by which diabetes affects periodontal tissues.

127

### The relationship between periodontal disease and indices of atherosclerosis

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**Background:** Epidemiological studies have shown that periodontal disease may be associated with an increased risk of progression of cardiovascular and cerebrovascular disease. Endothelial dysfunction would be present in patients with periodontal disease. The purpose of the present study was to examine the relationship between periodontal infection and endothelial dysfunction.

**Methods:** The study design was a cross-sectional study. Male subjects (average  $47.2 \pm 11.2$ -year-old, range 24–63) from same workplace with slight periodontitis ( $n = 38$ ), severe ( $n = 19$ ) and control ( $n = 41$ ) were enrolled for this study. Severity of periodontitis were classified with having at least one site of probing pocket depth more than 4 mm (slight), having more than 6 mm (severe), or control. For the indices of atherosclerosis, Brachial-Ankle Pulse Wave Velocity (baPWV) and the ankle brachial index that reflect the development of atherosclerosis were measured. Other indices known as risk factors for atherosclerosis, such as blood pressure and serum cholesterol were also measured. The ANOVA and post-hoc Scheffé test was used for statistical analysis.

**Results:** The baPWV were also higher ( $P < 0.05$ ) in slight periodontitis ( $1475 \pm 237$ ) and in severe periodontitis ( $1507 \pm 195$ ) than in control ( $1346 \pm 251$ ). After adjustment for age, a weak relationship was found between periodontitis and these indices of atherosclerosis. Periodontitis has the possibility of showing a relationship with atherosclerosis.

128

### Association of periodontal diseases with C reactive protein

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**Introduction:** C reactive protein is one of the acute phase proteins that its levels elevate during infectious diseases. The aim of this study was to evaluate the relationship between periodontal diseases and C reactive protein level.

**Materials and methods:** Fifteen patients (seven male, eight female, mean age  $34 \pm 11.6$ ) with chronic periodontitis and 15 ages and sex matched (seven male, eight female, and mean age  $29 \pm 10.4$ ) periodontally healthy subjects recruited from the patients referred to the department of periodontics, Mashhad faculty of dentistry. Periodontal probing depths of Ramfjord teeth were recorded for both groups. Peripheral blood samples were collected and sent to the laboratory to determine the amount of CRP using semiquantitative method.

**Results:** The amount of CRP in the test group was 4.1 mg/L. In the control group it was 0.18 mg/L. CRP in the test group was significantly higher than the control group ( $P = 0.008$ ). There was no significant correlation between the mean pocket depth and the CRP levels. Sex and age did not affect the amount of plasma CRP. **Conclusion:** Periodontal diseases can increase the amount of plasma CRP. This might be due to the infective nature of periodontal diseases.

129

### The effect of phase I periodontal therapy on blood lipid and CRP levels: a pilot study

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**Background:** Recently, it has been suggested that there is a link between the periodontal disease and blood lipid profiles and CRP levels. The aim of this study was to evaluate the effect of phase I periodontal therapy on blood lipid and CRP levels in gingivitis and periodontitis patients.

**Material and Methods:** Systemically healthy 46 subjects older than 30 years included in this study. The patients were divided into three groups according to their clinical recordings: gingivitis ( $n = 12$ ), the periodontitis ( $n = 17$ ) and control ( $n = 17$ ). Before and after 6 weeks of the phase I periodontal therapy, the clinical recording and blood sampling were obtained. Triglyceride (TRG), cholesterol (Chol), high-density lipoprotein (HDL), very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and C-reactive protein (CRP) levels were determined in the blood samples.

**Results:** After phase I periodontal therapy, all of the clinical parameters in both groups have improved. The levels of the blood lipids and CRP were not statistically significantly different, when compared in the groups and between the groups, before and after phase I periodontal therapy ( $P > 0.01$ ). No significant correlations were found between the clinical and blood parameters after therapy ( $P > 0.01$ ).

**Conclusion:** Within the limitations of this study, improvement in gingivitis and moderate periodontitis by phase I periodontal therapy did not affected the blood lipid levels and CRP levels in short term.

130

### Oestrogen receptor genotypes and alveolar bone loss in postmenopausal Malay women

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The polymorphisms of the oestrogen receptor gene (ERG) have been reported to be associated with systemic bone loss, which may also affect oral bone particularly mandibular bone resorption in postmenopausal women.

**Objectives:** To detect the association between ERG polymorphisms with alveolar bone loss and tooth loss in postmenopausal Malay women (PMW).

**Methods:** Sixty-four PMW (mean age  $57.06 \pm 4.69$ ) were recruited from Out Patient Dental Clinic, Hospital University Sains Malaysia, Kelantan. Oral panoramic radiographs were taken for dental assessment and measurement of alveolar bone loss using the technique as described by Wical and Swoope (1974). Genotyping was done through the restriction cleavage of polymerase chain reaction-amplified genomic DNA with the two restriction enzymes, *PvuII* and *XbaI*. Oestrogen receptor genotypes were represented as P or p (*PvuII*) and X or x (*XbaI*) with the lower case letters signifying the presence of the restriction site.

## Posters: Periodontal systemic interactions

**Results:** High frequency of heterozygous allelic variants (53% for Pp and 86% for Xx) was observed. There was no significant association between ERG polymorphisms with alveolar bone loss and tooth loss in PMW.

**Conclusion:** ERG polymorphisms may not be a genetic marker for alveolar bone loss in postmenopausal Malay women.

**Acknowledgement:** This study supported by USM Short Term Grant, 301 PPSG 6131300.

131

### **A comparative evaluation in pregnant women of the periodontal condition and low birth delivery incidence**

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The objective of this study was the evaluation of periodontal condition in pregnant women, the effect of the treatment of periodontal disease upon low birth weight. A total of 269 women in their first trimester were included in this study. Their ages ranged from 18–35 years, their height was above 150 cm and they have no history of any systemic disease. At the end of each trimester these measurements were performed: attachment level, probing depth, gingival index, plaque index, and bleeding on probing. According to the periodontal diagnosis the research population was divided into three groups: periodontally healthy women; periodontally diseased women, however rejection of treatment; periodontally diseased women, however acceptance of treatment. When parameters such as number of pregnancies, previous low birth weight, prenatal care, genital tract infection, use of antibiotics, smoking, tooth brushing habit were evaluated it was concluded that only the brushing habit was statistically significant ( $P < 0.001$ ). When such parameters as infant birth weight, gestational age, type of delivery and sex were evaluated, it was found that only the birth weight was statistically significant ( $P < 0.001$ ). The evaluation of the risk factors concerning low birth weight, prenatal care, irregular brushing and periodontitis found to be statistically independent risk factors. In this study one-way ANOVA, chi-square, conditional logistic regression analyses were used.

132

### **The prevalence of systemic diseases in Turkish urban population**

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**Background and Aims:** In Turkish population; hypertension, hypercholesterolemia, cardiovascular diseases (CVD) and diabetes are common diseases because of the traditional dietary habits. The aims of this study were to determine the prevalence of systemic diseases in patients with gingivitis and periodontitis and to explore the effects of periodontal disease in risk of systemic disease occurrence.

**Material and methods:** The study design was a retrospective recording review. The recordings were belonging to the period of 2001–2002. The study population included 2737 patients. The patient's self-reported systemic condition, age, gender, educational level, oral hygiene, smoking status were evaluated and periodontal status and the number of missing teeth were recorded.

**Results:** A total of 1887 patients were diagnosed as periodontitis while 850 patients were gingivitis. Periodontitis was frequently seen in male patients and patients older than 30 years. A total of 773/2737 subjects have reported their systemic diseases. A total of 331 patients have CVD, 196 have endocrine disorders, 79 have lung disease and 234 individuals are using regularly various drugs. Age, gender, presence of periodontitis, smoking, tooth loss and the

usage of prosthetic appliances were determined as risk factors for the presence of systemic diseases in this population.

**Conclusion:** Within the limitations of this study, systemic diseases were observed more frequently in patients diagnosed as periodontitis than gingivitis patients.

133

### **Coincidence between periodontal disease and coronary heart disease – verification of bacterial and cytokine's theories**

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The aim of the study was to evaluate the influence of periodontal disease on the inflammatory response in ACS and to evaluate incidence of selected anaerobic bacteria in subgingival and atherosclerotic plaques in patients treated surgically because of coronary vessels' obliteration. The study involved 50 patients with an initial diagnosis of acute coronary insufficiency, with diagnosed advanced chronic periodontitis (ACP) and 20 individuals with ACP, where subgingival plaque was collected from periodontal pockets, DNA test was used for marking eight pathogens responsible for periodontal tissues destruction. In the same patients material from atherosclerotic plaque was collected during by-pass implantation procedure, and identical DNA testing occurred. This material was used to ascertain the mean serum levels of CRP and TNF- $\alpha$ . The high mean values of clinical parameters measured attest the presence of active periodontal disease, which may affect the incidence of cardiovascular disease. Patients with ACS and with more advanced periodontal disease were characterized by higher mean values of serum concentrations of cytokine CRP in the acute phase of ACS and in the long term and of TNF- $\alpha$  in the long term. In DNA-tested group, in 13 of 20 patients pathogens most frequent in severe chronic periodontitis were found in coronary vessels. In 10 cases those bacteria were also present in atherosclerotic plaque.

134

### **Is preterm birth predictable in the case of pregnant women with early periodontitis?**

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The aim of our study was to examine if there was an association between early periodontitis of pregnant women and preterm birth in the South-East Hungary.

**Material and Methods:** In total 161 healthy women were included in this case-control study. Preterm birth was defined if the patient had a threatening premature labour during pregnancy, preterm rupture of membranes, or spontaneous preterm labour, and/or if the weight of the newborn was  $< 2500$  g. In the control group women had delivery after 37th gestational week and the newborn's weight was  $\geq 2500$  g. Early periodontitis was defined, if the patient had  $\geq 4$  mm probing depth at least at one site and bleeding on probing occurred at  $\geq 50\%$  of her teeth.

**Results:** A significant association was found between the weight of the newborns and the periodontitis of the mother. Deep pockets or gingival bleeding alone did not correlate with preterm birth, but having at least one  $\geq 4$  mm probing depth and BOP at  $\geq 50\%$  of the teeth at, had a strong correlation with preterm birth ( $P = 0.000$ ). Mean weight of the newborns was 2834.5 g in the periodontitis and 3180.3 g in the control group the difference was significant ( $P = 0.004$ ). Mothers with early periodontitis had 3.32 x greater odds of preterm delivery or low birth weight, than did women without periodontitis.

**Conclusion:** The results of the study showed, that the early periodontitis of the pregnant woman could be an independent risk factor for preterm birth.



135

### Occurrence of self-reported systemic medical conditions in patients with periodontal disease

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**Objectives:** The objective of this retrospective study was to investigate the occurrence of self-reported systemic disorders in patients referred to a specialist clinic for periodontal treatment.

**Materials and methods:** The study design was a case-controlled, retrospective chart review. Patient charts ( $n = 1044$ ) were selected from Department of Periodontology, University of Medicine and Pharmacy 'Carol Davila'. Two examiners collected the data.

**Results:** The most frequent disorders in patients with gingivitis were high blood pressure (35.85%), followed by coronary artery disease (15.741%), kidney and urinary tract disorders (15.71%) and allergic reactions (14.28%). Eighty percent of patients with gingivitis had at least one of these disorders. The most frequent disorders in patients with periodontitis were high blood pressure (29.51%) followed by digestive disorders (18.92%), coronary artery disease (16.54%), kidney and urinary tract disorders (16.24%), endocrine disorders (10.58%), and rheumatoid arthritis/rheumatism (9.68%). 81.96% of patients with periodontitis had at least one of these disorders. However, only rheumatoid arthritis was found to be more prevalent in periodontitis patients comparing with gingivitis affected individuals ( $P < 0.05$ ). Multiple correlations were found between the independent variables.

**Conclusions:** These findings support the results from previous investigators that a number of systemic conditions are closely associated with periodontal disease.

136

### Effects of simple periodontal therapy on periodontal status and glycaemic control in patients with diabetes

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Periodontal status of diabetic subjects has been shown to be affected by glycaemic control. Few studies have examined the effect of periodontal therapy on glycaemic control. The aims of the study were to compare the effects of different treatment modalities on periodontal parameters and glycaemic control in diabetic patients and to examine the effect of glycaemic control on treatment response. Ninety-eight adult subjects were randomly assigned to one of three groups: simple non-surgical periodontal therapy and oral hygiene (T;  $n = 36$ ), oral hygiene alone (OH;  $n = 32$ ) and no treatment (C;  $n = 30$ ). Blinded examiners carried out full mouth periodontal charting and blood samples at baseline, 3 and 9 months after therapy.

**Results:** Mean plaque scores were reduced at 9 months within T & OH ( $P < 0.05$ ). For bleeding scores, only T showed significant reduction at 3 and 9 months compared to baseline ( $P < 0.05$ ). At 9 months, T ( $19.4 \pm 19.6\%$ ) showed significantly better reduction in bleeding scores when compared to OH ( $7.1 \pm 14.3\%$ ) & C ( $3.8 \pm 18.2\%$ ). HbA1c levels were not significantly affected over time. Subjects with poorer glycaemic control (HbA1c  $> 8\%$ ) had poorer periodontal status. At 9 months, T & OH subjects with HbA1c  $> 8\%$  showed significant reduction in bleeding scores compared to subjects with HbA1c  $\leq 8\%$ . In this study, simple periodontal therapy improved the periodontal health of diabetic subjects, with little effect on their glycaemic control. Subjects with poorer glycaemic control benefited from some intervention.

137

### Prevalence and severity of periodontitis in individuals with metabolic syndrome

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Metabolic Syndrome (MS) represents a complex disease with several components from degenerative chronic diseases, like diabetes. These components include: high blood pressure ( $\geq 130/85$  mmHg); fasting blood glucose  $\geq 110$  mg/dL; abdominal circumference  $\geq 102$  cm (men); triglycerides  $\geq 150$  mg/dL; HDL  $\leq 40$  mg/dL (men). Some of those factors have been associated with periodontal disease.

**Aim:** The aim of this study was to describe the prevalence of periodontitis in a sample of individuals with MS.

**Materials & Methods:** Twenty-five males with MS, with mean age 49.6 (SD 6.2), all non-smoking, were examined for Clinical Attachment Level (CAL) and bone level using panoramic radiographs. Severe periodontitis was classified as  $\geq 10\%$  of sites with CAL  $\geq 5$  mm or bone loss  $\geq 2/3$  of the root length, and moderate periodontitis as  $\geq 10\%$  of sites with CAL  $> 2 < 5$  mm or bone loss between  $1/3$  and  $2/3$  of the root length.

**Results:** Seventy-two percent (18) of the individuals with MS showed signs of destructive periodontal disease – 36% (9) severe periodontitis and 36% (9) moderate periodontitis. Within the individuals with periodontitis, 83.3% (15) showed high triglycerides levels; 66.7% (12) showed high blood glucose levels; 66.7% (12) showed high abdominal circumference; and 55.5% (10) showed low HDL levels. No statistical differences could be observed in this small sample.

**Conclusion:** A rather high prevalence of periodontitis in individuals with MS was observed in this sample.

138

### Periodontal and serum lipid changes in diabetics following non-surgical periodontal therapy

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**Background:** Diabetics tend to have higher prevalence, more severe forms of periodontal disease and reduced treatment outcomes. Periodontitis is also associated with elevated blood lipid levels.

**Aim:** To determine the influence of glycaemic control and/or periodontal therapy on serum lipid levels.

**Materials and methods:** A total of 102 subjects were recruited from two diabetic centres, divided into well- and poorly-controlled diabetics and then randomly subdivided into test and control groups. Test group received oral hygiene instructions and scaling and/or root planning. Control group received no treatment. All subjects were examined at baseline, 3 and 9 months. Each examination included an assessment of HbA1c, serum lipid (total cholesterol TC, triglyceride TG and LDL) levels and full mouth periodontal assessment.

**Results:** Poor glycaemic control was associated with poorer periodontal condition and higher TC/TG/LDL at baseline. All except the control group with poor glycaemic control showed significant improvement in mean plaque score and BOP ( $P < 0.04$ ). Poorly-controlled diabetics who received periodontal treatment also showed significant improvement in LDL levels ( $P = 0.001$ ).

**Conclusion:** This study shows the beneficial effects of simple periodontal therapy in improving the periodontal health of diabetics. The relative improvement appears more marked in patients with poor glycaemic control. Diabetics with poor glycaemic control also showed significant improvement in LDL levels following non-surgical periodontal therapy.

139

### The evaluation of the oral and systemic parameters in diabetic patients: a pilot study

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**Background and aims:** The effects of diabetes on periodontal health are known. The aim of this study is to investigate the inflammatory and periodontal status of the diabetic and healthy individuals with the equal PD values and to determine the correlations between periodontal and systemic parameters in diabetic patients.

**Material and methods:** The study population was consisted of 32 diabetic (DM) and 17 healthy (C) individuals. The clinical parameters (gingival index-GI, plaque index-PI, gingival bleeding time index-GBTI, probing depth-PD and clinical attachment loss-CAL) and systemic parameters (total cholesterol, TG, HDL, LDL, cre and HBA<sub>1c</sub>) were obtained. The DM group was investigated about the presence of neuropathy, nephropathy, cerebrovascular stroke (CVS), chronic lung diseases (CLD) and retinopathy.

**Results:** All of the clinical parameters (except PD) and systemic parameters (except TG) were found significantly different between the groups ( $P < 0.05$ ). 9.4% of DM had grade 1, 18.8% grade 2, 12.5% grade 3, 3.1% grade 4 nephropathy. 3.1% of DM had neuropathy, 6.3% CLD, 34.4% retinopathy. The correlations between cre and PD and CAL were found significant in diabetic patients.

**Conclusion:** The significant differences between the groups in inflammatory markers and periodontal status have supported the relationship between the diabetes and periodontal diseases. Larger diabetic population is needed to determine the correlations between the clinical parameters and diabetic major complications.

140

### Association between periodontal health and hyperlipidaemia

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Recent studies have shown an association between increased plasma lipid levels and periodontal disease. The aim of this study was to determine whether a metabolic disorder such as hyperlipidaemia might be a risk indicator for periodontal disease. In this study body-mass index, plasma lipid levels, fasting blood glucose and C-reactive protein of 55 male and female patients aged 34–62 years who have evident hyperlipidaemia were measured and the results for 55 age- and sex-matched healthy controls' measurements were compared. For periodontal assessment, plaque index (PI), gingival index (GI), probing pocket depth (PPD), clinical attachment level (CAL), and bleeding on probing (BOP) measurements were evaluated. Hyperlipidaemic patients showed statistically significant increase in PI, GI, PPD, CAL and BOP values when compared to healthy controls ( $P < 0.05$ ). In conclusion, impaired lipid metabolism seems to be a risk indicator for periodontal disease.

141

### Systemic C-reactive protein and GCF IL-1 profiles in periodontitis

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**Background:** Elevated CRP levels in periodontal patients have been reported by several groups. Levels of IL-1beta in GCF are dependent upon a genetic influence and the clinical parameters at the site of collection. In this study, we examined whether CRP

plasma levels are in relation to levels of IL-1 in GCF evaluated from patients with varying degrees of periodontal disease.

**Methods:** Serum CRP (by radial immunodiffusion assay) and FCG-IL-1β (by ELISA) levels were assessed in 72 subjects, 24 with moderate mean clinical attachment loss (AL) ( $2.45 \pm 0.32$  mm) and 24 with height AI ( $4.27 \pm 0.68$  mm) as compared to 24 periodontally healthy controls (AL,  $1.79 \pm 0.24$ ).

**Results:** At baseline CRP plasma levels were correlated with IL-1β levels for each group. Statistical significant increases in CRP levels and in IL-1β levels were observed in subjects with periodontal disease when compared to healthy control. Subjects with high levels of mean clinical attachment loss had significantly higher mean CRP and IL-1beta levels ( $3.95 \pm 5.17$  mg/L and  $109.8 \pm 96.6$ ) than control ( $1.82 \pm 2.01$  mg/L and  $38.6 \pm 45.3$ ).

**Conclusion:** These data suggest that the levels of FCG-IL-1β and plasma CRP are closely associated with periodontal status. At baseline the extent of increase in CRP and IL-1β levels in periodontal groups depends on the severity of the disease after adjusting for age, smoking, cholesterol, etc. These markers expression can in part a host trait, and not strictly a function of clinical parameters.

142

### Are high-density lipoproteins associated with a lower risk of periodontitis?

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**Aim:** This study was performed to estimate the independent association between periodontitis and sociodemographic, overweight, high-density lipoproteins cholesterol and glycated haemoglobin (A<sub>1c</sub>) in diabetic and in non-diabetic subjects.

**Materials and methods:** We evaluated 158 subjects, 59 had type 2 and 20 had type 1 diabetes, and 79 were non-diabetic. For each participant we obtained information on pocket depth, clinical adherence level, bleeding on probing; age, sex, education, body mass index (BMI); analytical values: fasting glycaemia, low-density lipoproteins, high-density lipoproteins (HDL) cholesterol, triglyceride and A<sub>1c</sub>. Periodontitis with systemic repercussions was considered to be present when more than 50% of sites with PD  $\geq 4$  mm. To estimate the association between periodontitis and each of the evaluated variables we computed Odds Ratios and respective Confidence Intervals through multivariate logistic regression models.

**Results:** In a model including sex, age, smoke, prosthesis, education, BMI, HDL, A<sub>1c</sub> compared to non-diabetics the OR for type 1 diabetes was 2.00 (95%CI: 0.3–12.4), and 3.4 (95%CI: 1.3–9.2) for type 2 diabetes. Among non-diabetic subjects, the OR was 3.0 (95%CI: 0.4–24.3) when the BMI  $\geq 25$ , and the OR was 0.1 (95%CI: 0.01–0.8) when HDL  $\geq 50$ . Among the diabetic individuals the OR was 0.3 (95%CI: 0.1–1.2) when HDL  $\geq 50$ .

**Conclusions:** Regardless of the diabetic status, HDL values above 50 mg/dL are associated with a lower risk of periodontitis.

143

### Lipid profile in periodontal patients

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Cardiovascular disease and periodontal disease are some of the most common illnesses. They produce serious damage with high social and economic impact. Some papers have been published showing the epidemiological relationships between these processes. Atheroma is the main lesion of cardiovascular disease related with disordered lipid metabolism. Our objective was to the quantitative

152

plasma lipid profile, measured by gas chromatography, in a group of patients with periodontitis (PG) compared with non-periodontitis (NPG). Fifty-seven patients over 35 years, without systemic health problem were recruited for the study, previously approved by our Local Ethical Committee. Probing pocket depth (PD) and clinical attachment level (CAL) were recorded and blood and plasma were obtained. Periodontitis was defined on the presence of CAL  $\geq 6$  mm in two or more teeth and one or more sites with PD  $\geq 5$  mm (Machtei criteria). Total cholesterol: 195.04 (NPG), 217.67 (PG) ( $P = 0.04$ ); triglycerides: 92.93 (NPG), 144.63 (PG) ( $P = 0.005$ ); very-low-density lipoprotein-cholesterol (VLDL-C): 18.42 (NPG), 28.36 (PG) ( $P = 0.006$ ); total cholesterol/HDL: 3.56 (NPG), 4.58 (PG) ( $P = 0.01$ ); total saturated fatty acid: 80.51 (NPG), 109.12 (PG) ( $P = 0.0002$ ); total polyunsaturated fatty acid: 112.93 (NPG), 130.64 (PG) ( $P = 0.02$ ). Our data showed impairment in the lipid metabolism, with higher levels of plasma fatty acids, cholesterol and triglycerides. These alterations represent an increased risk for the development of cardiovascular disease.

144

#### Ten year longitudinal study of the relationship between periodontal status and coronary heart disease

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**Background:** To investigate: (i) the changes in periodontal evolution after etiological periodontal treatment and (ii) the influence of periodontal status in the evolution of cardiac health over ten years.

**Materials and methods:** Fifty-five patients (44 finished the study), two groups: Healthy Group (HG)  $n = 9$  and Cardiac Group (CG)  $n = 35$ , subdivided into Angor Pectoris Group (AG)  $n = 5$ , Acute Myocardial Infarction Group (AMIG)  $n = 22$  and Exitus Letalis Group (EG)  $n = 8$ . Gingival level (GL), probing depth (PD), clinical attachment level (CAL), plaque index (PI) and bleeding on probing (BOP) were measured to compare the periodontal status in both groups. The patients were examined and periodontal treatment was performed. They were examined at the end of 1 and 10 years. Statistical method: One way-ANOVA and MR-ANOVA were established ( $P < 0.05$ ).

**Results:** The CG patients showed a worse evolution of periodontal indices at the end of one year's periodontal treatment (PD  $P < 0.041$ , PI  $P < 0.043$ , BOP  $P < 0.007$ ). EG had the worst evolution ( $P < 0.008$ ). At ten years' treatment, it could be seen that although the CG continued increasing CAL on the second visit ( $P < 0.0385$ ), differences in PI ( $P < 0.023$ ) and BOP ( $P < 0.012$ ) were established at the end of 10 years.

**Discussion/Conclusion:** The coronary cardiopathy affected patients showed a worse evolution of periodontal indices than healthy ones. The patients with worse evolution of coronary cardiopathy showed the worst evolution of the aforementioned indexes.

145

#### Periodontal conditions in patients with coronary heart disease

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**Aim:** The aim of this study was to examine the periodontal conditions of a group of patients with severe coronary heart disease.

**Methods:** The study group comprised 161 patients aged 40–75 years recruited from University Hospital, Linköping. All patients had undergone percutaneous coronary intervention. Patients with diabetes were excluded. The control group comprised 162 subjects with no history of coronary heart disease.

Periodontal conditions were examined clinically and in full-mouth radiographs. Periodontal disease experience was classified into five groups according to Hugoson and Jordan (1982).

**Results:** Severe periodontitis (periodontal experience groups 4 and 5) was more prevalent in the test group, 25% vs. 8%. The patients had a mean bone level of  $3.0 \pm 1.0$  mm and the controls  $2.6 \pm 0.8$  mm. Mean tooth loss was 5.3 in the test and 3.4 in the control group. Periodontal pockets with a PPD 4–6 mm were more prevalent among heart patients. We found a significant association between coronary artery disease and the variables periodontal disease experience groups 4 and 5 and smoking. When controlling for smoking and a wide confidential interval, the odds ratio for having coronary heart disease when severe periodontal disease is present (periodontal disease experience group 4 or 5) was 2.4.

**Conclusion:** Severe periodontal disease seems to be more prevalent in individuals with severe coronary heart disease than among controls.

146

#### C-reactive protein, erythrocyte sedimentation rate and ferritin levels in humans with periodontitis

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**Introduction:** Periodontitis is frequently considered as a local inflammation. However, epidemiological studies have indicated that this disease may have a systemic effect.

**Aims:** The aims of this study were to determine if periodontitis effects inflammatory markers in the blood and if non-surgical periodontal therapy influences these markers.

**Material and methods:** C-reactive protein (CRP), erythrocyte sedimentation rate after 1 h and after 2 h (ESR) and ferritin (FER) were analysed in 35 generally healthy patients with periodontitis (test group) before treatment (baseline) and in 18 of those after non-surgical periodontal therapy. As control we recruited 22 periodontally and generally healthy adults and collected blood samples twice within fortnight.

**Results:** The comparison of the four parameters at baseline between control and test group showed no statistical significance except for ESR/2 h [ $P$ -value (Mann Whitney test): CRP = 0.2580; FER = 0.7121; ESR/1 h = 0.1538; ESR/2 h = 0.0378]. The differences within the test group before and after treatment were not statistically significant for any marker [ $P$ -value (Wilcoxon signed ranks test): CRP = 0.1477, FER = 0.6475; ESR/1 h = 0.3942; ESR/2 h = 0.7983]. Performing confidence interval for a single prediction using the two blood samples of the control group revealed that the reason for these results is based on the distinct intra-individual fluctuations within a person.

**Conclusion:** In our study the determined markers did not show statistical significance.

147

#### Genetic associations between rheumatoid arthritis and periodontitis

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Fc receptors for immunoglobulin G (FcγR) and interleukin 1 (IL-1) play a major role in the pathogenesis of rheumatoid arthritis (RA) and periodontitis. Both diseases are partly influenced by genetic component. The aim of this study was thus to evaluate whether FcγR and IL-1 genotypes could be a risk factor for periodontitis in RA. The study subject consisted of 46 RA patients, 83 periodontitis patients, and 104 healthy controls. RA patients fulfilled the American Rheumatism Association 1987 Revised

Criteria. Periodontitis patients were identified as having more than one diseased site with more than 4 mm in probing depth. Genomic DNA was isolated from peripheral blood, and FcgR and IL-1 genotypes were determined by the Invader method. The prevalence of periodontitis was found to be 76.1% in RA patients. No difference was observed in the FcgRIIA, FcgRIIIA, and FcgRIIIB genotype distributions among the subject groups. However, IL-1B + 3954 genotype distributions in RA patients were different from those in periodontitis patients and controls ( $P = 0.013$  and  $P = 0.04$ ), and also proved different between RA patients with and without periodontitis although this failed to reach statistical significance. These results document IL-1B polymorphism to be possibly associated with risk of periodontitis in RA patients.

148

### Gingival crevicular fluid levels of PGE<sub>2</sub>, IL-1B, T-PA and PAI-2 in type 2 diabetic patients with periodontal disease

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**Aims:** This study was conducted to evaluate gingival crevicular fluid (GCF) levels of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), interleukin-1β (IL-1β), tissue-type plasminogen activator (t-PA), and plasminogen activator inhibitor-2 (PAI-2) in type 2 diabetic patients with periodontal disease.

**Methods:** Seventeen type 2 diabetic patients with periodontal disease (DM), 17 otherwise healthy periodontally diseased patients (PD) and 17 systemically and periodontally healthy control subjects (H) were enrolled in the study. GCF samples were obtained from two randomly selected single rooted teeth and full-mouth clinical periodontal measurements were recorded at six sites/tooth. GCF levels of PGE<sub>2</sub>, IL-1β, t-PA and PAI-2 were analysed by appropriate ELISA kits. Data were tested statistically by ANOVA and Dunnett C test.

**Results:** GCF volume was significantly greater in DM group than PD and H groups ( $P < 0.05$ ). DM and PD groups showed significantly higher PGE<sub>2</sub> and t-PA levels than group H ( $P < 0.05$ ). DM group revealed significantly higher IL-1β levels than PD and H groups ( $P < 0.01$ ). PAI-2 level was significantly higher in DM group than H group ( $P < 0.05$ ).

**Conclusion:** Within the limits of this study, DM may be suggested to affect GCF levels of some major inflammatory mediators and to modify host response and eventually clinical periodontal situation. Further studies are required to better clarify mechanisms of interactions between DM and PD.

149

### Ligneous periodontitis-a destructive membranous disease

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Ligneous conjunctivitis is a rare form of chronic conjunctivitis characterized by the development of firm fibrin-rich, woody-like pseudomembranous lesions mainly on the tarsal conjunctivae. Less frequently, similar lesions may occur on other mucous membranes of the body (in the mouth it's been termed ligneous periodontitis), indicating that these manifestations are part of a systemic disease. Actually, systemic plasminogen deficiency has been linked to ligneous conjunctivitis in humans and mice. Histopathological findings from affected humans indicate that wound healing, mainly of injured mucosal tissue, is impaired and shows a thinned or eroded epithelium with superficial or subepithelial deposits of amorphous hyaline-like eosinophilic material and foci of persisting granulation tissue with accompanying inflammatory cells, mainly

lymphocytes, plasma cells, and granulocytes. The amorphous hyaline-like eosinophilic material of the pseudomembranes, which resembles amyloid, but is negative for Congo red stain, has been shown to contain mainly clotted fibrin (ogen), and other plasma proteins such as albumin and immunoglobulins (mainly IgG). This research is dealing with a short description of plasminogen activation and deficiencies, diagnosis and clinical characteristics of ligneous lesions mainly in the mouth, histological abnormalities and electron microscopical study of pseudomembranous lesions on some cases and current treatment approaches.

150

### Comparison of periodontal status in HIV-positive patients and controls: relationship with the immunological and virological parameters

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**Objectives:** To determine the periodontal status in HIV+ patients vs. a control group of HIV- patients, in relation with their immunological and /or virological state.

**Material and methods:** A group of individuals with HIV ( $n = 32$ ) and a control group of HIV- ( $n = 16$ ) were analysed. A health history was completed by each subject. Periodontal measurements included Ramfjörð teeth (16, 21, 24, 36, 41, and 44) and we recorded: locations with probing depths  $> 3$  mm (LPD  $> 3$ ), locations with loss of attachment  $> 3$  mm (LAL), gingival index (GI), the bleeding index (BI) and plaque index (PI). CD4 and CD8 lymphocyte counts and percentages and viral load (VL) were also recorded. The frequency of oral hygiene and visits to the dentist were noted. Statistical analysis was made by Kruskal Wallis test.

**Results:** There were no statistically significant differences in periodontal measurements between HIV+ patients and controls: PI (34.3% vs. 27.7%); BI (18.2% vs. 15.9%); GI (3.7 vs. 3.2); LPD  $> 3$  mm (2.9 vs. 2.9); LAL  $> 3$  mm (7.5 vs. 5.3). The average VL was 17596 copies/mL and the media of CD4 count was 537.6 cel/mm<sup>3</sup>; the CD4 percentage was 25.1%, and the CD8 count was 1123 cel/mm<sup>3</sup>, and the CD8 percentage was 50.3%.

**Conclusions:** The immunological and virological parameters showed a clinical HIV asymptomatic state. The high GI and PI were not related neither the immunological nor virological state but there may be concomitants factors, as poor oral hygiene, that could explain this matter.

151

### Influence of cardiovascular risk factors and glycaemic control on periodontal health in diabetes

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**Background:** There has been emerging evidence on the impact of cardiovascular risk factors on periodontal health. Patients with diabetes have been shown to be at increased risk to cardiovascular and periodontal disease. Few studies have been reported on the cumulative effects of cardiovascular risks and diabetes on periodontal health.

**Aim:** To investigate the effects of cardiovascular risks and diabetes on the periodontal status of a cohort of patients with diabetes.

**Materials and methods:** A total of 185 patients were recruited from two diabetic centres. All subjects had a full mouth assessment for plaque, bleeding on probing (BOP), calculus and probing depths. Blood serum analysis was also carried out for HbA<sub>1c</sub>, HsCRP and lipids. Subjects were divided into various risk categories based upon a combination of three serum risk profile targets. The acceptable targets for were: HbA<sub>1c</sub>  $< 8\%$ , HsCRP  $< 2$  mg/L and Cholesterol-HDL ratio  $< 4.5\%$ .

**Results:** More than one third of the patients had at least 2 of the risks above the target level. When the periodontal parameters were compared; subjects with lower systemic risks had lower BOP and probing depths as compared with those who had higher risk profiles (ANOVA  $P < 0.05$ ). Some differences were found between the racial groups.

**Conclusion:** The results highlight a need to consider the impact of systemic risks as well as demographic factors in risk analysis and control of periodontal disease in diabetes.

**Acknowledgement:**

The study is supported by NUS FRC Grant 222-000-017-112.

152

**Gingival bleeding in childhood: an update**

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Gingival bleeding in childhood is not only a sign of the plaque-induced gingivitis. According to various age periods it occurs in association with different local and systemic diseases with periodontal involvement. In deciduous dentition, gingival bleeding with teeth mobility and pocketing may represent periodontal manifestation of congenital, mostly hereditary systemic diseases leading to periodontal tissue damage because of immunodeficiency, enzymatic disturbances or tumorous destruction. Sudden gingival bleeding in children and adolescents associated with acute stomatitis and/or pharyngitis, cervical lymphadenopathy and fever may be a sign of primary herpetic gingivostomatitis and acute myeloid leukaemia. More often, gingival bleeding occurs as a clinical manifestation of plaque-related gingivitis, juvenile hyperplastic gingivitis and of early stages of aggressive or chronic periodontitis in periods of mixed and permanent dentition. Gingival bleeding associated with extremely painful necroses and inadequate oral hygiene in adolescence are typical signs of the necrotizing periodontal disease predominantly affecting immunocompromised individuals. Acute traumatic, chronic granulomatous and desquamative gingivitis are less common causes of gingival bleeding in childhood. The authors believe dental practitioners should be able to distinguish local and systemic diseases with gingival manifestations including bleeding and manage it.

**Acknowledgement:**

Supported by Research Project MZO 001 79906.

153

**Short-term effects of periodontal therapy on serum lipid levels in patients with hyperlipidaemia**

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The literature suggests that there is a relationship between periodontal disease and impaired lipid metabolism. We evaluated the effect of periodontal therapy on total cholesterol (TC), triglyceride (TRG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) in hyperlipidaemic patients. The study population included 50 patients aged 34–66 years with mild or moderate hyperlipidaemia. The subjects were divided into two groups and standard periodontal therapy was performed on the study group. Standard blood chemistry variables including plasma TRG, TC, LDL-C, HDL-C levels and periodontal parameters including plaque index, gingival index, probing pocket depth, clinical attachment level, gingival recession and bleeding on probing measurements were evaluated. Systemic and periodontal evaluations were performed at baseline and in third month of the periodontal treatment. Periodontal treatment resulted in a significant reduction of both periodontal parameters and plasma TC and LDL-C levels. For untreated and treated groups,

mean baseline and third month plasma TC levels were  $237.28 \pm 24.20$  and  $227.88 \pm 30.37$ , and  $244.88 \pm 21.22$  and  $213.60 \pm 32.59$  ( $P < 0.001$ ), respectively. Mean baseline and third month plasma LDL-C levels of untreated and treated groups were  $146.63 \pm 14.82$  and  $142.95 \pm 19.93$ , and  $155.22 \pm 19.02$  and  $115.80 \pm 37.07$  ( $P < 0.001$ ), respectively. This study indicates that periodontal therapy has beneficial effects on metabolic control of hyperlipidaemia.

154

**Correlation between the presence of periodontal pathogenic bacteria in dental plaque and in atherosclerotic arteries**

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Periodontitis has been associated with an increased risk of atherosclerosis and coronary artery disease. The aim of this study was to assess the presence of periodontal pathogens in subgingival plaque and atheroma samples of patients undergoing vascular surgery. 58 patients were enrolled in the study. A complete clinical and periodontal examination was performed, with the collection of plaque samples. Atherosclerotic plaques (eight aorta abdominalis, 11 carotid, 18 coronary, 16 internal mammary arteries, and nine A. femoralis samples) were obtained during surgery. In four cases, coronary and mammary artery specimens were taken from same patient. Specimens were analysed by PCR for the presence of *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia* and *Tannerella forsythensis*. Forty-two out of 58 subgingival plaque samples (72.4%) were positive for *P. gingivalis*, 20 (34.5%) for *A. actinomycetemcomitans*, 37 (63.8%) for *P. intermedia* and 15 (25.9%) for *T. forsythensis*. Bacterial DNA was found in 24 of 62 atheroma samples (38.7%): *P. gingivalis* in 21 (33.9%), *A. actinomycetemcomitans* and *P. intermedia* in 13 each (21%), and *T. forsythensis* in 1 (1.6%). There was a positive correlation between the presence of *P. gingivalis* in biopsy samples and pocket depth of 6mm and more ( $P < 0.05$ ). Periodontal pathogenic bacteria are frequent inhabitants of atherosclerotic plaques, and may play a role in the development and progression of atherosclerosis.

155

**Detection of periopathogens in subgingival and vessel samples in atherosclerosis affected patients**

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**Objective:** The aim of the present study was to investigate the presence of periopathogens in atherosclerotic and healthy vessel samples and try to correlate with both clinical and bacteriological periodontal assessments.

**Materials and methods:** Twenty-two patients treated with atherosclerosis repair surgery were included and allocated to either a moderate generalized chronic periodontitis group (mGCP;  $n = 11$ ) or a severe generalized chronic periodontitis group (sGCP;  $n = 11$ ) after a periodontal examination the day before vascular surgery. Bacterial samples were collected from four periodontal diseased sites in each patient. Specimens from atheromatous plaques, internal mammary arteries and saphenous veins were harvested during vascular surgery. Bacterial detection was done for 20 species using a modification of the 'checkerboard' DNA-DNA hybridisation procedure.

**Results and Discussion:** An increase of the total DNA probe counts and of the main periodontal pathogens appeared in the sGCP group compared to the mGCP group for both periodontal and vessel samples. Surprisingly, the percentage of internal mammary

arteries and saphenous veins colonized by periopathogens were similar than the percentage of atheromatous plaque in which the same pathogens could be detected. These results show clearly that

periopathogens enter the circulation system, which lead to bacteraemia, but call into question the contributing role of these pathogens in the development of atherosclerosis.

## Posters: Clinical tips and cases: Periodontology

156

### Self-reported oral symptoms poorly predict CRP-levels in general population of young adults

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**Objectives:** Previous studies have suggested that periodontitis associates with C-reactive protein (CRP) levels. In addition, stress, insulin resistance, inflammatory diseases, smoking, diet, physical exercise and obesity are known to associate with CRP-levels. Our aim in this study was to investigate the role of self-reported gingival bleeding, tooth loss and dental caries as sources of elevated CRP-levels.

**Materials and methods:** The study population consisted of a subsample ( $n = 8.463$ ) of 1966 Northern Finland Cohort. A health examination included CRP and serum lipid determinations. The participation rate was 71% ( $n = 6.033$ ). Gingival bleeding, tooth loss and dental caries were determined using a postal questionnaire. Potential confounders used in the multivariate regression model included smoking, alcohol use, physical exercise, obesity, angina pectoris, diabetes and rheumatoid arthritis.

**Results:** After controlling for the potential confounders, gingival bleeding and tooth loss had only a slight increasing effect on CRP-levels. The proportion of variation explained by them was low, being less than 1%. The most important determinant was obesity, followed by lipid profile and gender, which together explained about 90% of the explained variation in CRP-levels. The model explained 12% of the total variation in CRP-levels.

**Conclusion:** Self-reported gingival bleeding, tooth loss or dental caries poorly predict CRP-levels among a general population of young adults.

157

### Glycaemic control and periodontal disease: a case report of berardinelli-seip syndrome

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Total Generalized Lipodystrophy or Berardinelli-Seip Syndrome (BSS) is a rare hereditary disease characterized by absence of adipose tissue, insulin resistance and other manifestations like *acanthosis nigricans*, hepatomegaly, hyperandrogenism, muscular hypertrophy and diabetes *mellitus*. A 32 year-old woman with history of BSS presenting persistent uncontrolled glycaemic levels ( $> 600$  mg/dL) and many dental problems was evaluated. Periodontal and dental examinations were performed. Probing depth (PD), clinical attachment loss (CAL), gingival overgrowth (GO), gingival bleeding index (GBI) and presence of decays were assessed. Radiographic exam was performed using panoramic and periapical radiography. The patient had 20 teeth where only one was intact. Eighteen presented only the roots because the crowns were completely destroyed by the decay. A high degree of gingival bleeding (100%) was observed. Gingival overgrowth was present. Periodontal probing showed the presence of periodontal pockets with  $PD > 6$  mm and  $CAL > 7$  mm. Panoramic and periapical radiographies were analysed. Periapical lesions and an

osteoradionecrosis region were detected. All teeth were extracted and after two weeks the fasting plasma glucose (FGB) was 151 mg/dL. One month after the last extractions FGB was 151 mg/dl and 45 days after the last extractions FGB was 131 mg/dL. We conclude that dental infections like periodontal disease can compromise the glycaemic control of a diabetic patient.

158

### Phacomatosis pigmentokeratolica: oral and periodontal manifestations of a rare syndrome

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Phacomatosis Pigmentokeratolica is a rare genetic syndrome. It is supported by the genetic mechanism of twin spotting. Here the case of a young lady. She manifested a speckled-lentiginous nevus on the left shoulder and epidermal nevus spread on the right hemi soma, ipotrophy, dysesthesia, hyperpathia and hyperhidrosis of the right hemi soma; she shows torticollis, left facial hemiatrophy, dextroconvex scoliosis. She developed a primary lymphatic oedema. Radiographic and clinical periodontal examination confirmed a localized severe plaque induced periodontitis with bone loss associated with multiple epulises, oral dysesthesia expressed with monolateral hypersensitivity enhanced on the right side. The syndrome origin from a young post-zygotic mutation that results in twin-spotting phenomenon: the patient would be heterozygous for two different recessive mutations in the same chromosome and at an early stage of embryogenesis a postzygotic recombination would result in two homozygous daughter cell representing stem cells of the two types of nevi. We suppose a genetic substrate influencing pathogenetical response to periodontal pathogens with a different neurological distribution of pain in intraoral areas. We present the case for the specific oral findings: these include gingival localized enlargements and periodontitis, bone loss, dysesthesia. We treated the biggest epulises by surgery 2 years ago, but they relapsed. At the moment these epulises were solved by using a Nd:YAG laser.

159

### Association of radiomorphometric index and relationship with periodontal diseases. Valoration of vitamin D receptor gene polymorphism

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**Introduction:** The objective of this study was to investigate the relationship of periodontal diseases as a radiomorphometric index to measured mandible cortical density and to evaluate alveolar and basal bone in jaws of patients with periodontal diseases and compared with healthy population. Research has therefore focused to evaluate by panoramic radiographs that interaction by radiomorphometric index and then by informatics program compared measured jaw bone mass. In addition, we determined using a PCR-based method genetic polymorphism in the vitamin D receptor (VDR) associated with periodontal diseases.

**Patients and methods:** Selected clinical history between 1998 and 2004 approximately 8000 histories. Of the 480 patients chosen for

inclusion in the study 240 were periodontal disease patients and 240 healthy normal. A nearly even distribution between both sexes, racial groups, smoking habit and age from 30 to 45 years was obtained. Who had neither metabolic disease nor local lesion affecting skeletal metabolism was selected. Radiographs were obtained digitalis and captured by informatic program. We taken the morphometric and densitometry measurements of alveolar and basal bone, we classified periodontal diseases in panoramic radiography accordance with the ruler of Shei. Analysed the genotypes to determine the VDR: 25 patients of Chronic Periodontitis, 25 Aggressive Periodontitis and 25 controls. **Results:** Preliminary or final analysis of this study will be available in Europerio 2006.

160

### Oral lichen planus and desquamative gingivitis: analysis of 59 cases

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Desquamative gingivitis (DG) is characterized by a diffuse erythema of the marginal and attached gingiva, associated with several areas of desquamation. Desquamative gingivitis represents a reaction of the gingival, which conceals other pathological entities such as hormonal disturbances, chronic irritation, lichen planus and pemphigus vulgaris among others. In attempt to determine the frequency of desquamative gingivitis in oral lichen planus we present 59 cases of patients with oral lichen planus attending to the Oral Medicine Department of the Complutense University at Madrid. We found 59 cases of patients with oral lichen planus, 34 of them present reticular lichen planus, 24 atrophic lichen planus and only one bullous lichen planus. There are 30 patients coexisting desquamative gingivitis and oral lichen planus; we found 19 cases of DG and atrophic lichen planus, 10 cases of DG and reticular lichen planus and one case of DG and bullous lichen planus. We study clinical features, diagnosis and treatment of most common oral lesions.

161

### Evaluation of papillary blood flow using laser Doppler flowmetry

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**Objective:** Previous investigations have shown that there is an interaction between gingival blood flow and gingival health. The purpose of this study was to compare the papillary blood flow at sites treated with the blood flow at untreated sites.

**Design:** Twenty persons with resin faced, fixed partial dentures were enrolled in the study. The contralateral natural teeth of the site symmetrical to that of the restorations were used as controls. The blood flow was measured from the middle point of the papilla from both from test and control sites by laser Doppler flowmetry (LDF). The plaque index, papillary bleeding index, and probing depth measurements were recorded.

**Results:** There was a statistically significant difference between the test and control groups in papillary blood flow measurements ( $P < 0.05$ ). In contrast, no significant difference between two sites' clinical indices was found ( $P > 0.05$ ).

**Conclusions:** It should be emphasized that there is an important relationship between resin-faced, fixed partial dentures and papillary blood flow. Blood flow measurements provide information about the microcirculation of the tissues. It seems that it is necessary to evaluate, in detail, the effects of fixed bridges on proximal periodontal health conditions.

162

### Wegener's granulomatosis – report of a case with pronounced gingival alterations as a first sign

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Wegener's Granulomatosis (WG) is a triad involving primarily the vascular, the respiratory and the renal system. Necrotizing granulomatous lesions of the upper respiratory tract, generalized vasculitis in various organs and necrotizing glomerulonephritis are the classic findings. WG may also affect other tissues and atypical presentations have been reported, for example cutaneous and oral lesions. The awareness of these atypical or partial presentations ensures early diagnosis of a disease that is usually fatal if untreated. For the dentist the appearance of an enlarged 'strawberry-like' gingiva may be the first distinctive sign suggesting WG.

**Case:** A 48-year-old male was referred to the Dental Clinic by his dentist. Unusual generalized gingival alterations somehow 'granulated' persisted about 6 weeks and were refractory to treatment. The patient was reasonably suspected of having acute leukaemia. The result of an outpatient bone marrow biopsy performed at the Medical Clinic was negative. Next, a granulomatous disorder was supposed, e.g. Crohn's disease or WG, but the finding of the gingiva biopsy showed no specific indications. Several days after the first presentation the oral situation had worsened and the patient complained of having remittent fever. Within the next week symptoms at the paranasal sinuses appeared. Four days later the patient was hospitalized in the Medical Clinic. The diagnosis of WG was established by serologic findings and the cytostatic therapy started.

163

### Why periodontal patients come to dental office?

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**Aims:** Periodontal patients go to dental office due to different reasons. The purpose of this study is to know these reasons, in order to orient the type of information available in dental service, improving the communication with the patient.

**Materials and methods:** Clinical histories of 47 periodontal patients have been reviewed. These patients came during a period of one year to Faculty of Dentistry of Murcia demanding periodontal care. Special attention was paid to the reasons for consultation.

**Results:** The reasons because of periodontal patients came to the dental school were the following ones: dental cleaning (46.8%), dental revision (25.5%), gingival bleeding (14.8%), gingival recession (4.2%), others (8.5%).

**Conclusion:** Most of periodontal patients come to dental office by no periodontal symptoms. Gingival bleeding is the most frequent recognized by patients, followed of recessions. Any patients came by other periodontal symptoms like sensitivity, dental mobility, migrations or halitosis. For that reason, it seems suitable to have informative brochures in waiting room so that patients become aware from the importance of the periodontal disease, its symptoms and its treatment.

164

### Association between periodontal status and fertility parameters in males attending infertility and *in vitro* fertilization clinics

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More than 400 species of bacteria inhabit the human oral cavity. Some of these have been associated with bacterial endocarditis,

aspiration pneumonia, ischaemic heart disease and low birth weight. Recently, an association has been found between dental infections and infertility. The aim of the present study was to examine the possible association between infertility and periodontal condition. The study population consisted of men attending fertility and *in vitro* fertilization clinics. The test group consisted of men with diminished semen quality, according to the specifications of the World Health Organization. Patients requiring surgical procedures for the retrieval of semen (TESE, FNA or EEJ) were also included in the study. Control group included men with normal semen quality. Patients received a comprehensive clinical periodontal examination followed by routine sperm analysis. Subjects were diagnosed as either healthy (8%), with Gingivitis (48%), Chronic Periodontitis (40%) or Aggressive Periodontitis (4%). Sixty five percent of patients with Oligozoospermia, suffered from Gingivitis compared to only 46% of men with Normospermia. Eighty percent of patients with Azoospermia were diagnosed with Chronic Periodontitis compared to only 40% with Normospermia. These findings may implicate an association between infertility, diminished semen quality and periodontal infections.

165

### Solitary gingival lymphangioma: a case report

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**Background:** Lymphangiomas are rare, hamartomatous, lymphatic malformations that usually involve dermal and subcutaneous tissues. Oral lymphangiomas are most frequently encountered on the tongue and buccal mucosa. The purpose of this report was to present an extremely rare case of localized, superficial lymphangioma observed on the gingiva of a 16-year old girl.

**Materials and methods:** The lesion involved the buccal surface of the interdental papilla between the lower left incisor and canine and appeared as an irregularly erythematous small swelling with a vesicular texture. The patient was in good general health without other abnormalities elsewhere on the body. Local ultrasonic debridement had no beneficial effect and an excisional biopsy was performed to establish a definitive diagnosis.

**Results:** Histological analysis revealed several lymphatic vascular cavities of various size, lined with a flattened endothelium. Most of these lymphatic structures were located superficially, just underneath the overlying epithelium. The endothelial cells were negative for the CD34 antigen. Healing of the excision wound was uneventful and no signs of recurrence were observed.

**Conclusion:** Even though lymphangiomas are encountered very infrequently on gingival tissues, they should be considered in the differential diagnosis of related conditions. Differential diagnosis should include local inflammatory reactive lesions, acquired lymphangiectasia, and other vascular hamartomas or tumours.

166

### Actinomycosis-mimicking periodontitis : a report of two cases

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Actinomycosis is a suppurative systemic infectious disease concentrating in 50% of the cases in the cervico-facial region. It can cause sudden, acute and localized loss of periodontal tissue as encountered in aggressive periodontitis. Two patients have been diagnosed for both periodontal disease and actinomycosis (diagnostic confirmed by biopsy) mimicking localized aggressive periodontitis. Patients with periodontitis could be in certain circumstances more susceptible to actinomycosis because of the chronic mucosal barrier failure and the presence of anaerobic bacteria. Furthermore, considering the severity of the periodontal tissue destruction,

actinomyces species should be reconsidered as not being part of the « protective bacteria » such as described in the literature. The diagnosis based on clinical examination is difficult: the presentations can vary widely mimicking other diseases or even tumours such as lymphoma, less than 50% of the culture is positive and it can subsequently be largely underdiagnosed. The recommended diagnostic methods are biopsy or fine needle aspiration. Recurrence is not rare. The hypothesis of a relationship between these two separate entities could be based on a specific bacterial shift after brushing or periodontal treatment as actinomyces species has been shown as one of the first biofilm recolonizing the tissues a few hours after periodontal treatment.

167

### Hypophosphatemic vitamin D-resistant rickets. a case-report with 30-years follow-up

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The main effect of hypophosphatemic vitamin D-resistant rickets (HVDRR) is impaired mineralization of teeth, bone and cartilage resulting in rachitic changes at the growth plate and osteomalacia. The oral manifestations are multiple abscesses around the teeth, osteodystrophy, enlarged pulp chambers, elongated pulp horns that may reach the dentinoenamel junction and interglobular dentin. Literature on the dental condition in adult patients with this metabolic disease is absent. The long-term follow-up of a case of a HVDRR patient is reported radiographically and clinically. She was treated at the Dental school from the age of 20 to till 50 years. Initially the patient had 27 teeth and minor chronic periodontitis. During follow-up the mean plaque and bleeding on probing varied from 15–30%. No furcation involvement has yet been recorded. Despite of maintenance and good compliance she lost 14 teeth. Prosthetic treatment was necessary to keep the dentition functional. Gradually, horizontal bone loss converted into infrabony defects without signs of inflammation. The cost for keeping the dentition functional was overall 15.500; the yearly expense for periodontal maintenance was 185. Despite of good functional rehabilitation, endodontic and periodontal treatment the condition went gradually downhill due to attachment loss without clinical signs of infection. From a cost-benefit point of view this is disappointing.

168

### Neurofibromatosis with unilateral gingival hyperplasia – a case report

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Gingival hyperplasia is commonly associated with hormonal changes and medications. More unusual causes are systemic diseases like leukaemia and genetic syndromes. Neurofibromatosis (NF) is a group of genetic disorders (NF I-III) that may also manifest in the oral cavity. Oral lesions occur mainly as submucosal nodes but also gingival enlargement, intraosseal defects and vascular malformations have been reported. This case report presents diagnosis of neurofibromatosis in the mandibular ramus and cerebellum of a 13-year-old girl. She was referred to the Turku University Hospital because of unilateral gingival and alveolar hyperplasia. Anamnestically, she was healthy but had had prolonged bleeding after surgical exposure of the lower left molar. At age of seven plexiform neurofibroma had been removed from her left forearm. The face was symmetric and she had several pigmented nodules on the skin. The left side of the gingiva and alveolar processes in both maxilla and mandibular had prominent hyperplasia. Gingival biopsy showed no pathological



findings. Because of radiolucency seen in the panoramic tomography in the left mandibular ramus, wisdom tooth extraction including biopsy at medial side of ramus was performed. Histological diagnosis was neurofibromatosis. MRI examination showed lesions also in cerebellum, which later on were diagnosed neurofibromatotic foci. This case report shows that gingival hyperplasia can be a sign of severe systemic disorders such as neurofibromatosis.

169

### Localized lesions of cervical external root resorption: a case report

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Cervical external root resorption was reported at natural teeth involving one or more teeth in the same patient. The incidence of these lesions appears to be random and the aetiology is still unclear. The aim of the present case report is to present the therapeutic approach and the 2-year results following treatment of multiple external cervical resorptions in a 46-year old Caucasian male patient. At baseline, the clinical and radiographic examination showed circumscribed lesions in the cervical region of the teeth 13, 23 and 33. The affected teeth were vital and the x-ray examination revealed no signs of periapical pathology. Following initial periodontal therapy periodontal surgery was performed involving flap elevation and removal of the granulation tissue, which occupied the resorption cavity. During surgery, an invasion of the bone into the defect was observed and osteotomy performed to expose the cervical defects. The cavity was debrided, shaped and filled with glass-ionomer cement. The histological examination of the removed granulation tissue revealed the presence of multinucleated cells similar to osteoclast indicated a resorptive activity. At 6 months after therapy the soft tissue measurements did not reveal any significant changes compared to baseline values (PPD less than 3 mm) and clinical/radiographic re-examination at 2 years indicated a stable periodontal situation and without any further deterioration of the cervical defects.

170

### The phenotypic overlap of syndromes associated with hereditary gingival fibromatosis: a follow-up of a family for five years

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**Background:** Hereditary gingival fibromatosis (HGF) is characterized by the slowly progressive, fibrous enlargement of gingival tissue. It usually develops as an isolated disorder but can also be one feature of various syndromes. The currently preferred terminology of these syndromes mainly describes the clinical features of the disorder without identifying the cause. In this report, we describe a family with HGF and features of three previously described syndromes: Jones syndrome, Zimmerman-Laband syndrome and HGF-hypertrichosis syndrome.

**Methods and results:** The 45-year-old father had HGF, hypertrichosis, hearing loss and short stubby fingers and toes with hypoplasia of the terminal phalanges, hypoplasia of the nails on the thumbs. The features of 13-year-old son were almost identical to those of his father except for hypertrichosis but in addition he was mentally retarded. While the 10-day-old son had HGF and defective fingers, the mother and 7-year-old daughter was unaffected.

**Conclusions:** Due to the overlap of these syndromes, we argue that the identification of the genetic pathways and mechanisms will be the most important factor in classifying these disorders, with the phenotype playing a minor role.

171

### Clinical evaluation of free gingival graft shrinkage in two dimensions: fifteen consecutively treated patients

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Free gingival graft (FGG) is a predictable method for obtaining a satisfying amount of keratinized tissue. Shrinkage of FGG is a well known clinical phenomenon but there are limited studies demonstrating the dimensional changes during healing in FGG and there is no published study on determining shrinkage of the graft in either two dimensions. Aim of study is to examine shrinkage of FGG in both horizontal and vertical dimensions, calculate changes in surface area of the graft and record the complaints of the patients. Mandibular anterior areas of 15 patients were treated. Graft sizes and areas were measured and shrinkage of graft was calculated at baseline and 10, 21, 180 days. Haemorrhage, sense and pain symptoms were also examined. Five, six and seven patients demonstrated surface area changes at first, second and third follow-up visits respectively. When dimensional changes of graft were analysed, observed changes in horizontal direction were not statistically significant during study period. However there were statistically significant reductions in vertical direction at all visits except 10 days. Calculated area was also significantly reduced during the study period. Five patients had postoperative bleeding, five patients had sense alterations. Eight patients had slight postoperative pain in their both recipient and donor sites. Our findings suggest that reduction in vertical dimension seems to be the major factor affecting the calculated graft area and clinical outcomes of the FGG procedure.

172

### Oral health in diabetic and not diabetic subjects: with especial reference to periodontal diseases

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**Background:** Diabetes is a pathology with a high prevalence in our society. Some authors relate the diabetes with an increase both severity and number of injuries in the oral cavity. The relation between oral pathology and diabetes is for many authors bidirectional.

**Aims:** To value the level of oral health in a group of diabetic patients and other one of not diabetic patients, to compare the level of oral health in both groups and to value the existing relation between diabetes and disease periodontal.

**Materials and methods:** An exploration was realized to a group of diabetic patients and to other one of not diabetics. The variables that were object of study were the following ones: Age, Gender, Consumption of Tobacco, Consumption of Alcohol, Type of treatment of the diabetes, Presence of other associate pathologies, Pathology in oral mucous, dental state by means of CAOD, Community Periodontal Index, Glycated haemoglobin (diabetic patients).

**Results:** We find statistically significant differences between both groups as well as the presence of disease periodontal as associate systemic diseases.

**Conclusions:** Diabetes is not related to the number of caries. Diabetes is related to the disease periodontal. The diabetic subjects present major frequency of other systemic diseases: arterial hypertension and stroke accident. We find major number of oral

mucous injuries in the diabetic patients, although this information does not statistically significant.

173

### Legal aspects and risk management in periodontal practice: case report

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The failure to detect, diagnose, treat or refer a patient for treatment of periodontal disease may be negligent and such failures are often litigated in dental malpractice actions. Failing to diagnose periodontal disease may be responsible of an untreated progressive destruction of the periodontal tissues up to severe or irreversible clinical conditions, frequently resulting in the loss of one or more teeth. Moreover, failing in providing adequate periodontal care may lead to fixed and/or removable prosthetic rehabilitations on teeth with poor or hopeless prognosis. On the other hand, since bacterial plaque is by far the most important etiologic agent for the occurrence of periodontal disease, it is evident that patient compliance in the daily plaque control is necessary to obtain and maintain successful treatment outcomes. It is noteworthy that a noncompliant patient may be adjudged negligent in court, but to support a claim of contributory negligence, the dentist must be able to show that the patient did in fact contribute to his or her injury. In our litigious society, a complete periodontal charting results necessary to document diagnosis and treatment or referral in the case of a lawsuit, inasmuch as only clear and accurate clinical records may prove the best defence in a malpractice suit. From the analysis of a professional liability case, attention is focused on the legal standard of periodontal care, essential to avoid a malpractice claim and to prevent a lawsuit.

174

### Evaluation of the clinical results of the surgical correction of gingival recession 4 years results

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**Introduction:** It has long been one of the objectives of the corrective periodontal surgery to cover the denuded root surfaces. There are several surgical techniques on the field of aesthetic mucogingival surgery. The most widely used techniques are the subepithelial connective tissue grafts and the epithelialized free gingival grafts. The long-term evaluation of the results after surgical correction of gingival recession cases is scarce in periodontal literature.

**Material and methods:** Between 2000 and 2001, 18 epithelialized and 18 subepithelial connective tissue graft operations were performed at the Department of Periodontology, 75% of cases were Miller III-IV defects. The mean age of the patients were 34 years. Only patients with excellent oral hygiene were surgically treated.

**Clinical results and discussion:** The average improvement of the gingival recession after 1 year was 42% with the subepithelial connective tissue graft and 72% with the free gingival graft. The mean width of the keratinized gingiva increased by  $3.15 \pm 2.01$  mm and  $5.25 \pm 2.00$  mm with the subepithelial connective tissue graft and with the epithelialized graft respectively. The follow-up of our cases showed a sustained gingival coverage after 4 years and only minimal additional denudation and gingival shrinkage occurred. The success and the durability of surgical results were dependant on the morphology of the gingival recession, the surgical techniques and the periodontal supportive therapy.

175

### Combined treatment with GTR and connective tissue graft of a periodontal necrosis after RCT with 'radiosurgery' – a case report

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**Background:** The surgical techniques utilizing radiofrequency has had a wide publicity recent years. A 28-year-old female patient - referred to our out-patient service - had root canal treatment with a 'radiosurgical' instrumentations. Around the treated tooth suddenly developed serious gum and alveolar bone necrosis.

**Material and methods:** Around her #12 tooth a necrotized alveolar crest, complete lack of marginal gingival, severe tooth mobility and an opened access cavity were seen at admittance. The treatment was started with wound debridement and provisional Ca (OH) 2 medication was placed in the root canal. The mobile lateral incisor was temporally splinted. Subsequently SRP and definitive root canal filling was performed using step back and lateral condensation techniques. Two months later a full thickness mucoperiosteal flap was elevated and after defect's debridement NBM graft covered with barrier membrane were used. Before suturing a subepithelial connective tissue graft was transplanted under the gingival flap to augment the attached gingival.

**Results:** Both the clinical and the radiological parameters showed CAL gain at 6 months and 1-year controls. The temporary splint was removed at month 18 postsurgically and the tooth became firm.

**Conclusion:** Certainly radiosurgery in dentistry has its well-defined indications. It should be kept in mind that the inadequate application can lead serious periodontal cosequences.

176

### Comparison of genetical, microbiological, immunological findings and periodontal status of dizygous twin

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In this report twin female patients one of who had advanced periodontal disease and the other had initial localized periodontal problem were compared from the view of genetical, microbiological, immunological findings and periodontal status. Type of the HLA antigen was determined by PCR for immunological evaluation. Immunophenotype was examined including CD3, CD4, CD8, CD19, CD3-CD16+CD56, CD45, CD14, CD4/CD8. HCMV, HSV-1, EBV viruses and *A. a.*, *F. n.*, *C. r.*, *P. g.*, *T. f.*, *P. i.* bacteria were quantiated by real-time PCR in the subgingival plaque samples for microbiological evaluation. IL-1A, IL-1B, IL-1RN, IL-6, VDR and OPN gene polymorphisms were determined. HLA antigens were found the same and immunophenotype results were recorded in normal limits. EBV-1 virus was found as  $3.5 \times 10^5$  in only the patient with advanced periodontal disease. In the same patient *C. r.* ( $3.3 \times 10^6$ ), *F. n.* ( $5.1 \times 10^7$ ), *P. g.* ( $4.3 \times 10^7$ ), *T. f.* ( $4.3 \times 10^7$ ), *P. i.* ( $6.1 \times 10^8$ ) were determined. In the other twin no virus detected. However *F. n.* ( $6.1 \times 10^8$ ), *T. f.* ( $2.1 \times 10^7$ ), *P. i.* ( $7.1 \times 10^6$ ) were observed in this patient. Although the genotype analyses of IL-1A, IL-1B, IL-1RN, and OPN gene polymorphisms were the same, VDR and IL-6 gene polymorphisms were found to be different. Even the environmental factors were shared and the immunological response can be thought to be the similar due to HLA antigens match, the microbiological and genetical factors seem to play important roles in the initiation and the progression of periodontitis.

177

# **Labial piercing and localized periodontal destruction – partial periodontal regeneration following periodontal debridement: a case report**

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**Background:** Localized periodontal destruction has been reported as a rare complication of intraoral piercing. The purpose of this case report is to illustrate the destructive nature of a lip stud and to describe the successful treatment of this case.

**Case:** A 26-year-old female patient attended to the emergency dental department with the chief complaint of 'inflamed lower front teeth'. Clinical examination revealed a labial lip stud with an intraoral closure in region 31/41, isolated probing depths up to 9 mm on teeth 41 and 31, increased mobility, recessions, and the presence of calculus. Probing depths of the remaining dentition were within normal limits (1 to 3 mm). The lip stud was removed and supra- and subgingival debridement was performed. Due to a shallow vestibule, the absence of keratinized gingiva, and the strong frenulum insertion at the gingival margins, a free gingival graft was placed.

**Results:** Subsequently the patient demonstrated a significant amount of osseous regeneration and partial coverage of the recession, which has been clinically and radiographically (computer tomography) documented.

**Discussion/Conclusion:** It is generally accepted that periodontal wound healing is age-dependent and is faster in younger individuals than in older individuals. Consequently, since body piercing is popular among young people, a higher potential for osseous regeneration might be expected in this patient population after removing the etiologic agents.

178

# **The aggressive periodontitis in Morocco: heterogeneity of the clinical forms**

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Periodontologists have been studying and listing the various clinical forms of periodontal diseases. Many classifications have been made. Because of difficulties in standardizing, the choice of these classifications, the consensus of the world workshop of 1999 was to go back and through some specific parameters of classifications to simplify the diagnosis of the periodontal diseases: The aggressive periodontitis was born, a new form that has regrouped several entities. Therefore, there are a variety of clinical forms of the aggressive periodontitis observed in Morocco. The confrontation of these aggressive periodontitis, regarding to specific standards of classification, will allow emphasizing the heterogeneity of these pathologies and difficulty of putting a definite diagnosis.

179

# **Periodontal plastic surgery for the orthodontic treatment**

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The orthodontic movements act on all the supporting tissue of the dental organ. The anatomical interdependence makes the superficial periodontal affect the deep periodont. In a way that of the orthodontic therapy implemented comes to a failure. Because of an orthodontic treatment to the child, the adolescent or the adult, the plastic periodontal surgery can have either a preventive or curative results according to the different elements of the superficial and deep periodont. Not to forget the periodontal typology, the

topography of the frenom or the existence of a muco-gingival injury. A careful periodontal clinic examination is therefore a required and essential for a better orthodontic treatment. It helps the patient combine with a therapeutic form that is adapted to his periodontal morphology as well as and to the proposed orthodontic movements propose. The periodontal plastic surgery can then implemented in three levels: in pre, per and post orthodontic. The clinic cases, which represent the different situations, are added. They will be worked on so as to show the situations that need a pre-orthodontic surgical preparation, to define the serious risks of the superficial periodont during the orthodontic treatment and to illustrate specific techniques, aiming to recover some aesthetic damage that could get the periodont after the orthodontic treatment.

180

# **Laser assisted periodontal therapy with the use of ND-YAG laser**

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**Purpose:** To evaluate the use of ND-Yag laser for the treatment of ulcerative gingivitis, early, moderate and advanced Periodontitis. Periodontal defects form as a result of a disease process that is initiated by the presence of subgingival microorganisms. The toxins from the microorganisms provoke a constant inflammatory state that gradually breaks down the epithelial attachment and leads to resorption of the alveolar bone. If the disease is not untreated, periodontal bone loss will progress to tooth mobility and eventually to tooth loss. The ND-Yag laser has bactericidal effect, deactivates the endotoxins, alters the root surface and biostimulates the cells of the host for tissue and bone regeneration. The ND-Yag laser has a penetration depth up to 1100 µm.

**Materials and methods:** In this study we used the ND-Yag laser to treat four patients with ulcerative gingivitis, early, moderate and advanced Periodontitis. We performed conventional and surgical therapy as needed. In conventional therapy no anaesthesia was necessary. In the surgical phase and especially at gingivectomy topical anaesthesia was used and no stitches were needed.

**Results and conclusion:** The case involving ulcerative gingivitis had immediate improvement clinically and systemically, due to the reduction of fever and lymph glands swelling. At the intrabony defects we found: reduction of periodontal pockets, gain of attachment level, no bleeding on probing and reduction of tooth mobility.

181

# **Soft tissue grafts to manage lesions of oral pemphigus: a case report and review of the literature**

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**Introduction:** Pemphigus is a rare cause of desquamative gingivitis. Description in the management of a patient with periodontitis and multiple oral lesions of pemphigus vulgaris is presented.

**Material and methods:** A 40-year-old female with a 3-years history of painful, ulcerated, and bleeding gingiva, diagnosed of pemphigus vulgaris 7 years ago. Patient was treated with systemic and topical corticosteroids. Due to side effects, physicians suspended their use. Other treatments were tried (dapsone) with no success. After clinical and radiographic evaluation the patient was diagnosed of moderate periodontitis and oral pemphigus lesions. The patient received oral hygiene instructions and the use of a 0.2% chlorhexidine mouthrinse for 15 days. Instrumentation with local anaesthesia was performed to eliminate supra and subgingival calculus. Six weeks after treatment

pocket depths were reduced in general, but the patient still referred soreness, spontaneous bleeding, and difficulty to brush in several areas. After her consent, soft tissue autografts in those areas were performed.

**Results:** After 24 months, the patient shows the excellent clinical appearance of the gingival tissues and no bleeding and soreness is referred.

**Conclusion:** This report demonstrates the successful treatment with soft tissue autografts of multiple buccal gingival pemphigus-associated lesions in a patient with pemphigus vulgaris with relative contraindication to corticosteroids.

182

**Clinical crown lengthening in high aesthetic areas: a new surgical technique**

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The objective of this work is to illustrate a variation of the clinical crown lengthening surgical procedure for the restoration of dental elements of aesthetic interest. Our department treated 17 cases of single teeth (15 upper and two lower) with this modified surgical technique. Following a sulcular incision both in the buccal and palatal/lingual side of the compromised tooth, a total thickness debridement reveals the bone crest. The osteotomy and the osteotomy are performed with burs and scalpels. Two vertical buccal incisions, with a very long internal bevel are made, which start at the papilla's base and extend beyond the mucogingival line. The flap is divided in three parts (two papilla and one intermediate sector) and cut to partial thickness to be repositioned apically at different levels. The intermediate part is stabilized more apically to increase, in the apical coronal sense, the gingival margin, minimizing the formation of interproximal spaces and the flattening of the gingival profile. Stabilizing stitches are positioned. This technique has specific indications for the frontal upper area, for defects that are more accentuated buccally and the great advantage of treating all gingival biotypes. A small correction of the roundness of the gingival profile sometimes becomes necessary later. This modified clinical crown lengthening procedure in aesthetic areas is a valuable solution since it is easily performed, it has good predictability and great aesthetic results.

183

**Treatment of amlodipine-induced gingival enlargement: a case report**

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Gingival enlargement may be seen in patients taking amlodipine, a relatively new drug belonging to the calcium channel blocker group applied to hypertension and angina. This is a report of periodontal management of a 54-year-old hypertensive male who had been using this drug 10 mg/day for about 10 years. His complaints were gingival swelling, spontaneous bleeding, difficulty in chewing and speaking, and poor appearance. Moderate to severe gingival enlargement was observed on both mandible and maxilla, with more pronounced enlargement at the upper right molar area. Gingiva was also oedematous, red, smooth and shiny, and bled easily on probing. The amlodipine was replaced with an angiotensin II receptor blocker after consultation with his physician. Oral hygiene instruction, scaling and root planning with adjunctive use of chlorohexidine and, extraction of 16, 26, 28, 48 teeth were performed. After initial treatment, gingival enlargement was so reduced that surgical removal of overgrown gingival tissues was performed in certain areas only. Change from amlodipine to another drug and periodontal treatment resulted in significant

improvements in clinical parameters (1 year changes, plaque index 2.3, sulcus bleeding index 3, probing depth 2.8 mm, gingival hyperplasia score 2.3), function and aesthetics. A combined approach of periodontal treatment and suspension of amlodipine was effective in the management of gingival enlargement up to 1 year after completion of treatment.

184

**Connective tissue grafting on resin ionomer in localized gingival recession: a case report**

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One of the main objectives of periodontal reconstructive surgery is the coverage of exposed roots that occurred due to gingival recession. In some occasions, where caries, root resorption or an amalgam filling exists on the exposed root surface, the treatment planning becomes more complex. This case report describes the use of a subepithelial connective tissue graft on resin ionomer-restored root surface to treat a gingival recession that is complicated with all the abovementioned handicaps. Amalgam filling and caries lesion were removed following full thickness flap reflection and the cavity was restored with glass ionomer cement. A subepithelial connective tissue graft was placed onto the restoration and the flap was coronally positioned. Porcelain crown restoration was performed 9 months after surgery. At 3 and 9 months follow-ups, probing depths were reduced and gain in attachment level was obtained with no clinical signs of inflammation in gingiva. Monthly periodontal controls revealed that creeping attachment has occurred on the restoration during the follow-up period. This single case report serves as a good example to show that subepithelial connective tissue grafting can be successfully performed to treat gingival recession associated with glass ionomer-restored root surface.

185

**A multidisciplinary treatment of idiopathic gingival overgrowth in a class iii malocclusion: a case report**

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**Background/Aims:** Idiopathic gingival overgrowth is a rare disorder, characterized by proliferative fibrous increase of gingival connective tissue, teeth mal-position and retention. These clinical features associated with a Class III malocclusion cause difficulties to orthodontic therapy. Recurrence of the gingival overgrowth after periodontal surgery may occur. The aim of this presentation is to show, after 3 years, the results of a multidisciplinary approach to treat the combine disorders.

**Materials and methods (Treatment Approach):** A 16-year-old male presenting generalized severe gingival overgrowth, covering almost all teeth, with a Class III malocclusion was treated with a reverse bevel gingivectomy, without osteotomy, under general anaesthesia, mainly to allow the orthodontic therapy. At the end of treatment, gingival contour was corrected using the same surgical technique. Histological analysis of the excised tissues was performed.

**Results/Discussion:** The surgical postoperative course was uneventful and, after 3 years, no recurrence of the overgrowth was found. After orthodontic and periodontal therapy a good aesthetic outcome was achieved.

**Conclusion:** This multidisciplinary treatment of idiopathic gingival overgrowth in a Class III malocclusion seems to have good results. Teeth sequestrum may complicated the orthodontic and periodontal therapy however a good occlusal and aesthetic outcome was accomplished.

186

### A split – mouth study to determine the effect of bioactive glass (novamin®) in the treatment of teeth hypersensitivity post periodontal surgery

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**Introduction:** The dentinal hypersensitivity is a usual problem that affect among 8–30% of the adults, especially with periodontal disease. In the market, there are several agents to treat this problem. Bioactive Glass (Novamin<sup>®</sup>) is a new product, which reduce the hypersensitivity, by forming crystals of hydroxyapatite that occlude dentinal tubule.

**Objective:** The aim of this study using a placebo control is to assess the therapeutic value of Bioactive Glass (Novamin<sup>®</sup>) in dentine hypersensitivity after periodontal surgery.

**Material and methods:** The study is a single-centre, randomized, placebo controlled, split – mouth design with active and placebo preparations applied to test teeth in contralateral arches, after a periodontal surgery. 30 patients with at least two similar periodontal surgery in contralateral arches are recruited into the study. Sensitivity was scored by the subjects on a 0–10 visual analogue scale, before the surgery, and one week and one month, after the surgery.

**Conclusions:** All the recorded results will be analysed to determine if Bioactive Glass (Novamin<sup>®</sup>) is effective in the treatment of the dentine hypersensitivity.

187

### The effects of topical doxycycline in the periodontal management of Down syndrome patients

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Individuals with Down Syndrome (DS) have an increased prevalence of periodontal diseases. Our previous investigation found that mechanical debridement alone in DS patients largely failed to prevent tooth loss (Tenorio et al., 2003). The aim of this study was to test the efficacy of adjunctive topical Doxycycline (8.5% Doxycycline oral gel, Atridox<sup>®</sup>) for the treatment of DS-associated periodontal disease. Subjects received full periodontal assessment and a split mouth design was adopted. OHI was given and the test side received nonsurgical therapy and a single application of doxycycline gel to pockets >5 mm. The control side received nonsurgical therapy alone. After 1 week there was an overall reduction in gingival inflammation in test sites clinically and reduction of DI. BI score remained unchanged. At baseline, mean figures for pocket depth (45 sites) were 5.9 mm in test and 5.8 mm in controls. At 12 weeks mean test sites were 4.7 mm against 5.3 mm in controls. The results show an average decrease in pocket depth of 1.2 mm in test as compared with 0.5 mm in controls. Mobility was also decreased. DI and BI figures remained unchanged. Our findings are in agreement with previous studies on the efficacy of adjunctive topical antimicrobials. The results support the use of Doxycycline as an adjunct to conventional therapy in DS patients although longer term follow-up would be useful to determine the potential benefits of this treatment.

188

### Recurrent ulcerations on attached gingiva in a patient with iron deficiency anemia

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This is the case report of a 24-year-old female patient suffering from bleeding gums and recurrent oral ulcers (ROU), who was

referred to our clinic. Her clinical examination revealed chronic marginal gingivitis due to poor oral hygiene and mouth breathing. Recurrent minor and major ulcerations were present in any location of the oral cavity, atypically on attached gingiva and hard palate close to gingiva. Initial periodontal therapy performed to remove plaque and calculus resulted in resolution of present inflammation. At 3 and 6 months recall, ROU were still present. Because of these observations, Behçet's disease or systemic effect of a haematological disease was suspected and the patient was referred to dermatology and haematology clinics for further examinations. Pterygia test and serological analysis of Herpes Simplex Virus type 1 and 2 were negative. However, haematological analysis demonstrated anaemia of iron deficiency. After treatment with oral iron supplementation, anaemia resolved and major oral ulcers healed. Minor ulcers were observed rarely in menstrual cycles during 5 years follow up period. Although the relationship between these ulcers and iron deficiency anaemia is not clear, this association needs to be further evaluated.

189

### Benefits of laser treatment as an adjunct to conventional periodontal treatment: a case series study

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Recently Nd:YAG laser is used for enhancing the results of conventional phase I periodontal treatment, which consists of scaling and root planning (SRP).

**Aim:** To evaluate the benefits of laser treatment as an adjunct to conventional periodontal treatment in clinical means.

**Materials and methods:** Four periodontitis patients who had moderate or advanced were included in the study. Plaque Index (PI), Sulcus Bleeding Index (SBI), Pocket Depth (PD) and Clinical Attachment Level (CAL) were used for clinical evaluation. After SRP in all sites, patients' 1st and 4th quarters received Nd:YAG laser treatment (SRP + L) in this split mouth study. The laser treatment was performed three times in the same week. The Nd:YAG laser that was used had 1064 µm wave length, 100 mj/pulse energy, 20 Hz frequency, power of 2 W and was used in very short pulse mode. No statistical method was performed.

**Results:** In the SRP received sites PI scores reduced from 1.74 to 0.82; SBI scores reduced from 3.06 to 2.15. In the SRP + L received sites PI scores reduced from 1.76 to 0.95; SBI scores reduced from 3.25 to 2.30. SRP received sites with 4–6 mm. PD showed a reduction of 1.72 mm, whereas SRP + L received sites decreased 1.91 mm. The difference of PD in SRP received sites with 6–8 mm was 3.45 mm, whereas SRP + L received sites showed a reduction of 4.55 mm.

**Conclusion:** Laser treatment in addition to conventional periodontal therapy showed positive effects on clinical values.

190

### Vitamin complex on periodontal wound healing

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Nutrition is one of the modifying factors that can influence the immune response and the integrity of host's hard and soft tissues. Vitamin-B complex may be important for periodontal wound healing. Vitamin-C can influence on gingivitis early stages and on decreasing gingival bleeding. Vitamin-C deficiency could alter the permeability of the sulcular epithelium. Low levels of calcium could influence a more aggressive progression of periodontal disease. Vitamin-D stimulates osteoclasts activity, which mobilizes bone calcium. Vitamin-E can accelerate wound-healing process

during early stages of granulation and epithelialization. Vitamin-E and Selenium inhibit oxygen free radicals from phagocytic inflammatory cells that can participate on collagen destruction.

**Objectives:** The aim of this study is to assess if nutritious complex LACER VIT improves tissues response to periodontal surgery flap.

**Material and methods:** This study is a randomized, double-masked, placebo-controlled clinical trial. A total of 30 patients with generalized moderate to severe chronic periodontitis are being studied. All of them presented two or more teeth in the same quadrant with probing depth equal or  $> 5$  mm and bleeding upon probing in need of access flap surgery. Fifteen subjects will be instructed to take two capsules a day of Lacer Vit (test group) and the other subjects will take a placebo (control group). Measurements of PD, CAL, BOP and GI will be taken at baseline and 4, 8, 12, 24 and 48 weeks.

191

**The menopause and the climacteric when the periodontal diseases occur**

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The periodontal disease is characterized by its multi faceted aetiology, which has the presence of the bacterial plaque, as its own determinant condition. Currently, the knowledge about this disease elucidates the acting of others associate elements, among them the hormonal alterations present in menopause or the climacteric, which influence in the severity and the progression of the disease. The purpose of this article is to discuss over the relationship of menopause with the periodontal disease, because during this period of the life of women there is reduction of estrogenic and progesterone hormones, resulting in physiological alterations, with manifestations also in the mouthpiece cavity such as xerostomia and desquamated gingivitis. Another common discovery in this phase is osteoporoses and osteopeny, which occur in the mouthpiece cavity such as accentuated osseous wastage, endangering the dental stability, which can induce to the premature lost of the units. All these alterations are worsened by the emotional instability, which characterizes menopause and climacteric, being this subject of essential importance within the context of the periodontal medicine, because the function of the psychosocial factors is already well documented in the immune answer, which guarantees the integrity of the general health of the individuals.

192

**Incidence of periodontal manifestations in mucocutaneous diseases**

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In the 1999 international workshop on the classification of periodontal diseases, the non-plaque-induced gingival lesions include the manifestation of systemic conditions. Mucocutaneous disorders in gingiva used to show as redness and persistent gingival soreness that is named as desquamative gingivitis (DG). Diagnosis is based on the history and clinical features, but biopsy and direct immunofluorescent staining may be needed.

**Material and Methods:** A total of 27 patients with DG were enrolled in this study, their average age was 61.2 years. The diagnosis of the disease was made after take a biopsy and histopathologic study. The patients were evaluated at two and four weeks and two months, and the results were classified as partial remission if they improved the symptoms or the clinical signs, and as complete if they disappeared both.

**Results:** Of the 27 patients, 66.6% corresponded to OLP; 22.2% to mucous membrane pemphigoid, 3.7% to pemphigus and 3.7% to

linear IgA disease. The treatments were: topical steroid therapy to 77.7% of the cases, systemic steroids to 7.4% of the cases, chlorhexidine digluconate to 7.4% of the cases, 3.7% with systemic sulfone and a 3.7% was not treat for being asymptomatic. After treatment with topical steroid therapy resulted in complete remission of 57.1% of the patients and in partial remission of 42.8% of the patients. The patients treated with systemic therapy resulted in partial remission.

193

**Guided tissue regeneration following treatment with two bioabsorbable membranes in combination with DFDBA**

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The purpose of this study was to compare the clinical efficacy of a combination of calcium sulphate barrier and DFDBA to collagen membrane and DFDBA for the treatment of human periodontal defects. Using a split-mouth design, eighteen pairs of intrabony periodontal defects were treated and surgically re-entered six months after the initial surgery. Periodontal defects were randomly treated with either a combination of DFDBA with calcium sulphate barrier experimental and DFDBA with a collagen membrane control. Clinical measurements were recorded at the baseline and after six months when all study sites were surgically re-entered for evaluation. Preoperative probing depths, clinical attachment levels and intra-operative bone measurements were similar for the experimental and control sites at baseline and showed statistically significant improvement six months after the surgery ( $P < 0.001$ ). Between experimental and control sites there were no statistically significant changes in pocket depth (PD), clinical attachment level (CAL) and bone fill (BF) six months after the surgery (mean PD reduction  $3.16 \pm 1.42$  and  $3.34 \pm 1.51$ , mean CAL gain  $2.63 \pm 1.60$  and  $2.85 \pm 1.68$ ) ( $P > 0.05$ ). Re-entry evaluation revealed a mean BF  $2.38 \pm 1.32$  experimental and  $2.41 \pm 1.26$  control sites ( $P > 0.05$ ). Improved clinical measurements were achieved with DFDBA and CS as well as DFDBA and collagen membrane, which indicate that DFDBA with CS represents a therapeutic combination that can be highly effective in periodontal regeneration.

194

**A randomised study to value the efficacy of the diode laser application with a bioadhesive gel of potassium nitrate 10% in dentine hypersensitivity**

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To compare the efficacy on dentine hypersensitivity (DH) evaluated by the examiner using Evaporative Stimulus (ES), Tactile Stimulation (TS) and Global Subjective Evaluation (GSE) of two treatments: application of the diode laser with another treatment based in the application of a bioadhesive gel of NK 10%, taking as reference a placebo laser and a placebo gel. A consecutive sample of 60 patients were informed and were willing to participate in a pilot triple blind study, controlled with placebo and a random distribution treatment for 60 days. The volunteers suffer DH and will be randomly divided in three homogeneous groups: test LASER: Diode laser and placebo gel. Control +: Placebo laser and NK10%. Control -: Placebo laser and placebo gel. Once in the research, the patient will be checked in the clinic in order to take the baseline data and next it will be applied an only one session of the diode laser or placebo laser on the teeth that suffer DH. At 15 and 30 min after putting the laser irradiation it will be taken

another data and it will be written down. From there on, the patient will use each 12 h/day, NK10%gel or placebo, applied by him/herself on the teeth with DH for 15 days. New data will be taken after 2, 7, 14, 30 and 60 days. The experimental phase will be finished January 2006.

195

### Localized aggressive periodontitis in a prepubertal patient: case report

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Periodontal diseases are among the most frequent diseases affecting children and adolescents. These include gingivitis, localized or generalized aggressive periodontitis and periodontal diseases associated with systemic disorders. A 12-year-old boy presented with generalized gingival inflammation, extensive localized bone loss, and mobility of 32, 31, 41, 42, as well as the beginning of bone loss in the first molars. Clinical and RTG examinations led us to the diagnosis of aggressive periodontitis. Neither unusual infection, nor traumas were detected. Also, there was no sign of the periodontal disease in the child's parents. Microbiologic examinations revealed the presence of *Actinobacillus actinomycetemcomitans*. Treatment consisted of supragingival and subgingival debridement, devitalizing and splinting mobile teeth, root scaling and planning combined with 10 days prescription of amoxicillin and metronidazole. Basic immunological tests revealed decline in Natural Killer cells and CD 56 deficiency. Clinical and microbiologic follow-up was continued over next year. After six months *A. actinomycetemcomitans* could not be isolated, and although the situation is at present stable, the long-term prognosis is questionable, because of the late diagnosis, the great amount of bone loss initially presented immunologic status.

196

### Designing Brazilian smiles: experiences from periodontology

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Ideally the smile should expose minimal gingival, therefore patients with gummy smile and passive eruption altered or excessive marginal gingivae, usually excessive gingival display because incomplete anatomical crown exposure is present. If the maxillary incisor show at rest is optimal, active upper incisor intrusion should not be initiated. To achieve a smile with minimal gingival exposure, the anatomic crown should be fully exposed by surgical crown lengthening. Precise determination of the location of cemento-enamel junction prior to surgery, precise placement of incisions and correct establish of biological width are necessary in order to achieve this goal. One protocol is described and clinical results from 20 Brazilian subjects, after three years post surgery are showed.

197

### Manifestation of systemic diseases in the periodontium – a report of two cases

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A variety of systemic diseases could manifest in the periodontium especially in the gingiva. These include mucocutaneous disorders such as lichen planus, pemphigoid, pemphigus vulgaris, erythema multiforme and lupus erythematosus; gastro-intestinal disease such as Crohn's disease, ulcerative colitis and haematological disorders such as leukaemia. The periodontist could play an important role

in the diagnosis of these conditions as they might first present in the oral cavity. Early diagnosis would ensure appropriate management are instituted as soon as possible. This is a report of two cases, acute myeloid leukaemia and pemphigus that manifested in the gingiva.

198

### Investigation on the use of a micro-endoscope for open and closed periodontal treatment

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**Aims:** The use of a newly developed micro-endoscope (Gyrus, Tuebingen, Germany, according to a design established by the author) for the optical control of open and closed periodontal treatment was investigated.

**Materials and methods:** Under direct view, a flexible micro-endoscope with a 200-µm fibre and a 1.00-mm optics was used in 16 patients. The endoscopic examination occurred in the closed pocket for visual control before and after supra- and sub-gingival scaling and root planning as well as within the scope of an open periodontal-surgical treatment. Clinical documentation was performed by video or digital storage.

**Results:** Due to the small optics and additional flexibility new perspectives arise for the periodontology with respect to documentation and therapy control also in the distal area of posterior teeth. The endoscopic support of periodontal-surgical treatments permits further findings such as caries (radiologically not verifiable) and concretions residuals. The extent of bone defects, the identification of root fractures and root fissures as well as insufficient crown margins can be diagnosed and documented intra-surgically.

**Conclusion:** The endoscope used therefore proved suitable for secure pre- and post-surgical diagnosing and documentation in conservative and surgical periodontology.

199

### Sliding overlap pedicle flap design in mandibular second molar surgery

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Reduction of periodontal pocket in the mandibular retromolar region has been clinically challenged by difficulty in access, osseous anatomy, quality and quantity of the gingiva, and by the presence of vital structure in the immediate surroundings. Distal wedge procedure and variations have been utilized commonly, however, the respective procedure does require abundance of gingiva pre-operatively, which may not be common. The lingual nerve, according to the literature, may lie within the retromolar triangle. The Sliding Overlap Pedicle (SOP) Flap was devised to reduce redundant tissue, but to ensure preservation of gingiva, and to honour vital structures. An oblique distal releasing incision is made from the disto-lingual line angle of second molar. It is approached facially as it advances distally as to follow the bony anatomy. Once reflected, the facial aspect of the flap is thinned appropriately. The lingual flap is raised in full thickness. On completion of osseous surgery, the flaps are reproximated, and the amount of tissue overlap assessed. The superficial aspect of the lingual flap is depithelialized via means of fine haemostat and surgical blade or scissors. The periosteum is left intact as this is most likely region where lingual nerve is housed. The excess buccal flap is overlapped, sliding over the depithelialized lingual flap and sutured appropriately. Predictable clinical results were observed in 164 consecutive cases, over a four-year period.

200

### **Nonsurgical periodontal therapy in smokers: a concept to redefine?**

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Cigarette smoking is a strong predictor of progressive periodontitis, the effect of which is dose related. Given the effect of smoking on the prevalence and severity of periodontal disease, and its multitude of effects on the host, it has been also demonstrated a poor response to periodontal treatment in smokers compared to non smokers. Smokers showed less gain of attachment and remain culture positive for periodontal pathogens after therapy compared to non smokers. The objectives of this presentation are to determine through a literature revue and under the light of clinical cases, an evidence based therapeutic which combine the effect of smoking cessation on response to conventional scaling and root planning, the association of topical subgingival applications of antibiotics and the use of a new generation of ultrasonic instrumentation.

201

### **Evaluation of morbidity after periodontal surgery**

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Evaluation of morbidity after periodontal surgery.

**Introduction:** Postoperative course after surgery is a mayor concern for dentists and patients. Also, it is well known that the professional experience is one of the most important factors that can affect the postoperative days.

**Aim:** Evaluate the postoperative morbidity after periodontal surgery comparing the results between experienced periodontists and postgraduate students of periodontics.

**Material and methods:** The number of patients evaluated will be of at least 30, all of them attending different offices from experienced periodontitis and also the university clinic. A questionnaire will be delivered to the patients the day the sutures will be removed. The next parameters will be recorded: systemic diseases, usual medication, toxic habits, medications used after the surgery and its compliance, teeth sensitivity to percussion, thermal sensitivity, swelling, pain experience, and other.

**Results:** The results will be obtained with an analysis of variance statistic program in which the responses from the patients will be entered. Relation between professional experience and the postoperative course after the surgery will be determinated.

**Discussion and conclusion:** The experience of the professional is one of the main factors that affect the postoperative course of the periodontal surgery.

202

### **Optimum timing of performing periodontal surgery in the adult orthodontic patient**

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*Priv. Clinic ltd to Periodontics and Implants*

The control and elimination of gingival inflammation through conservative periodontal treatment is the prerequisite for the initiation and the progress of the orthodontic treatment in adult patients with periodontal problems. Some patients will benefit from the initiation of periodontal surgery before orthodontic treatment and others may receive benefits through a reversal of these procedures. Proper sequencing may not only improve the periodontal stability of the affected area, but also be critical to the esthetic considerations in the patient's treatment. The purpose of this oral presentation is to define the factors leading to the selection of the optimum time for the performance of periodontal surgery in

relation to orthodontic treatment. Eight cases with combined periodontal – orthodontic problems will be presented in order to define the advantages and limitations of performing periodontal surgery prior or after the removal of orthodontic appliances. Long term results with attractive, esthetic dentitions and smiles, which provide the stability of combined therapy result, are present in most of the cases. In conclusion, good prognosis of therapeutic results after a combined periodontal – orthodontic treatment in adult patients is directly related in many cases to the choice selection of optimum time for the performance of periodontal surgery in connection with the application of orthodontic treatment.

203

### **Aggressive periodontitis: multidisciplinary approach**

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Aggressive periodontitis (A.A.P 1999) are characterised by inflammation, gingival recession, tooth mobility, sometimes suppuration and dental migration. One aim of periodontal therapy is to arrest further loss of periodontal attachment and to ensure an aesthetic and functional outcome (Van Winkelhoff 2000). Rehabilitation of dentition with aggressive periodontitis disease is a complicated treatment procedure which requires skills in several disciplines of dentistry. The functional, esthetic and post treatment stability demands of each case dictate the need for a team approach including periodontist, orthodontist and prosthodontist. There must be an agreed upon treatment plan by all disciplines involved with treatment objectives and limitations clearly understood (Gazit, Lieberman) we will illustrate our work with clinical cases of aggressive periodontitis with multidisciplinary approach.

204

### **Histological analysis of palatal healing following connective tissue graft harvesting**

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The purpose of the study was to histologically evaluate the healing of the soft tissues following the harvesting of a palatal connective tissue (CT) graft. Eight patients requiring multiple root coverage or soft tissue augmentation procedures were harvested the second, third or fourth CT-graft at 4–6 months following the first or second harvesting procedure. No prolonged paresthesia of the palate following either first or second harvesting was observed in any of the patients. Patients were harvested a second CT-graft from the previously harvested area and submitted to histological analysis. Clinically, all CT-grafts harvested a second time revealed a slightly more fibrous consistency than that of a normal CT-graft. Tissue volume was always more than adequate at the second harvest. Clinical performance was identical to tissue from the first harvest. Histologically, all reharvested CT-grafts presented as being more fibrous than normal palatal soft/connective tissue. The tissue was less vascularised and reduced innervation was observed. Glandular and adipose components were limited. There was slight scarring following CT-graft harvesting at a timeframe of 4–6 months post-operatively. It was concluded that: (a) the palate can be used successfully for a second harvest (from the same side/region) of a CT-graft 4–6 months after the first harvest and, (b) clinically, the second harvest performs equally well to a first harvest CT-graft.



205

### Use of glass fiber reinforced composites as part of the treatment options in periodontally compromised patients

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Periodontal splinting has been successfully used as part of periodontal treatment although there is no actual scientific evidence of its effects on the health of periodontal tissue, or its role in the preventing the progression of the periodontal diseases. It has been shown that teeth with healthy but severely reduced periodontium can be maintained for long time with the aid of splint or fixed bridges. Traditional metal-frame bridges and splints are good but expensive and time-consuming treatment option. For chair-side use splinting materials such as metal wires, aramid, polyethylene and glass fibers have been used widely. There are considerable differences between the different fibers available, depending mainly on whether they have been pre-impregnated with monomer resin or polymer. Proper bonding between the fibers and composite and correct design of splints are key factors for the success. Pre-impregnated glass fiber reinforced composite (FRC) splints have gained popularity due to their minimally invasiveness, handling properties and esthetics. Therefore, FRC can offer dynamic and cost effective alternatives in stabilizing periodontally involved teeth or replacing single or multiple missing teeth. This presentation describes the advantages of novel pre-impregnated glass fiber reinforcement (everStick®) and its use in the treatment of periodontal patients. Patient cases will be presented and discussed to illustrate different treatment solutions and designing principles.

206

### Corticotomy-facilitated orthodontics on patients with chronic periodontitis

S. Clotter\* and R. Poturak

*Unicare Privat Praxis*

Frequently Orthodontic therapy is equivalent to bone loss and root resorption. Hence, it can be assumed that the treatment of patients with chronic periodontitis is challenging. This presentation describes the influence of inflammation-mediated osteopenia on the regional acceleratory phenomenon during healing and regeneration of bone by using a corticotomy-facilitated and augmentation technique in conjunction with orthodontic therapy. 18 Patients with chronic periodontitis were treated with corticotomy-facilitated orthodontic procedure (AOO). Plaque scores, probing depths, and probing attachment levels were recorded before and after the completion of the orthodontic treatment. During the orthodontic treatment of adults with advanced periodontal diseases; it is probable that a further bone and tooth loss will take place. Also the reflection of full-thickness flaps result in future bone resorption. It is therefore important to combine the surgical procedure with local bone augmentation and find a constructive way for a successful orthodontic therapy. It appears corticotomy-facilitated orthodontics in combination with bone augmentation makes it even possible to treat patients with a remarkable bone loss and greatly decrease the overall treatment time. From our point of view, tooth movement can be achieved into a bone graft made of demineralized bone matrix (Grafton®) and with a biomaterial (Bio-Oss®). Further studies are necessary to examine the various effects of this procedure.

207

### Tacrolimus is not related to drug-induced gingival overgrowth in renal transplant patients

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**Aim:** The aim of this study was to examine the prevalence and severity of gingival overgrowth (GO) in patients using tacrolimus and to compare demographic, pharmacological and periodontal parameters associated with GO of these patients with those using cyclosporine (CsA).

**Methods:** Oral examinations were performed on 46 patients receiving tacrolimus and 97 patients taking CsA. All renal transplant patients were at least 12-month post transplant, medicated with either CsA or tacrolimus. Tacrolimus group had been taking tacrolimus since transplantation. Demographic and pharmacological data including age, gender, time since transplant, calcium channel blocker, diabetes, azathioprine and prednisolone dosage were recorded. Periodontal parameters including plaque index (PI), papillary bleeding index (PBI) and hyperplasia index (HI) were recorded for whole mouth. Patients with HI index scores 2 or 3 at  $\geq 6$  sites were considered as having severe GO.

**Results:** Age and gender distribution were similar between tacrolimus and CsA group. PI and PBI scores of the two groups were also similar. None of the patients in the tacrolimus group had GO (HI = 0). On the other hand, 32% of CsA group had severe GO. Pharmacological data were not different between both groups.

**Conclusion:** These results indicate that tacrolimus is not associated with GO in renal transplant patients. Therefore, this drug could be considered as an alternative drug in patients who are susceptible to CsA-induced GO.

208

### Orthodontic treatment of Moroccan patients with aggressive periodontitis

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The aggressive periodontitis causes localized breakdown of periodontal attachment. The common symptom is rapid attachment loss (AAP 1999). The attachment loss can result in pathologic migration of the teeth. Orthodontic treatment of this disease not only improves esthetics and function but also helps prevent inflammation and the recurrence of periodontal breakdown. In Morocco, the frequency of the aggressive periodontitis is increased. Orthodontic therapy may be needed for alignment of teeth which have pathologically migrated following severe bone loss associated with aggressive periodontitis. Migration was present on anterior teeth as flaring (fanning out labially), diastema, rotation, and extrusion. The potential detrimental affects to the periodontium from moving teeth with existing active disease must not be outweighed by the desire to improve aesthetics (Zachrisson, 1997). Initial and corrective periodontal therapy should be completed before initiating orthodontics therapy. Plaque should be controlled. There has to be sufficient bone for orthodontic treatment to be undertaken, and permanent retention of the aligned tooth/teeth may be required. Treatment planning should be undertaken jointly between the orthodontist, periodontist, and patient. Supportive periodontal maintenance is essential during orthodontic treatment (Clerehugh and Tugnait, 2001). Through our work, we will approach our daily experiment in the assumption of responsibility of these patients.

209

### Orofacial granulomatosis as a manifestation of crohn's disease: a case report

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**Background:** Crohn's disease is an inflammatory disease with chronic diarrhoea and/or abdominal pain, and may involve the oral cavity.

**Summary:** We report an 18-year old anorexic woman under severe stress, and severe oral pain. The patient had been diagnosed with Crohn's disease and on systemic corticosteroids. The patient was unemployed and on social welfare. She presented with fever, generalised acute gingivitis with spontaneous bleeding, ulcerations, and severe lesions on the palate. Emergency care included disinfection (betadine) and pain control. No probing depths > 5 mm, but clinical attachment loss was found. At the follow up, microbial testing showed a unique presence of *Campylobacter rectus* and *Micromonas micros* (95%). Additional debridement was performed. Zithromax 500 mg once daily for five days, and Ibuprofen (500 mg tid) was prescribed. Following treatment the oral conditions improved drastically. Palatal lesions have disappeared but there is a tendency of recurrence of ulcerative gingivitis. The microbiota from pockets, and palatal areas have normalized but *M. micros* and *C. rectus* are consistently present. At the most recent visit the subgingival microbiota was dominated by *C. rectus*, *M. micros*, *Fusobacterium* spp. *Porphyromonas gingivalis*, *Tannerella forsythia*. *Actinobacillus actinomycetemcomitans* was not found.

**Conclusions:** In spite of frequent dental recalls, the use of antibiotics, and medical attention have only partly controlled the oral conditions.

210

### Treatment of periodontal-endodontic lesions – long-term results

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**Aims:** Because of close anatomical relationships between the periodontium and the pulp a bacterial infection originating in one of these tissues may involve the other. The aims of our study were to find out prognostic parameters and to recognize the reasons for tooth loss by evaluation of the long-term results after treatment of periodontal-endodontic lesions (PEL).

**Methods:** Fifty-nine teeth (37 molars, 13 premolars, 5 incisors, 4 canines) with PEL received first endodontic and -if indicated- 6 months later periodontal therapy according to a special treatment plan. Questioning of patients, clinical and radiographic examinations were performed 5–10 years after endodontic treatment.

**Results:** 52.5% ( $n = 31$ ) of PEL were found in patients with generalized periodontal disease. Mandibular molars ( $n = 28$ ) were mostly affected. Teeth restored by crowns ( $n = 35$ ) developed PEL more often than teeth with ( $n = 12$ ) or without ( $n = 12$ ) fillings. 61% ( $n = 36$ ) of all PEL teeth could be maintained more than 5 years after therapy. 13 of 23 teeth with primary endodontic lesions, 2 of 7 with primary periodontal and 9 of 28 with true combined lesions could be maintained throughout the observation period. The main reasons for tooth loss ( $n = 35$ ) were periodontal problems ( $n = 12$ ), endodontic complications ( $n = 9$ ) and vertical root fractures ( $n = 5$ ).

**Conclusion:** The long-term prognosis after treatment of PEL seems to be determined by correct primary diagnosis, general periodontal conditions and careful endodontic treatment.

211

### Clinical guidelines improving the outcome of regenerative procedures using deproteinized bovine derived xenograft (BDX)

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**Initial Background:** Regenerative treatment using BDX is not commonly accepted to yield to successful long-term results. The aim of the presentation is to give step-by-step clinical guidelines for predictable and stable long-term outcomes of regenerative procedures with BDX based on the data of retrospective studies in our private practice.

**Materials and methods:** The guidelines to be presented have been developed during the years 1999–2004 while more than 900 infrabony defect sites have been regeneratively treated using BDX. The validity of the established specified standard protocol is underlined by a high success rate of more than 95%. Success was defined as absence of inflammatory signs, reduction of tooth mobility and PPD, high radiographical CAL gain as well as predictable esthetic outcome.

**Results:** The systematic management includes a standard protocol as following: Presurgical: Initial therapy including FMD, microbial DNA-testing if indicated, OH instruction, re-evaluation and therapy design decision. Surgical: Microsurgical approach, flap design, membrane coverage if indicated, primary closure using microsurgical sutures. Postsurgical: medication options, chemical plaque control at regeneratively treated sites, splinting in case of severe tooth mobility, short recall interval.

**Conclusion:** The standard protocol to be presented improves the predictability for successful long-term clinical outcome in the treatment of infrabony defects using BDX.

212

### Periodontal treatment and placement of a dental implant in a case of hadju-cheney syndrome (HCS)

T. P. Vrahopoulos\* and E. Bazopoulou et al.  
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HCS is an inheritable rare disorder of bone metabolism associated with rapidly progressive periodontitis. The aim of this case report was to present the periodontal treatment and the placement of a dental implant in a 22 year-old female with HCS. Physical examination revealed a short stature, small face, prominent epicanthal fold, thin lips, small mouth and short hands. There were no abnormal biochemical, hematological or hormonal data. Tests for Bone Mineral Density were indicative of osteoporosis. Local findings included hypoplasia of the midface, pathological mobility of all standing teeth and a generalized horizontal bone loss of about 50%. The gingival was mildly inflamed. Periodontal treatment included supra- and sub-gingival scaling and root planning. 4 teeth were extracted. A dental implant was inserted surgically in the upper jaw in order to test its ability for osseointegration. The patient was enrolled in a maintenance program with recall visits every 3 month. Periodontal recordings registered during the last 18 months showed an overall improvement on all clinical parameters. Radiographic evaluation of the submerged implant at 3 and 6 months post-op were indicative of a successful course for the osseointegration process. In conclusion, non-surgical periodontal management combined with a strict maintenance program proved to be effective in HCS periodontitis. Implant therapy may also be considered as a recommended approach for such patients.

213

**Surgical correction of hereditary gingival fibromatosis – case series**

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**Introduction:** The hereditary gingival fibromatosis inherited by an autosomal dominant or recessive trait and develops in the early ages. The treatment is definitively surgical but the too early operation can lead to rapid recurrence. Conventional gingivectomy was preferred in the past, but today mostly reversed beveled gingivectomy techniques are used.

**Cases:** The first case was corrected with conventional gingivectomy techniques more than two decades before with acceptable esthetical outcomes. The second case was first detected at the age of 7 and followed up for over another 7 years. Because of patient's young age the surgery was postponed till the rapid phase of the skeletal development will have been finished. The excessive mass of fibrotic tissue in the maxilla was removed by internal beveled incision and the palatal and buccal gingival flaps were united with sutures resulting in uneventful healing in ten days. The third case is a 18 years old young adult who had been orthodontically treated for more than 1.5 years to extrude his teeth without any success but he had gingival fibrosis.

**Results and discussion:** Today the more conservative internal beveled incision is preferred over the conventional gingivectomy providing more predictable healing and better esthetics. The timing

of the surgery is critical to avoid recurrences. Postoperatively all patients need perfect plaque control and comprehensive orthodontic rehabilitation.

214

**Effect of oral contraceptives pill on the gingival and periodontal health**

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*KHMC royal medical services, Amman, Jordan*

**Abstract object:** To study the effects of oral contraceptives (Lo-femenal) on the periodontium.

**Material:** This study includes 155 women who were using (Lo-femenal) as the study group. Two hundred women who never used contraceptives, as the control group. Both groups were clinically examined for plaque levels, gingival condition and loss of periodontal attachment. Structural questionnaire for the purpose of this study was filled for each woman.

**Results:** Gingival index was significantly higher among contraceptives users than non-users ( $P < 0.05$ ), which was correlated with the duration of usage ( $r = 0.8$ ). Loss of attachment was significantly higher among users of contraceptives for more than 2 years while no significant difference in plaque index was found between both groups.

**Conclusion:** The use of contraceptive pill (Lo-femenal for long period's increases the likelihood of gingival inflammation and loss of attachment.

# Posters: Clinical tips and cases: Implantology

215

**Follow up of plaque and gingival index in patients with dental implants**

M. Serrano\*, M. Moya, A. Sanchez, M. Calvo and E. Wojtovic

*Universidad de Murcia, España*

**Background and aims:** Much have been written about the association between mucositis and peri-implantitis. In this study, we sought to determine the efficacy of early diagnosis of mucositis in avoiding the development of peri-implantitis. To detect mucositis we basically look for the presence of clinical signs related to plaque accumulation and bleeding on probing.

**Materials and methods:** For a period of three years 150 patients underwent dental implantation. Plaque index (Silness and Loe) and gingival index (Loe and Silness) were recorded in the follow-up appointments. Patients were recalled for appointments after 15 days, 1 month, 3 months, 6 months and 9 months following the implantation. All patients received oral hygiene advice after the first visit.

**Results:** An increase in the score was observed in plaque index at the first recall visit because of the fear experimented by patients at the time of brushing. After that, on the first month, patients got a lower score which continued decreasing till the sixth monthly revision after which increased slightly. The gingival index followed a similar pattern.

**Conclusions:** Both indexes present a high correlation showing a worsening after two weeks but improving later from the first the sixth. Afterwards they follow a stable situation whenever the

patients receive hygiene advice and/or periodontal maintenance. Recall visits should not exceed six months, in order to allow an adequate control of peri-implant mucositis.

216

**The art and science of precision implant placement: the incision and position guide**

J. Y. Kim\*, H. J. Lee and R. Ju

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The importance of precise surgical placement of dental implant according to its functional and esthetic needs cannot be overstated. Various protocols and techniques for fabricating a surgical guide exist. A surgical guide has to be: (1) simple and cost-effective to fabricate, (2) good retention (adjacent teeth or landmark), (3) ability to translate pre-surgical work-up information accurately to surgical site, and (4) allow easy access of drills / guide pins / osteotomes intra-operatively. The presentation will cover the steps involved in planning a surgical case from a restorative stand point, including location and direction of the anticipated implant and of the specific incision design anticipated. Utilizing a surveyor and several specifically designed guide pins and jigs, and a choice of materials, a simple guide can be constructed within minutes that can double as radiographic guide as well as incision template. The authors have been utilizing this protocol coined, "EZ Guide, over a period of 4 years on several hundred cases, with great degree of accuracy.

217

### Flapless, one piece implant surgery

T. S. Dung\*, Kim-Choy Low, Hunghui Chi and Hsuan-Po Chou  
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The mainstream of periodontal therapy in the 21st century is simple, convenient, and minimum invasive. Recent case studies indicated that the success rate of one-piece implant surgery is similar to that of two piece one. Flapless implant surgery is to place implant without opening a flap and with no needs for sutures. While there are not many controlled clinical documentations, flapless one-piece implant surgery can be a treatment alternative at appropriate condition. In general, it requires enough keratinized mucosa, ridge width and height, and good bone density to provide initial implant stability and ideal implant position for flapless approach. Six case reports are presented to demonstrate the indications and treatment procedures of flapless implant surgery. The advantages of single stage, flapless, one piece implant are to reduce surgical time and post-operative discomfort, to eliminate secondary surgery, and to reduce post-surgical maintenance care, prosthetic works, and treatment time and cost. However, flapless surgery of one-piece implant is more difficult to perform. In case of meticulous patient selection and proper treatment planning, the success rate of one-piece implant shall improve dramatically in the near future and thus become a popular treatment option among dental implant candidate. More clinical studies are required to validate the predictability and long-term success of one-piece implant.

218

### Implant treatment in the edentulous mandible: a 5-year follow up report

A. Arab Oghli, Ch. Mertens\* and H. Steveling  
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This prospective study reports the clinical and radiographic performance of osseointegrated implants which support mandibular over denture over than 5 years. The implants were placed in eleven edentulous patients between 1996 and 1999. Orthopantomograms were used for radiological evaluation. For each implant, the radiographs were evaluated regarding marginal bone height, and its changes over time using the upper edge of the fixture as a reference point. The cumulative implant survival rate was 100%, and the survival rate of the superstructures was 100%. Of the sites were no signs of inflammation and all implants were plaque free. The mean bone level was 1 mm below the reference point after 5 years. The mean total marginal bone loss was 0.04 mm in the second year, and 0.34 mm in the fifth year. The present study has demonstrated that Astra Tech implants offer a reliable and appropriate method for rehabilitation of mandibular edentulism.

219

### Preservation of remaining labial ridge around immediately placed implants into fresh extraction socket

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**Aims:** The Aims of this study were to compare three procedures for preservation of the labial plate following immediate implantation; bone graft, e-PTFE membrane, titanium reinforced e-PTFE membrane (TR membrane).

**Materials and methods:** (1) A 21-year old female with coronal fracture on upper right lateral incisor. Thin labial plate (about 1 mm) and a 3 mm gap remained on the coronal aspect following

implantation. Cerasorb®(β-TCP) alone was grafted at the coronal defect. (2) A 32-year old male with root rest on upper left lateral incisor. The defect was treated by a regenerative procedure with e-PTFE membrane. (3) A 43-year old female with root rest on upper left lateral incisor. The defect was treated by a regenerative procedure with TR membrane. At second surgery after six months, all of the implants were evaluated for the thickness of labial plate and the resolution of the coronal gap.

**Results:** In the case of bone graft alone, labial ridge showed considerable resorption with appositional bone growth within the defect. The thickness of remaining labial plate was reduced to 1 mm. The two procedures using ePTFE and TR-membrane maintained original thickness of labial plate. All coronal gaps were completely filled with bone.

**Conclusion:** If thin labial plate remains following immediate placement of implant, it would be helpful to use regenerative procedures with the ePTFE or TR-membrane for preservation of the labial plate.

220

### Healing of the bone defect after immediate dental implant placement

D. Katanec\*, T. Kuna and D. Gabric  
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Immediate implantation of the dental implant after tooth extraction has great advantages over postponed implantation, i.e. fewer surgical operations, shorter period up to prosthetic restoration, prevention of bone resorption and better positioning of implant. The aim of the report is evaluation of clinical value of immediate placement of the dental implant with augmentation of the bone defect by autologous bone transplant following tooth extraction. In a group of 15 patients with indication for tooth extraction, a dental implant (Replace Select by Nobel Biocare) was inserted immediately after extraction and remaining bone defect was augmented with autologous bone transplant taken from the chin symphysis. Follow up was performed on RVG images 1, 6 and 12 months after implant placement and clinical monitoring was included measuring implant stability by analysis of resonant frequency. Results were assessed on the basis of computer guided densitometric analysis on previous RVG images at 8 points, 1 mm in diameter placed on the cervical, middle and apical part of the newly formed bone around the implant. The results show great density of the newly formed bone around inserted implants supported by intraoral bone autotransplant. Primary and secondary stability was between 70–90 Hz. No implant after prosthetic reconstruction and follow up in period of 12 months was lost in all 15 cases.

221

### Periotest utility in diagnostic of factors affect to implantology stability

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*Universidad De Murcia, España*

**Introduction:** Primary stability is the most important factor in order to achieve immediately load as well as delayed load. Moreover, secondary stability depends on some variables different of primary stability.

**Aim:** To establish what factors affect to values of implantology mobility in primary or secondary stability.

**Material and methods:** They underwent to the study a total of 40 implants, which were inserted with a dynamometric key (torque of 30 Ncm) until the polished ring was located to the height of the bony crest. Once inserted, we placed on the implant a transfer, applying a torque of 20 Ncm in the prosthetic screw. After that, we proceeded to measure the mobility of the implant with the

Periotest® (mobility 1), striking from a perpendicular way to the transfer, in its half part. This procedure repeated 2 months after the implantation (healing period), obtaining the mobility 2 of the implant.

**Results:** The mobility 1 depends on variables like type of bone and implantology location. However, the mobility 2 does not depend on factors like type of bone, location, diameter or longitude of implant, age or gender.

**Conclusions:** The Periotest® provides an useful method to value the stability of the implant and it would help us to decide the load protocol.

222

### Immediate loading of dental implants in the edentulous mandible

T. Eccellente, M. Piombino\*, A. Rossi and M. Ortolani

*Private Practice, Grumo Nevano (NA)*

**Aim:** To present the clinical results and the protocol of a simplified technique of immediate loading in edentulous mandible.

**Material and method:** In 19 patients, a total of 76 implants (Ankylos, Friadent, Mannheim, Germany) were placed in edentulous mandibles (four intraforaminally implants in each jaw) and immediately loaded. 41 implants were immediately inserted into fresh extraction sockets; 8 were placed as delayed implants. Following the surgery all implants were connected with prefabricated conical abutments that are manufactured with a precise fit to secondary conical copings. These copings were polymerised into the denture base chairside as described by May & Romanos 2001. Panoramic radiographs, mSBI and mPII were recorded in different time intervals. Patient' satisfaction was evaluated.

**Result:** During the healing period 1 implant was removed due to mobility. After a total observation period of 27.3 months (range 12–46 months) the remaining implants presented healthy peri-implant hard and soft tissue conditions (mSBI > 1; mPII = 1). Cumulative success rate was 98.6%. Suppuration was not observed. All patients appreciated function, aesthetic and retention of the rehabilitation.

**Conclusion:** The present data validates the predictability of immediate loading in edentulous mandible, showing a high success rate in accordance with literature. This simplified technique reduces treatment time and costs of dental rehabilitation, with relevant satisfaction for the patients.

223

### Immediate loading of dental implants in the edentulous maxilla

T. Eccellente, M. Piombino, A. Rossi and M. Ortolani\*

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**Aim:** To present the clinical result of 23 cases of immediate loaded implants in the edentulous maxilla with a new treatment concept.

**Material and method:** In 23 patients, a total of 92 implants (Ankylos, Friadent, Mannheim, D) were placed in edentulous maxillae (four implants in each jaw) and immediately loaded. 21 implants were immediately inserted into the fresh extraction sockets; 25 were placed as delayed implants. Subsequently all implants were connected with prefabricated conical abutments, which are manufactured with a precise fit to secondary conical copings. These copings were polymerised into the denture base chairside as described for the edentulous mandible by May & Romanos 2001. Panoramic radiographs, mSBI and mPII were recorded in different time intervals. Patient satisfaction was evaluated.

**Result:** During the healing period 2 implants were removed due to mobility. After a total observation period of 27.6 months (range 15–42 months) all remaining implants presented healthy peri-implant hard and soft tissue conditions (mSBI > 1; mPII > 1). Cumulative success rate was 97.8%. Suppuration was not observed. All patients appreciated function, aesthetic and retention of the rehabilitation.

**Conclusion:** Basing on the present data it was concluded that four implants with high primary stability, may support immediate loading in edentulous maxilla. Additionally this simplified technique reduces treatment time and costs of dental rehabilitation, with relevant satisfaction for the patients.

224

### Periodontal compromised patients: full immediate placement for optimal immediate function

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**Aim:** Periodontal compromised patients, specially those young adults needing a full arch implant-supported rehabilitation, are, some times, candidates to immediate implant placement followed by immediate function. In this poster presentation we will describe step by step, all the clinical fazes of our protocol, making its illustration with a clinical case with 2-year follow-up.

**Materials and methods:** A 40 year-old, female, non-smoking patient was referred to us presenting all her 11 maxillary teeth with severe bone loss and mobility. The treatment plan was to extract every teeth and immediately place 10 implants. In the same surgical step, bilateral sinus lifting was done with the simultaneous placement of 2 implants in each one. An acrylic provisional fixed implant-supported full bridge was made and adapted to the 6 implants in the first 24 h. After 4 months a definitive ceramic fused-to metal complete implant-supported bridge was placed.

**Results:** None of the 10 immediate placed implants was lost. The 2-year follow-up radiographic control, showed great bone-implant contact of the total 10 implants and a perfect adjustment of the prosthetic components. The clinical 2-year follow-up showed an optimal aesthetic final stable result.

**Discussion/Conclusion:** The rationale to use the concept Immediate Placement & Immediate Function in periodontal full arch compromised patients is to minimize the psychological and functional consequences of a full arch natural dentition extraction.

225

### Rationale of the surgical approach for different types of alveolar ridges with GBR using astra tech implants

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*Private practice*

**Introduction:** In the last years, there has been a lot of concerns about marginal recession in the zenith of implant-supported crowns in the esthetic zone. This recession occurs because of deficit of bone at implant placement or bone resorption occurring during the biological width formation. Astra tech implant-supported restorations have shown stability of marginal bone and gingival levels along the years.

**Objective:** To expose a rationale for treatment with guided bone regeneration of alveolar ridge on the esthetic zone, according to bone amount, in such a way that implants can be placed with good esthetic outcome.

**Material and methods:** Three single tooth cases in the esthetic zone to be restored by implants. One case with proper amount of bone to receive an implant. Another case with a limited amount of bone to receive an implant but producing a dehiscence defect and a last case with extremely limited bone amount vertically and

horizontally that could not receive an implant. An approach based on GBR is proposed to treat each case.

**Results:** All three cases were treated successfully with good esthetic result. All implants showed total stability of marginal bone and gingival level one year after loading.

**Conclusion:** Treatment of the single tooth anterior edentulous ridge under this GBR approach show stability of bone and gingival levels and good esthetic result.

226

### Increasing the attached gingiva around endosseous implant using acellular dermal matrix allograft

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The aim of this study is to investigate the clinical efficacy of the acellular dermal matrix allograft to achieve increased attached gingiva around implant.

**Materials and methods:** Ten male patients having attached gingiva less than 2 mm on the facial aspect of implant treated site were included in this study. The raised partial thickness flap was positioned apically and secured to the periosteum by the absorbable sutures. Then the patients received acellular dermal matrix allografts, which had been rehydrated in sterile saline for a minimum of 10 minutes. The grafts were placed on the firm periosteal bed with the connective tissue side against the periosteum. Patients were seen at 3-month follow-up to monitor healing and plaque control. Differences in PI, GI, PD, and ATW between baseline, 2 weeks and 3-month post surgery were assessed by Wilcoxon signed rank test.

**Results:** The soft tissue healing was uneventful and no patients had postoperative complications besides mild pain and/or swelling. The change in PI, GI, and PD was not statistically significant. ATW increased from a mean of  $1.0 \pm 0.6$  mm to  $5.0 \pm 0.9$  mm at 2 weeks, and  $3.0 \pm 0.9$  mm at 3 months. The mean graft width was  $4.5 \pm 0.8$  mm and the shrinkage rate at 3 month was  $39.2 \pm 17.2\%$ .

**Conclusions:** Within the limit of my study, it is concluded that the ADM grafts could be applied as a substitute for autogenous connective tissue grafts to increase the zone of the attached gingiva.

227

### Computer assisted implant surgery with an image guided navigational system: 18 months clinical and radiographic follow-up

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*University La Sapienza, Rome, Italy*

**Aims:** Description of the adoption of an Image Guided Navigational system (Den X®, Moshav Israel) to place dental implants in 10 patients.

**Methods:** Ten healthy non-smokers patients (age 40–55 years) were selected to insert a total of 32 fixtures (Imtec Implants® U.S.A.) using the Den X System® that tracks the actual position of the hand piece in real-time. The tracking method used by this system is an optical three linear camera tracker Flashpoint 5000 system (Image Guided Technologies. Boulder®, Colorado USA). The type, size, position and orientation of the implants were planned based on CT data with IGI Software from DenX Ltd. adopting a Dentascan CT protocol. No intra or post-operative injuries or accidents were reported.

**Results:** All the CT Scan and Orthopantomograms control examinations performed after 1 week showed the correct implant position and a complete radiographic superimposition. The peri-implant parameters (PPD, CAL, BOP, Mobility, and Essudate)

showed healthy conditions around all the rehabilitations. All the patients reported complete satisfaction and minimal swelling, pain and discomfort in the immediate and 1-week post-operative interviews.

**Conclusions:** Adoption of image-viewing software and motion tracking devices during the implant placement allows the correct insertion of fixtures improving the functional and esthetic results, minimizing intra-operative risks and prosthetic complications.

228

### Four-year follow-up of 174 implants supporting fixed bridges in maintenance therapy

T. Eccellente, M. Piombino, A. Rossi\*,<sup>1</sup> and E. Conserva<sup>1</sup>  
<sup>1</sup>Private Practice, University of Genova, Napoli

**Aim:** To present the clinical result of 174 implants in maintenance therapy.

**Methods:** Between February 2000 and March 2001, 174 implants (Ankylos, Friadent, Mannheim, D) were inserted in 62 patients. After a conventional healing period, 38 implants were loaded with single crowns, 93 implants with 38 bridgework and 43 implants were used for 6 full arch bridges. After the prosthetic treatment, all patients were submitted to a quarterly control. In different time intervals mPII, mSBI, standardized peri-apical radiographs, technical complications, patients' satisfaction were recorded.

**Results:** After a total loading period of 52.4 months (range 44–59 months), no implant was lost. All implants presented healthy peri-implant soft tissue conditions (mPII = 1, mSBI > 1) and a stable peri-implant bone level. No patient reported suppuration. During the observation period, six patients reported ceramic fractures. Seven patients were not satisfied with the aesthetic result.

**Conclusion:** The long-term success of implant therapy depends undoubtedly from osseointegration; hence the soft tissue integration is not less important. The characteristic design of the implant-abutment connection leads to negligible microgap which significantly influences the peri-implant soft tissue and stability of the bone level. It can be concluded that a sufficient oral hygiene, the presence of keratinized mucosa and the microgap design, could influence the stability of peri-implant tissues and the longevity of implants.

229

### Inlay-onlay grafting of autogenous mandibular bone for augmentation of the atrophic posterior maxilla. clinical results

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**Background:** Few papers describe the results of posterior maxilla reconstruction when combined vertical and horizontal alveolar ridge augmentation is required. Three-dimensional reconstruction of the resorbed posterior section of the maxillary arch has been achieved using different surgical methods: a combination of sinus floor elevation and vertical ridge augmentation with barrier membranes, sinus floor elevation and onlay bone grafts harvested from the iliac crest. The aim of this study was to evaluate the clinical results of alveolar reconstruction of the posterior maxilla with autogenous mandibular bone.

**Methods:** Twelve patients were consecutively treated with a combination of inlay particulate grafts after elevation of the sinus membrane and vertical or horizontal bone block grafts secured with titanium screws in a single procedure. The clinical and radiological results were evaluated. 34 implants were inserted (4 at the time of reconstruction and 30 in a second stage). Follow up varied from 6 to 30 months after loading.

**Results:** At implant placement mean vertical inlay augmentation was 8.4 mm, mean vertical onlay augmentation (8 cases) was 3.4 mm and mean horizontal augmentation was 4.2 mm. All implant survived, two implants showed marginal bone loss of more than 2 mm and were considered partial success.

**Conclusions:** The use of mandibular inlay-onlay bone grafts has shown excellent results in the reconstruction of composite defects in the posterior maxilla.

230

### Periodontitis and periimplantitis: microbiological relationships. Review and practical observations

S. D. Aspriello, M. Piemontese and F. Antenucci\* et al.  
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Successfully osseointegrated dental implants are colonized principally by facultative Gram-positive cocci and rods. Periimplant sites of partially edentulous subjects harboured higher percentages of *Bacteroides* and *Capnocytophaga* than do edentulous sites. Also periodontal pathogens are present in periimplant sites. Periodontal status of subjects (probing depth) and microbiota on periodontal pockets and remaining teeth are the major influence on periimplant microbiota. No differences were observed in the microbiota between implants or abutments with different surfaces. In edentulous subjects *A. actinomycetemcomitans* and *P. gingivalis* are not detected. In experimental and in vivo periimplantitis the periodontal species detected principally are *P. intermedia/nigrescens*, *A. a.*, *P. g.*, *T. forsythensis*, following by spirochete (as *T. denticola*), *Fusobacterium*, *staphylococcus*, *Enterics* and *streptococcus* in a variable frequency. Failing dental implants have been associated with periodontal pathogens such as *T. f.*, *Fusobacterium*, spirochetes and *P. micros*. Evidence and ours observations support the strictly relation between periodontal status and the risk for periimplantitis and a different detection of periimplant pathogens between partially edentulous and edentulous subject. Periimplantitis in edentulous subjects have microbiological features peculiar, in fact in these cases perio-pathogenes such as *A. a.*, *il P. g.*, *P. i./n. e* *Bacteroides e* *Capnocytophaga* are decreased or not found.

231

### Peri-apical implant pathology: etiology, diagnosis and treatment options

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Osseointegration is the healing of alveolar bone around dental implants so that the direct anchorage of the implant is then maintained allowing functional loading without the growth of fibrous tissue at the bone-dental implant interface. Although a high success rate has been reported, many reports demonstrated implant failures. Peri-implantitis is an inflammatory process affecting the tissues around an osseointegrated implant in function, resulting in loss of supporting alveolar bone, where a bone defect arises in the marginal portion of the dental implant site and has the shape of a crater that may cause implant failure. As well as peri-implantitis, peri-apical implant pathology has been recently defined where the loss/removal of the implant is suggested. The etiology of peri-apical implant pathology is speculated as overloading and excessive tightening of the dental implant, bone overheating during surgical procedure, fenestration of the supporting bone, presence of pre-existing bone pathology, contamination of the dental implant surface, pre-existing microbial pathology, or poor bone quality. A limited number of reports are published about peri-apical implant pathology, and most of them are treated with resective surgery. The present report aims to introduce the diagnosis and the treatment of peri-apical implant pathologies with regenerative surgery. Further,

a detailed literature review will be provided about the etiology, diagnosis and the possible treatment options.

232

### Evaluation of implant treatment from a position of risk factors. Biological and prosthetic complications

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**Purpose:** The purpose of this article is to present clinical results and evaluate the types of risk factors associated with complications after placement of dental implants with the following implant-supported fixed partial prosthetics (FPP).

**Materials and methods:** This study generalized the clinical results that were received after treatment by FPP. There were 74 patients, who received HiTech and NobelBiocare implants between 2000 and 2004. Totally 248 implants were inserted. Prosthetics: 203-metal porcelain and 45- cast metal crowns. Complications: biological (inflammatory and surgical) and prosthetic complications.

**Results:** Total loss - 20 implants (8%) Visible displays of mucous's inflammation (reddening) were around of 32 implants (12.9%). Sufficient marginal bone loosening was registered with 35 implants (14.11%) after 1-year follow-up. Only 10 (4%) abutments were lost. Fractures of metal framework - 5 (2.06%), of porcelain veneers - 10(4%) crowns. Loss of cement bond - 28 crowns (11.3%). Hygienic condition of an oral cavity after implant treatment were not sufficient with most patients.

**Conclusion:** The results of this study shows, using of HiTech and Radix implants in our department is a safe and good-prognosable method for the treatment of partially edentulous patients. It's important to stress the level of oral care for patients with FPP plays a significant role for further successful functioning of implant-supported FPP.

233

### Osseointegrated implants in a patient with Papillon-Lefevre syndrome : (a 10-year follow-up)

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The 25-year-old female reported in present case was diagnosed with Papillon-Lefevre' syndrome. Oral function, phonation and aesthetics were supplied with implant treatment after the conventional periodontal treatment were completed. The 10-year follow-up dental records, including full-mouth radiographs, intra-oral photographs, microbiological samples, leukocyte function tests, and clinical indexes were evaluated. The treatment of the periodontal disease and implant treatment in our case was successful. Initially, the patient had depressed polymorphonuclear leukocyte (PMN) chemotaxis and adherence as well as evidence of periodontal infection with *Actinobacillus actinomycetemcomitans* (*A. a*), *Capnocytophaga*, *F.nucleatum*, *E.corodens*. But 10-year follow-up showed normal PMN function treatment must be considered a different mode of treatment for maintenance of oral function and aesthetics of patients with Papillon- Lefevre syndrome.

234

### Peri-implantitis treatment in an HIV-infected patient

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*Eastman Dental Institute, University of London, UK*

**Objective:** Report the findings of GBR treatment for a peri-implant defect in an HIV+ patient.

**Materials and methods:** An HIV+ patient with reduced CD4-counts and a history of chronic periodontitis was referred to the EDI for treatment of a peri-implant defect in position 26. The

patient received non-surgical therapy under antibiotic coverage (Metronidazole). The reassessment at 8 weeks showed a reduction in PPD in all sites except for the area 26 where residual PPD  $\geq$  6 mm were encountered. The peri-implant defect was treated with a combination of Bio-Oss® and Bio-guide®. Following the removal of the granulation tissue and calculus from the implant surface with ultrasonics and thorough rinsing of the surface with saline and CHX, Bio-Oss® granules were placed and covered by two membranes. Primary closure was achieved and antibiotic therapy for 2 weeks was prescribed. Follow-up appointments were carried out for 1 month on a weekly basis. Following 6 months of healing, overall PPD of 4 mm was achieved around the implant and the bleeding on probing was reduced. A three-month recall program was established and the clinical measurements were maintained for 12 months after surgery, where radiological bone fill of the defect was also seen.

**Conclusion:** This case indicates that in HIV + patient with reduced CD4-counts, the GBR principle might provide clinical improvements when used for the treatment of peri-implant defects.

235

### **Implant prosthesis treatment in angulated posterior sector. Two techniques to improve the protetical emergency profile**

C. Ivorra\*, J. Novell, F. Novell, Perramon and D. Romero  
*Univ. Santiago de Compostela, Spain*

**Objective:** The objective of this study is to explain two techniques to achieve a correct emergency profile in the replacement of the first molar when the second molar is mesioangulated compromising the prostodontical restauration.

**Materials and methods:** The second molar mesioangulation has negative consequences for the first molar prostodontical rehabilitation like the food impactation between the first and second molar. We have treated 18 patients from this pathology. The patients were distributed in two aleatory groups. The patients from group A had a definite implant to do the orthodontic treatment. The patients from group B had one or two microimplants distal to the second molar to do the orthodontic traction.

**Results:** All the patients except one received the full treatment. The patient left lost the implant positioned in the place of the first molar. In group B the mean treatment time was longer than the one from group A. Two patients of group B lost one of the microimplants. The most grave problems appeared in the orthodontical treatment. All the patients were screened during one year. They presented a good gum health of the second molar and also a good health of our implant.

**Conclusions:** The mesioangulation of the second molar will always have to be treated before or during the rehabilitation of first molars with implants. Both techniques that have been described have some inconvenient and some advantages. To extract more conclusions we have to do a study with bigger groups.

236

### **Treatment of a hypodontia patient with implants and tooth supported fixed partial dentures: a case report**

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Patients who are congenitally missing one or several teeth are frequently encountered in routine practice. Hypodontia is defined as the developmental absence of one tooth or more and may present with varying degrees of severity. This condition is frequently combined with hypoplasia of alveolar bone in the sites of missing teeth. In such cases in addition to functional

insufficiency and esthetic compromise, psychosocial development is another important concern for the young patients. Traditional therapeutic approaches for the rehabilitation of these abnormalities have included removable partial dentures, fixed prostheses with minimal tooth preparation and orthodontic movement of teeth to close spaces. Dental implants represent an alternative to the traditional treatment methods, especially in patients who have moderate/severe hypodontia. This case report demonstrates the rehabilitation of a dysfunctional malocclusion caused by a moderate/severe hypodontia utilizing a combination of orthodontic, prosthodontic, surgical treatment and, dental implants to replace the missing teeth. An 18 year-old woman patient with hypodontia of the permanent teeth and partially retained primary teeth was decided to receive implant and tooth supported by multidisciplinary team that included an orthodontists, a prostodontist and a periodontist.

237

### **Success of dental implants placed by inexperienced dentists. Results at 36 months**

M. Pastor\*, S. García, C. Subirà and J. J. Echeverría  
*School of Dentistry, University of Barcelona, Spain*

**Introduction:** Although, it is generally accepted that implant surgery has a long learning period, very few studies have considered, whether the lack of surgical experience of the operator has an impact on the short term success after implant placement.

**Objective:** To determine the success rate and the cumulative survival rate of endosseous implants placed by general practitioners without previous experience in implant surgery.

**Materials and methods:** During three consecutive years, a one-week implant training program was offered to general dentists without prior experience in the surgical phase of implant therapy. 251 Astra Tech® implants were placed in 84 patients. All implants were installed under closed supervision. The success rate and the cumulative implant survival rate were analysed up to 36 months.

**Results:** Twenty-four (29.5%) patients received single implants, 46 (56.7%) patients received between 2-4 implants and 14 (16.6%) patients received five or more implants. Implants failures occurred in five patients, who received more than one implant. Of the 251 implants placed, six failed (2.4%), all of them before occlusal loading. The success rate and the cumulative implant survival rate was 97.6%.

**Conclusion:** Within the limits of this study, clinician previous experience did not seem to be an influencing variable on implant success.

238

### **A prospective cohort study of the dental implants systems in patients with and without a history of periodontal disease - long-term implant prognosis**

V. Nicolaescu\*, M. Damian, C. Mocanu  
*University of Apollonia, Iași, Romania*

**Aims:** The aim of this 5-year study was to compare the failure, success and complication rates between patients having lost their teeth due to periodontitis or other reasons.

**Material and methods:** Eighty-five patients who received 112 hollow screw implants of the Nobel Biocare and Alpha Bio Dental Implant System were divided into two groups: group A – twenty three with 21 implants having lost their teeth due to periodontal disease; group B – sixty two patients with 91 implants without a history of periodontitis. The incidences of periimplantitis were noticed during of a regular supportive periodontal therapy.



**Results:** Success criteria at 5 years were set at: pocket probing depth (PPD)  $\leq$  5 mm, bleeding on probing (BoP), bone loss  $<$  0.2 mm annually. The survival rate for the group with a history of periodontal disease (group A) was 90.5%, while for the group with no past history of periodontitis (group B) it was 96.5%. Group A had a higher incidence of peri-implantitis than group B (28.6% vs. 5.8%). With the success criteria set, 52.4% in group A and 79.1% of the implants in group B were successful.

**Conclusions:** Patients with implants replacing teeth lost due to periodontal disease demonstrated lower survival rates and more biological complications than patients with implants replacing teeth lost due to reasons other than periodontitis during a 5-year maintenance period.

239

### Horizontal ridge augmentation by simultaneous use of guided bone regeneration (GBR) and osseointegrated implants in edentulous mandible jaw

V. Piperias\* and E. Sinanioti

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Ideal architectural structure of the alveolar crest facilitate the optimal prosthetic placement of implants from an aesthetic and functional point of view. The reconstruction of deficient alveolar ridges through Guided Bone Regeneration (GBR) is capable of promoting new bone formation in horizontal or vertical dimensions or both. The purpose of this poster was the presentation of the therapeutic approach to two cases with large alveolar ridge deformities in lower jaw in a bucco – lingual dimension. In the first case the placement of three implants in an ideal prosthetic position has resulted in a large buccal implants exposure outside the bony housing in non-space making bone defects because of the insufficient crest width for implant placement in the mandible. Titanium - reinforced membrane was applied supported by bone graft materials following perforation of the surrounding cortical bone plate to achieve a bleeding bone surface. 6 months later, after the removal of the membrane the results were excellent. An overdenture provided a satisfactory solution for the patient. In the 2nd case the placement of four implants in an ideal prosthetic position has resulted in a moderate buccal implant exposure in the mandible. A collagen membrane was applied supported by bone graft materials. The result was excellent and a fixed prosthesis provided a satisfactory long- term result. The primary stability of the implants was of paramount importance for the successful results in both cases.

240

### Modification of the index of oral hygiene in patients carrying mandibular implant overdentures on bars

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**Aim:** Create useful tool in health and hygiene evaluation in patients carrying mandibular implant overdentures on bars. Modify the plaque index Silness & Loe and calculus index to the characteristics of this treatment.

**Material and method:** Forty patients, with two implants mandibular overdentures on gold or titanium bar. Modified plaque index is measured: Score 0, no plaque. Score 1, no plaque at first sight, the presence is demonstrated slipping probe on abutment and/or on bar. Score 2, moderate plaque at first sight on abutment and/or on bar. Score 3, plaque at first sight, abundant, that occupies more than 1/3 of abutment and/or of bar. Modified calculus index: Score 0, no calculus. Score 1, supragingival calculus only on the abutment and/or on bar. Score 2, moderate subgingival

calculus, existing or not supragingival. Score 3, abundant subgingival calculus, existing or not supragingival.

**Result:** 22.5% presented plaque score 0; 50% score 1; 25% score 2, 2.5% score 3. 10% presenting visible plaque on the bar, not on abutments, described traditionally like score 0 but according to this modification is included within score 2. Calculus index: 75% non-present, 22.5% score 1 2.5%, score 2.

**Conclusion:** Plaque and calculus index are valid for studies of periimplant health. Avoid bar hygiene suppose an allocation of plaque and calculus values lower to the real ones, poor hygiene is undervalued. Necessary include oral hygiene protocol to avoid subsequent periimplant complications.

241

### Immediate implants and immediate function clinical considerations

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The aim of this presentation is to discuss the clinical aspects of this kind of treatment, and the limitations that we still have to take in account. The sociologic and cultural advances of the society in the last years has influenced Dental Medicine. The concern about 'perfection in aesthetics' is nowadays one of the objectives of our treatment. The new techniques and materials have changed and we can offer to the patients' better results in a reduced time. In fact the final results of these treatments are not only health and function but also aesthetics and in the fewer time possible. In this presentation we will discuss the indications and limits of immediate implants in concern to 'perfection in aesthetics', and also the immediate loading, their biological principles, indications and limits. We will present the clinic protocols with clinical cases and discuss the results published in the literature. From our point of view immediate loading and immediate function can be a predictable treatment option if pre-operative factors are favourable.

242

### The use of platelet-rich fibrin in the osteotome sinus floor elevation technique: preliminary results

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The purpose of this prospective study is to document radiographically tissue remodeling patterns around implants placed according to the bone-added osteotome sinus floor elevation technique with the use of platelet-rich fibrin as bone graft material.

**Materials and methods:** Three men and 10 women, aged between 42 and 73 years (mean age 51.7 yr), were treated with 23 implants placed according to osteotome sinus floor elevation technique. Platelet-Rich Fibrin (PRF) membranes was prepared during the surgery from a blood sample of the patient and served as bone graft material. Intraoral radiographs were obtained presurgically and postsurgically at 12 months.

**Results:** The implant survival rate was 100% after 12 months. The mean preoperative distance between the sinus floor and the crest was 5.4 mm (range 2.7–9.4 mm). The mean distances between the implant apex and the initial sinus floor were:  $4.63 \pm 1.92$ . 12 months postchirurgically, the mean bone height increase of the new bone reaching apically to the implants was  $0.02 \pm 0.22$  mm.

**Conclusion:** PRF is an autologous fibrin matrix with a high platelet concentration. The grafted area apical to the implants undergoes resorption which is stopped by the implant extremity. PRF could be used as a bone graft in the osteotome elevation with simultaneous implant placement.

243

# **Aetiological hypothesis and treatment for an implant periapical lesion: a clinical case**

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There are two categories of complications that occur in implant therapy: biological and technical (mechanical) 'Biological complications' refer to disturbance in the function of the implant characterized by biological processes that affect the tissues supporting the implant. A particular group of biological complications are the implant periapical lesions. These lesions can be similar to periapical sear found at the tooth apex and characteristically could presents no clinical symptomatology, as well as infected lesion usually accompanied by fistula development. The scientific literature reports an incidence next to 0.2% but probably this percentage is very far from the real frequency. The occurrence of these lesions is also influenced by an unscrupulous analysis of bone characteristics of the site suitable to implant insertion. The purpose of the presentation is to investigate the aetiopathogenetic factors that are responsible of such peri-implant pathologies by means of a clinical case exhibition. It is also suggested a modified classification and the strategies of treatment related to the different forms. The required condition to achieve the treatment successful is an advanced diagnosis. It can be reach by a well-organized supportive care program.

244

# **Multidiscipline management of oligodontia (multiple agenesis): the relationship between an interdisciplinary team**

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**Initial background:** Oligodontia « few teeth » this term designates the lack of at least ¼ of the permanent teeth.

**Aims of the report:** Multidisciplinary treatment has improved our ability to restore patients with oligodontia using orthodontic, maxillo-facial surgery and implants for aesthetics and functionality. The use of a combination treatment approach may facilitate predictable success.

**Materials and methods:** Amongst the numerous oligodontia cases that we have treated, three cases were chosen. A 22 year old patient presenting 26 missing teeth, a 16 years old patient presenting 25 missing teeth and finally a 15 years old patient presenting 17 missing teeth. All three patients were treated by an interdisciplinary team composed of a dental surgeon, an orthodontist, a maxillo-facial surgeon and an implant surgeon. The first patient was treated by orthodontics, bi-cortical parietal bone grafts and dental implants. The second patient was treated by orthodontics, orthognatic surgery and dental implants and the third patient was treated by orthodontics, orthognatic surgery, bi-cortical parietal bone grafts and finally dental implants.

**Discussion/conclusion:** The more severe the Oligodontia case, the more obvious is the need of a multidiscipline approach. This hand in hand cooperation of the overall specialists is essential in order to obtain the patient's motivation as well the one of his or her entourage.

245

# **Vertical and horizontal ridge augmentation with resorbable barriers, a simpler and more predictable technique**

F. Llambés\*

*Private practice, Valencia, Spain*

Guided bone regeneration with non-resorbable membranes has been well documented, studies have shown that horizontal and

vertical alveolar bone augmentation is possible. However, resorbable collagen barriers seems to be an interesting alternative because they are easy to manipulate, well accepted by oral tissues and they don't have to be removed after the surgery. The aim of this presentation is to show that vertical and horizontal bone augmentation is possible with collagen membranes in implant dentistry. Comparison between different types of collagen barriers will be exposed and advantage of resorbable membranes will be discussed. Guided bone regeneration was performed in several clinical cases with a collagen membrane that resorbs in 6 months. Autograft and xenograft were used to support the membrane and avoid collapse. Membranes were not fixed to alveolar bone and gingival tissues were sutured with horizontal mattress and interrupted sutures. Results have shown that vertical and horizontal ridge augmentation is possible with collagen membranes. This technique seems to be very predictable and incidence of complications is very low. Other clinical reports that use resorbable barriers can be found in the literature, but there are not scientific studies with a high number of patients. In conclusion, this membrane seems to be a promising alternative to expanded polytetrafluorethylene barriers, because they could simplify the technique and increase its predictability.

246

# **Success, biological and technical complications with screw retained fixed partial dentures on implants in the edentulous mandible; 3–6 year results**

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**Aim:** To assess in a period of 3–6 years the success, biological and technical complications of screw retained fixed partial dentures (FPD) on AstraTech implants.

**Materials and methods:** In 42 patients a total of 238 implants were placed in edentulous mandibles. Of the 238 implants 75 were immediate, 22 were placed after 6–8 weeks, others were placed in a standard procedure. Of the 42 patients 32 received 6 implants, 3 received 5, while 7 received 4. In 38 patients the implants were placed in the inter-foraminal region, in four patients the implants were placed in the entire mandible.

**Results:** One implant failed to osseointegrate (0.4%). 28 implants had exposed implant necks (11.7%), while 7 implants had periimplantitis (2.9%). FPD: 28 were metal-ceramic (66.6%), 14 were metal-acrylic (33.3%), and there were 131 cantilevers. Five FPD experienced metal framework fractures (11.9%) and were remade, porcelain fractured in 11 cases (26.2%), acrylic fracture occurred once (2.3%). Screw fractured in seven cases (2.5%), abutment fracture occurred once (0.4%).

**Conclusions:** Implant borne screw retained FPD are a reliable method of treating complete edentulous patients. A relatively high incidence of technical complications for the screw retained FPD could be because of the problems with the passive fit of the metal framework leading to the deformation of the suprastructure. With the new developments such as the Cresco system for the passive fit a number of complications could be eliminated.

247

# **Cad cam drilling guides for transferring CT-based digital planning to flapless placement of oral implants in complex cases**

F. Valente\* and A. Sbrenna

Two of the most notable trends in modern surgical specialties are minimally invasive surgery and the integration of computerized diagnostics and computer-guided surgery. In oral implantology

these two trends are now mainstream in the form on one side of the so-called flapless surgery and on the other side of the computer-aided implantology. Even though flapless surgery can be performed in some cases without the aid of the computer and a computer-aided approach not always allows a flapless procedure, these two approaches can be often combined with great advantage. Flapless surgery reduce pain almost to zero and should be used whenever possible. However, no matter how advantageous it is, flapless implant surgery remains a blind surgical technique, difficult to perform and prone to errors leading to potentially serious complications. It can be transformed in a relatively simple procedure thanks to the computer-aided implantology. There are now numerous CT-based software available for 3-D implant planning. The one shown in the present lecture is the SimPlant® software (Materialise, Leuven, Belgium). Computer assisted surgery is an exciting field whose popularity is rapidly increasing among implantologists all over the world. This approach can be used with great advantage not only in good bone ideal cases, but also in so called complex cases, where bone dimensions are less than ideal, entailing the use of tilted implants.

248

### Soft tissue management of post-extraction sockets as a pre-implantological concept in the aesthetic zone

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DGI, EDA, APW, DGZMK, Nippon Dental Univ., Niigata

**Background:** Three dimensional soft and / or hard tissue deficiencies after tooth extraction often require significant manipulation of the mucosa which can compromise the aesthetic outcome and increase the risk for post-operative wound dehiscence's.

**Aim:** The aim of this evaluation was to analyse whether filling of the fresh extraction sockets with a space maintaining material (Bio-Oss Collagen®) and subsequent healing without primary wound closure will result in the formation of an adequate amount and appearance of soft tissue as a pre-implantological or pre-augmentative procedure.

**Materials and methods:** In our private practice we performed until now an evaluation in 45 patients and 59 sites. At least one anterior tooth (14-24) per patient had to be extracted and was planned to

be replaced by a dental implant. The fresh extraction sockets were filled with Bio-Oss Collagen®.

**Results:** The results in soft tissue amount and appearance were adequate to an aesthetic acceptable situation. In some cases only in the apical defect region one could find bone formation. This was the fact where only the Bio-Oss Collagen® was used and no membrane had been applied to cover the socket.

**Conclusion:** Soft tissue management by filling the socket with a bone substitute can lead to adequate soft tissue amount and appearance at the time of augmentation and / or implant insertion. Clinical, histological and restorative results of the clinical evaluation will be presented and discussed.

249

### ALL-ON-4 immediate function in edentulous patients

J. Espirito Santo\*, H. Franco and J. Suarez

Hugo Franco Jose Suarez Quintanilha

Dental implants are used increasingly in dental treatment, as they can often offer many advantages for the replacement of the lost tooth. The ALL-ON-4 technique represents an alternative treatment when the amount of bone available in the posterior area of the maxilla is insufficient. For this purpose inclination of the posterior or distal implants is used. In this way it is possible to create an ideal support for an acrylic bridge that can be designed and placed within a few hours after the surgical intervention.

**Objectives:** To present the indications and contraindications for the ALL-ON-4 technique, and the diagnosis, treatment plan, surgical techniques, types of implants and prostheses for the technique.

**Clinical implications:** The surgical procedure can be performed by the dentist with appropriate surgical experience. The dentist should be trained in diagnosis, in performing the treatment plan. They should have knowledge of the surgical technique, of post-operative follow up, and should be able to evaluate their results in the medium to long term.

**Conclusions:** ALL-ON-4 is an impressive advance for the treatment of the edentulous maxilla. In addition to its simplicity and predictability, from a surgical point of view the protocol allows the placement of implant-retained prostheses with immediate function.

## Posters: Clinical tips and cases: Aesthetics

250

### Supraperiosteal envelope in soft tissue grafting for multiple root coverage. Technique and case report

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The search for predictable clinical solutions to the problem of gingival recession has led to several important surgical advances in the last years. Free connective tissue grafting for root coverage combines an overlying flap and an underlying vascular bed for graft nourishment. Langer developed the subepithelial connective tissue graft technique to treat in both isolated and multiple adjacent areas of recession. Raetzke in 1985 described the envelope technique to manage isolated areas of root exposure. Allen in 1994 described the rationale and technique of the supraperiosteal envelope to include multiple adjacent areas of recession. The presentation will show two case reports with multiple adjacent areas of recession were covered with the supraperiosteal envelope in soft tissue grafting described by Allen.

251

### Enhancing aesthetics using astra's implant system abutments

G. Eid\* and A. Berberi

Private Practice

In the past ten years, Implantology has made great progress. In the beginning, "function" was the main objective. However, due to the significant achievement of osseointegration, 'aesthetic' becomes the main goal and is increasingly asked for by our patients. Above all it now represents a social phenomenon. Most Dental implant systems have improved their components or developed new devices and procedures that allow for the production of more natural and aesthetically pleasing restorations. Astra dental implant system has greatly contributed to the revolutionary and improvement of implantology by its conical design which offers a measureless stability between abutment and fixture. On the other hand, the Microthread design allows even distributed forces around the implant head resulting in marginal soft tissue stability, thus long

## Posters: Clinical tips and cases: Regeneration

lasting aesthetic results. These properties allow Astra dental implant system to develop new prosthetic components such as Titanium Abutment with different heights and widths, which permit the production of appropriate restorations replacing missing teeth, regardless of implants diameter, simultaneously making the maintenance easier in posterior regions as well as empowering aesthetics in anterior region.

252

### **Aesthetic reconstruction of impacted maxillary canine associated with AOT**

S. M. Lee\*, J. H. Park, H. M. Kim, J. Y. Lee and M. Y. Kim

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Adenomatoid odontogenic tumour is classified as an odontogenic epithelial tumour. It is predominantly found in young and female patients, located more often in the incisor-canine -premolar region in most cases associated with an unerupted permanent tooth. The management of unerupted tooth associated AOT is important to the patient in aesthetic and functional purposes. So the reconstruction is considered a multi-disciplinary entity requiring the expertise of a number of specialists. It included not only rehabilitation of the occlusion but also periodontally esthetic aspects. Otherwise, reconstructed teeth is accompanied with unfavourable gingival condition; root recession, lacked attached gingiva, and gingival concavity and surgical scar. This case report is the repair of unesthetic gingival condition resulted from orthodontic eruption and surgical excision of impacted canine teeth related to AOT using subepithelial connective tissue graft in a 21-year-old woman. It has followed up for 6 months and maintained a favourable gingival esthetics.

253

### **Periodontal management and role of CAD-CAM technologies in esthetic dentistry**

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The aim of the present work is to evaluate from a clinical point of view the relations existing between soft tissues, surgically treated, and prosthetic therapies performed by means of CAD-CAM technologies. We considered clinical cases treated with different kinds of surgical techniques. The final prosthesis was given by the system CAD/CAM Procera, and the material used was the densely sintered alumina. We also used a CAM technology to obtain metal-free temporary crowns. The relationship between biological widths, placing of the finishing line, gingival biotype, type of preparation and the working area are elements to be considered. But these factors are strictly correlated to the type of material used for restoration. The clinical experience matured through the use of the auro-galvanic crowns (AGC), and many different metal-free methodologies, has shown us that there is a significant difference in tissue response and in the consequent natural gingival trophism obtainable, part of it intrinsically linked to the core utilized. In fact, in optimal conditions of oral hygiene, the constant factors are high tissue naturalness, and gingival health with the absence of irritation around the prosthetic restoration. An ulterior element of interest is the observation of the extreme naturalness of transmission of light through the deep cervical gingival tissues. CAD-CAM technologies are ready for our use, but it's realistic for us to consider whether we are really ready to use them.

## Posters: Clinical tips and cases: Regeneration

254

### **A treatment of severe periodontal and periodontal-endodontic lesions without a membrane**

Dr. Nik. V. Pandya\* BDS, MSc, DGDp

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The objective of this study was to regain clinical attachment for teeth that would normally have been extracted due to severe periodontal disease or periodontal-endodontic lesions. The material used was synthetic and resorbable, which has a bio-engineered surface charge that up regulates the gene factor, and has added benefit that no membrane is required. The aim was to review the stability of the newly formed tissues over a five-year period. CIP disease may present as pockets with/without suppuration. The affected tooth may exhibit tenderness to percussion, increased mobility and presence of a bony defect. Such symptoms may also be present due to irritants in the root canal(s) of the tooth. The success of both periodontal and endodontic therapy depends on the elimination of both disease processes, whether they exist separately or as a combined lesion. The main constituent of the bone substitute is a multi-porous b-Tri-Calcium Phosphate. A Calcium Sulphate matrix allows formation of mouldable material which sets hard and acts as a cell-occlusive barrier. It also allows space maintenance into which bone will grow when placed in a bony defect. The material also contains other ions and a bio-engineered surface charge to enhance bone growth. The matrix has additional benefit of being bacteriostatic. This may be an important factor in the treatment of the periodontal-endodontic

lesion. Successful clinical outcomes have been reviewed for a period of five years.

255

### **Evaluation of bone healing after odontogenic cyst enucleation with and without guided bone regeneration with CT**

S. Kahraman\*, S.Gultekin, S.Cetiner and S.Yucetas

*Gazi Univ, Ankara, Turkey*

This prospective, controlled and randomized study was planned to evaluate bone regeneration by using allogenic bone graft (ABG) and collagen membrane (CM) after removal of large odontogenic cysts. Twenty-four patients with odontogenic cysts selected and divided into two, as control and study groups. The control group was treated by enucleation and primary closure, whereas ABG and CM were applied to the cavities of the study group after enucleation. CT scans were taken postoperatively on the tenth day and in the third, sixth and twelfth months to measure residual cavity volume and density of the newly formed tissue. Initial and remaining cavity volumes were compared on CT images and density was measured in Hounsfield units. Means and standard deviations were calculated for all variables in the groups. Similar results were observed in control and study groups. A progressive reduction in the volume of the cavities both with and without graft material was apparent. Findings for the mean density of the newly formed tissue in the third, sixth and twelfth postoperative months

were also similar. The mean residual volume at each observation period did not show statistical differences between the groups. 12th month CT scans showed a significant increase in the average density of the newly formed bone in the mandible when compared to the maxilla. Results of this study suggest that using grafts doesn't contribute to increased bone regeneration and large cyst defects can regenerate spontaneously.

256

### The long-term effect of infra-osseous defects treatment with EMD and bone substitutes

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**Aims:** The purpose of this study was to evaluate the long-term treatment outcome of the use of Emdogain and BioGran in periodontal treatment of infra-osseous defects in conjunction with surgical procedures in order to obtain higher clinical attachment levels.

**Method:** The protocol included 20 patients with at least two interproximal sites in the same jaw, appropriately separated and randomly distributed in pair test and control sites. The morphologic characteristics were pocket depth  $\geq 6$  mm, infraosseous defects with at least 4 mm depth and 2 mm width. Clinical parameters included API, PBI, periodontal pockets depth, clinical attachment level, gingival recession and radiographic evaluation. Statistical analysis used *t*-Student test (SPSS 10.0) with the patient as statistic unit, considering the greater attachment level value, mean and standard deviation.

**Results:** The average values of the clinical attachment level in the test sites were 1.8 mm at 6 months, 2.1 mm after 12 months and 2.5 after 18 months, for the group treated with Emdogain and Biogran and respectively 1.1 mm at 6 months 1.2 mm at 12 months and 1.4 mm after 18 months for the group treated with Emdogain only.

**Conclusions:** Periodontal regeneration using EMD and bone substitute can be used in the areas with severe bone loss resulting from periodontitis. The results are first observed in the reduction of probing depth, while the bone gain is evident after 2 months and it continues to improve.

257

### Effects of demineralized bone matrix implantation on repair of alveolar socket of diabetic rats (type I)

M. Kadhodazadeh\*, F. Mollaverdi and S. H. Ahmadpoor  
Tehran University, Iran

**Aim:** The aim of this study was to determine the effects of DBM on repair of alveolar bone of diabetic rats treated with insulin.

**Methods:** This study was carried out on 80 adult 8 weeks old rats (Albino) with average weight 200–250 gram; The animal are divided in to four groups (N:5) as follow: first group; control and second, third and fourth monohydrate through veins of tail. Diabetes was only controlled in group 4 with insulin NPH, 1 unit daily. After 10 days upper right incisor extracted by an adapted instrument, and socket was filled with DBM in groups 4, 3. In the end of first, second, third and fourth weeks animals scarified. Specimen's were prepared and stained with H.E.

**Results:** In group 4; in the end of first week osteoblastic activity and bone formation invicnty of DBM with collagenfibers was observed in the end of second, third and fourth. Thick trabecular bone formation was observed whereas in group 3(untreated with insulin) bone trabecular formed dispersly around the DBM particles. In group 2 (untreated, no DBM) in the end of first and second weeks haematoma and inflammation was the dominant

histological feature, in addition bone formation showed poorly in the third and fourth weeks.

**Conclusion:** The results of this study showed that osteogenesis and trabecular formation in alveolar socket of group 4 that received DBM and insulin, was better than other groups. It seems DBM stimulated osteoprogenitor cell to produce osteoblastic cell.

258

### Osteoconductive potential of collagen/hydroxyapatite/chondroitin sulphate biomaterial in alveolar bone reconstruction

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**Background:** The aim of this study was to describe the clinical and histologic outcome of reconstructive procedure for a critical-size, post-extraction alveolar bone defect with a collagen/hydroxyapatite/chondroitin sulphate graft (Biostite®) and a collagene bioabsorbable membrane (Paroguide®).

**Materials and methods:** After tooth removal and socket degranulation, Biostite® was implanted to fill the defect. A Paroguide® membrane was trimmed and adapted to completely cover the defect and the implanted graft. Flaps were mobilized and sutured to ensure primary closure. After 12 and 36 months, hard tissue biopsies were harvested.

**Results:** Healing progressed uneventfully, no esfoliation or infective complication were recorded. Radiographic follow-up along the observation period showed a progressive reduction in diameter of the grafted biomaterial and, parallely, an increase in radiopacity of the defect. Histologically, a mature, well-vascularized, newly formed bone was present. Particles of biomaterial integrated with surrounding bone were still evident at both observation intervals. A scarce number of CD68-positive cells was observed at 12 and 36 months, suggesting a slow resorption rate of the biomaterial.

**Conclusions:** This case report supports the osteoconductive potential of a collagen/hydroxyapatite/chondroitin sulphate graft. This study was partly supported by VEBAS s.r.l., Italy and Research Center for the Study of Periodontal Diseases, University of Ferrara.

259

### Surgical treatment of advanced periodontal defects by combined use of GTR-barriers and bovine bone. a follow-up of 29 patients for up to 5 years

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**Background:** Long-term clinical data on the combined use of GTR-barriers and filler materials in periodontal surgery are scarce. This report comprise up to 5-year follow-ups of advanced periodontal defects treated with GTR-barriers and bovine bone mineral.

**Material and methods:** Twenty-six patients, consecutively treated are reported. After examination and oral hygienic treatment the extensive periodontal defects of various topography included in the study were surgically exposed, debrided, filled with bovine bone mineral (Bio-Oss®), and covered with bioresorbable GTR-barriers (Guidor® or Bio-Gide®). Postoperatively, patients were advised to refrain from brushing in the surgical area, instead rinsing with 0.1% chlorhexidine for 6 weeks. After 6 months of close monitoring, supportive periodontal therapies were given with individual re-call intervals. Clinical and radiographic examinations were then made annually.

**Results:** 26, 17 and 17 patients have so far been examined at one-, three- and five-years follow-up respectively. Mean probing depth (PPD) was  $9.9 \pm 2.0$  mm at baseline,  $4.7 \pm 1.9$  mm at year one,

4.3 ± 1.3 mm at year three and 4.6 ± 1.9 mm at year five. Gain of mean clinical attachment level (CAL) was 3.8 mm at year one, 4.3 mm at year three and 4.0 mm at year five.

**Conclusion:** The study shows that, extensive periodontal defects can be successfully treated by the combined use of GTR-barriers and bovine bone mineral resulting in a substantial reduction of PPD and achievement of a stable, long-term gain of CAL.

260

### **Radiographic anatomy of intrabony defects as a predictor of the response to periodontal treatment**

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**Introduction:** The presence of periodontal intrabony defects is a main indication of regenerative therapies. Although the recent regenerative procedures are more predictable, the disparity of the results orient us to consider the influence of unknown variables.

**Aim:** To study the possible influence of morphological radiographic variables of bone defect over the bone response to the treatment with open flap debridement, valued radiographically.

**Material and methods:** We have compiled a total of 430 periodontal patients of a dental office dedicated to the treatment of periodontal disease. They were treated with SRP, open flap debridement and participate regularly in a supportive periodontal therapy program each 3–6 months during at least 12 months. As radiographic variables we have considered the bone fill, rate of bone fill, crown-root ratio and type, depth and angle of the defect.

**Results:** There have been analysed by a computerized method 55 intrabony defects of a minimum depth of 2 mm. The average time of participation in the supportive periodontal therapy program was of 69 months. We have found a significant statistical relation between bone fill and depth and angle of the defect.

**Conclusions:** Our results suggest that the radiographic morphology and more concretely the depth-width ratio are valid predictors of the response to the therapy employed. This reinforces the contemplation of the characteristics of the defect as main factors that indicate the use of regenerative therapies predictably.

261

### **Periodontal and bone cicatrisation after the rescue surgery (surgical repositioning) of impacted lower second molars**

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The rescue surgery includes a group of surgical techniques that, alone or aided by other dental techniques allow the reposition of an included or retained tooth in its normal situation inside the dental arch. Inside these techniques is the surgical repositioning, that pursues the placement of the tooth in their correct position by means of a slight elevation of the same one, elongating, but not breaking, their vascular-nervous package. In the case of the impacted lower second molars, this elevation, besides repositioning correctly the tooth, create an empty space in mesial of the relocated tooth. They have intended different treatments with the purpose of that in this space are regenerated the normal structures of support of the tooth (materials of bone filler, bone regeneration or tissular guided regeneration, etc.). In our work we apply this technique on ten retained second lowers molars, valuing the protocol used in the bone and periodontal cicatrisation of our sample, and comparing our results with those of other published studies.

262

### **Early orthodontic movement in periodontally involved teeth treated with GTR: report of three cases**

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**Background:** The orthodontic needs in case of periodontal disease with deep infrabony defects, require both orthodontic and regenerative therapy. The sequence and the timing of these therapies is still unclear and lack of information exist in the literature on this issue, moreover, few data are available about the best clinical periodontal approach in this cases.

**Aim:** The aim of this study was to evaluate the influence of the early orthodontic movement after GTR.

**Material and methods:** Three patients were treated with regenerative procedure in aesthetic area. Clinical parameters such as PD, CAL and REC, were recorded at baseline, 3, 6 and 12 months. X-rays were taken at baseline, and at the end of the orthodontic therapy. The orthodontic treatment started after twelfth months after surgery in one case, sixth months in the second case and three months in the last case.

**Results:** Clinical results showed a significant mean reduction of the baseline parameters in terms of PD and CAL with an improvement of the soft tissue conditions, such as gingival margin and interdental papillae high.

**Conclusions:** Within the limits of the study we observed that in all three cases the regenerative procedure combined with the orthodontic movement gave satisfactory results in terms of PD, CAL and REC gain and as aesthetic final outcome. The different timing of the beginning of the orthodontic force application did not influenced the final result. The reduction of the therapy duration resulted in less discomfort for the patients.

263

### **Bone regeneration in surgical and no surgical cases**

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**Introduction:** The aim of this study is to compare the clinical and radiological results of the treatment of deep periodontal pockets accompanied by bone defects after using surgical and no surgical therapies.

**Materials and methods:** Calcium carbonate biomaterial (Biocoral) as bone substitute and Metronidazole 25% (Elyzol) antimicrobial medication for local use. Studies demonstrate that porosity of natural coral, constituted fundamentally of calcium carbonate, is important for bone graft regeneration. The absorption of the material and newly formed bone are also demonstrated. Antimicrobial release on the infection site supplies a higher concentration on infected spot with advantage of minimizing side systemic order effects.

**Results:** Improvement in radiological and clinical parameters which indicates osseous regeneration in all cases.

**Conclusion:** Despite technique followed the most important thing is that therapy is actually effective as a function of a correct patient selection, an adequate indication and execution of technical procedure and a periodic maintenance.

264

### **Treatment of a perio-endo complicated tooth by intentional replantation after emdogain® application a case report**

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Root-canal infection has been shown to provoke an inflammatory reaction in the periodontium. The prognosis of teeth with

periodontal problems combined with root-canal infection is often doubtful. The application of EMD to replanted teeth may enhance regeneration of the periodontal ligament and prevent ankylosis. The aim of this case report is to present the treatment of a 45-year-old female patient displaying a combined periodontal-endodontic pathology. At baseline, a deep periodontal pocket (PPD = 12 mm) was detected at the palatal medial region of tooth 15. The tooth had already received endodontic treatment 5 years ago. Radiographic examination indicated periapical radiolucency. A diagnosis of periapical endodontic lesion with suppurative apical periodontitis was established. The treatment consisted of initial periodontal therapy followed by periodontal surgery. After flap elevation, visual examination revealed a draining sinus tract on the palatal bone plate associated with an angular bony defect. The tooth was intentionally extracted and the buccal and lingual cortical bone plates remained intact. The periapical lesion was removed and Emdogain® was applied to the root surface. The tooth was then replanted back into its socket and splinted. At 1-year following therapy, the PPD was reduced to less than 3 mm whereas the radiographic examination revealed an almost complete resolution of the apical radiolucency indicating a stable clinical situation.

265

### **Influence of osteoporosis on periodontal regeneration**

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The aim of present study is to evaluate new bone density following periodontal surgical therapies, and to analyse significant factors associated with clinical outcome. Material comprised 40 female patients with chronic periodontitis separated into two groups (group A,  $n = 20$  without any systemic disease; group B,  $n = 20$ , with osteoporosis without systemic therapy). Before surgical therapy periodontal indexes were measured together with radiography and radiodensitometry. Bone density was measured by computer program 'THROPHY-RWG-UI', and expressed as a part of grayscale (0–256). Following re-evaluation, they were treated with surgical therapy with mucoperiosteal full thickness flap. Infrabony defects were filled with Bio-oss spongiosa and covered with Bio-gide. Control measurements were done after 6 months and after 1 year. After 6 months post surgical measurements of bone density in both groups showed bigger density values (Group A,  $+84 \pm 50$ ,  $P < 0.0001$ ; Group B,  $+12 \pm 14$ ,  $P < 0.002$ ), and difference between group A and group B was in favour of group A ( $P < 0.0001$ ). After 1-year post surgical measurements showed minimal increase compared to measurements after 6 months (Group A,  $+14 \pm 30$ ,  $P = 0.0002$ ; Group B,  $+1 \pm 1$ ,  $P < 0.0001$ ). This minimal increase was in favour of group A ( $P = 0.005$ ). This shows usefulness of periodontal surgical therapy, and draws attention to the fact that regeneration of new bone is rather inferior in female patients with osteoporosis, but without systemic therapy.

266

### **Allomatrix injectable putty in periodontal regeneration: a preliminary study**

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Demineralized bone matrix (DBM) with a synthetic calcium sulphate has demonstrated clinical success in periodontal regeneration. A new evolution of this biomaterial was achieved in orthopedic surgery through the combination of human DBM in a medium of surgical grade calcium sulphate and pharmaceutical grade sodium carboxymethylcellulose. The present study aims to

assess the clinical and radiographic effect of this combination (Allomatrix Injectable Putty) in periodontal treatment. Eight chronic periodontitis patients who had radiographical interproximal defects with an associated probing depth (PD) of  $\geq 6$  and an intrabony component of  $\geq 4$  mm, were included. Prior to surgical treatment and at 8 months, plaque and sulcus bleeding indices, PD, marginal soft tissue and relative attachment levels, probing bone and radiographic bone levels were measured. No adverse event related to the use of Allomatrix was noted. Favourable clinical outcomes in soft and hard tissue measurements were achieved when compared to baseline ( $P < 0.01$ ). Considering the deepest site of the defects,  $4.0 \pm 1.41$  mm of PD reduction,  $2.5 \pm 1.31$  mm of attachment gain,  $1.33 \pm 0.65$  mm of recession, were obtained. The mean clinical and radiographic bone gain was  $2.12 \pm 0.90$  mm and  $2.67 \pm 1.3$  mm, respectively. Within the limits of this study, it was concluded that Allomatrix Injectable Putty is promising for clinical and radiographic improvements in the treatment of intrabony periodontal defects.

267

### **Enamel matrix proteins and bovine derived xenograft in the treatment of intrabony periodontal defects**

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The aim of the present study was to present cases and assess the clinical and radiographical outcome of the EMD + BDX therapy in intrabony periodontal defects. Fourteen chronic periodontitis patients who received non-surgical periodontal therapy and had radiographical interproximal defects with an associated probing depth (PD) of  $\geq 6$  and an intrabony component of  $\geq 4$  mm, were included. Prior to surgical treatment and at 8 months, plaque and sulcus bleeding indices, PD, marginal soft tissue and relative attachment levels, probing bone and radiographic bone levels were measured. Favourable clinical outcomes in soft and hard tissue measurements were achieved when compared to baseline ( $P < 0.001$ ). Considering the deepest site of the defects,  $4.32 \pm 1.31$  mm of PD reduction,  $3.21 \pm 0.97$  mm of attachment gain,  $0.82 \pm 0.54$  mm of recession, were obtained. The mean clinical and radiographic bone gain was  $2.64 \pm 0.60$  mm and  $2.57 \pm 0.39$  mm, respectively. Within the limits of this study, the combined application of EMD with BDX in intrabony periodontal defects significantly improves the clinical and radiographical parameters.

268

### **Decision making guidelines for grafting in sinus floor elevation procedures**

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**Purpose:** The average dimensions of the adult maxillary sinus are 25–35 mm (width), 36–45 mm (height) and 38–45 mm (length) so a majority of the sinus augmentations can be performed with 2–3 cc of autogenous bone in a composite graft. We propose a decision-making guidelines for grafting in sinus floor elevation procedures. **Materials and methods:** We evaluated the results obtained in 62 sinus lift procedures. In 46 sinus lift procedures (70%) the grafts were autologous bone grafts obtained from oral cavity harvested using a safe scraper device. In 16 sinus lift procedures (30%) tibial and calvarial grafts were harvested. Survival rates for implants placed in the augmented sinus were 95% after the one year follow-up period.

**Results:** In all cases new bone formation was confirmed radiologically and implant placement was performed successfully. The analysis of samples obtained by biopsy revealed the presence of mature bone. No healing problems were observed in any case.

**Conclusions:** Those sinuses presenting normal pneumatization in the radiologic examination can be augmented with a mixture of bovine hydroxyapatite and autogenous bone obtained from oral cavity with the use of a bone scraper. If the pneumatization of the sinus is high, the requirements of bone graft become increased and other donor sites for autogenous bone such as the iliac crest, tibia or calvaria must be taken into account.

269

### Use of rhBMP-2 and demineralized bone matrix in periodontal surgery in humans

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**Aims:** Clinical trials in animals and humans have demonstrated the high osseoinductive potential of rhBMP-2. Since rhBMP-2 became registered medication for specific treatments of bones in 2002 thousands of patients have successfully been treated for these indications. In comparison to the carrier material ACS as part of the medical kit DBM meets the requirements of a more stable consistence. In individual cases it was expected to observe the ability of rhBMP-2/DBM (a) to repair periodontal bone defects by de novo bone and (b) to create vertical bone without the presence of surrounding bony walls.

**Material and methods:** In four individual cases rhBMP-2/DBM was used as a new strategy in patients with periodontal bone lesions who could not successfully be treated in the conventional way. RhBMP-2 was applied on DBM and implanted in the periodontal defect using a modified operation technique. The evaluation was conducted by measurement of pocket depth, clinical observation and x-ray control.

**Results:** The probing pocket depth of 5 to 8 mm could be reduced in all cases to the physiological standard of 2 to 3 mm. The x-ray control clearly shows vertical bone growth without prior existing bony walls.

**Discussion:** The newly developed method of augmentation with the high osseoinductive rhBMP-2 on DBM shows good and promising

results in four individual cases concerning vertical de novo bone growth in periodontal bony defects. Following clinical trials should confirm these results.

270

### Evaluation of effectiveness between deproteinized bovine derived bone graft alone and combined with an autogenous bone graft in intrabony defects

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**Aim:** This study was designed to evaluate the effectiveness between deproteinized bovine derived bone graft alone and combined with an autogenous bone graft in periodontal intrabony defects.

**Materials and methods:** Forty-two interproximal intrabony defects in 17 systemically healthy patients with chronic periodontitis have been included in the study. The control group, in which Bio-Oss® alone has been used, consisted of 21 defects. The test group, in which Bio-Oss® has been combined with an autogenous bone graft, consisted of 21 defects. In this study, Silness-Löe plaque index, Löe-Silness gingival index, location of the gingival margin, periodontal probing depth, clinical attachment level, sounding measurements have been performed. Besides re-entry measurements have been used to measure the formation of new mineralized tissue in 25 defects.

**Results:** The gain of clinical attachment level in the control group 2.26 mm, in the test group 2.12 mm was observed at the end of a 6-month healing period. In the transgingival measurements; bone fill of 2.5 mm in the control group, 2.76 mm in the test group was obtained. During the re-entry surgery, bone fill of 3.13 mm in the control group, 3.81 mm in the test group was gained. In all the clinical measurements the differences between two groups were found statistically insignificant.

**Conclusion:** These findings indicated that both two treatment choices have been found to be effective in the treatment of intrabony defects.

## Posters: Clinical tips and cases: Restorative aspects

271

### Aesthetic rehabilitation using mixed metallo-nonmetallic prosthetic appliances

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The dento-somatho-facial aesthetic field has as main purpose to create the illusion of the natural aspect. The aim was to evaluate the possibilities of the aesthetic rehabilitation by using metallo-ceramic/metallo-composite prosthetic appliances. We realized 12 metallo-ceramic and 15 metallo-composite prosthetic appliances used for different partial edentations treatment. The metallic frameworks made up of Ni-Cr-Mo alloy, realized on working casts mounted into an Whip-Mix articulator, were conditioned for an optimum link between the constructions elements. The ceramic (IPS d.SIGN) or the composite (SR ADORO) aesthetic element covered the entire metallic framework. In both technologies the aesthetic material application was possible in successive layers, corresponding to the dental tissues components. While metallo-ceramic constructions detain the supremacy in the individual aesthetic elaboration, metallo-composite technology is characterized by a

shorter working time, a lower price compared to metallo-ceramic constructions and chromatic effects similar to those of ceramic. The new biomaterials and the performant technologies create the conditions to obtain a superposition between the optical, mechanical and biological properties of prosthetic constructions and those of natural teeth. Metallo-ceramic and metallo-composite therapy makes possible a morpho-functional recovery of the affected dental arches by partial edentations and an exceptionally dento-somatho-facial aesthetic rehabilitation.

272

### 'Restorative alveolar interface (RAI)' revisited

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**Background:** There are a lot of ways of treatment in case of the crown root fracture. The surgical crown lengthening procedure, the surgical extrusion, the forced eruption and extraction are possible treatments, but their cons exist. Ross et al. (1982, 1983) showed the surgical management of the restorative alveolar interface (RAI). These cases are the modifications of the RAI concept to overcome



the shortcoming of the Andreasen's method (1994) in the crown root fracture.

**Materials and methods:** The four cases (two incisors, two molars) of the subgingival crown root fractures were managed by the following method. After the flaps were made, the fractured subgingival root surfaces were prepared with the high speed and low speed diamond burs. If the fracture lines were in the alveolar bone, the osseous surgery was done. The flaps were repositioned or apically positioned if the positive architectures were possible. After the soft tissue healing was completed, the margins of restoration were made in the newly created shallow sulcus.

**Results:** In all cases the BOPs were negative, the PDs were 2 to 3 mm and esthetics was obtained.

**Conclusion:** This method is easy, fast and clinically highly predictable in compared with the other methods in case of the crown root fracture, especially in molars. If the fractured line is in the bone, the combined treatment with the forced eruption or the surgical extrusion is recommended to avoid the osseous surgery.

273

### **An alternative to immediate loading for implant cases in totally edentulous patients**

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In recent years there have been many articles and presentations about totally edentulous patients treated with immediate loaded fixed prostheses over implants. It is obvious that the results of this type of treatment, as seen in the literature, are quite similar to that of delayed loading, when respecting a few basic rules. Nevertheless, there are several factors (the inexperience of the clinician in immediate loading, the busy professional life of some patients that

prevent them attending for the numerous appointments for this kind of treatment) that can lead us to offer our patients other alternatives to immediate loading. We present some cases of totally edentulous patients to which a sufficient number of implants were co-located in order to restore them with a fixed restoration after the osseointegration period. During the time taken to make this final restoration, patients were restored with a provisional metal-resin fixed prosthesis made on a few strategic teeth that, despite their bad periodontal prognosis, were retained during the osseointegration period to serve as abutments for the provisional restorations.

274

### **Porcelain laminated veneer restorations in periodontal patients**

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One of the most common problems when completing periodontal treatment, especially after surgical procedures, is the creation of broad interdental spaces that, in the anterosuperior area, means an important esthetic defect. In this context, one of the most esthetic restorative treatments used in Prosthodontics, the porcelain laminated veneers, has not been used enough to hide these black triangles. Probably, this fact has been due to the frequent association between this type of treatment and very conservative dental preparations that are not going to be done in periodontal patients because of the need of locating the contact point closer to the new location of the osseous cresta. In this abstract, we present some clinical cases of periodontal patients restored with porcelain laminated veneers in which we can check not only the esthetic improvement of the anterior region, but also the excellent adaptation of periodontal tissues to the restorations.

## Posters: Miscellaneous

275

### **Immunohistochemical evaluation of plasminogen activators and their inhibitors in drug-induced gingival overgrowth**

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**Aim:** To evaluate expression of plasminogen activator enzymes and their inhibitors by immunohistochemical techniques in drug-induced gingival overgrowth tissue samples.

**Methods:** Fourteen cyclosporin-, three phenytoin-, five nifedipine-induced overgrown tissues, 10 control tissues from gingivitis patients, and 14 control tissues from healthy subjects were evaluated. Clinical periodontal recordings and tissue sampling were performed at baseline. Expression of tissue-type (t-PA) and urokinase-type (u-PA) plasminogen activators, plasminogen activator inhibitor-1 (PAI-1) and PAI-2 were determined in formalin-fixed/paraffin-embedded tissue sections. Data were evaluated by ANOVA, post-hoc Sidak test with modified Bonferroni correction and Pearson correlation analysis.

**Results:** The t-PA expression was significantly more in cyclosporin, nifedipine, and gingivitis groups than in healthy control ( $P < 0.01$ ). All drug-induced gingival overgrowth groups exhibited significantly more expressions of PAI-2 than healthy control group ( $P < 0.01$ ). u-PA expression in cyclosporin- and phenytoin-treated groups was significantly higher than gingivitis and healthy control groups ( $P < 0.01$ ). Cyclosporin group

revealed significantly more expression of PAI-1 than nifedipine and healthy groups ( $P < 0.05$ ).

**Conclusion:** In conclusion, significant increases in gingival tissue expressions of plasminogen activators and their inhibitors may indicate involvement of this system in drug-induced gingival overgrowth pathogenesis.

276

### **Endocannabinoid, anandamide in gingival tissue regulates the periodontal inflammation through NF- $\kappa$ B pathway inhibition**

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**Aims:** Anandamide (AEA) exhibits anti-inflammatory effects. However, its role in the periodontal field remains unknown. In this study, we investigated the roles of AEA and its receptors in the pathogenesis of periodontal disease.

**Materials and methods:** The levels of AEA in gingival crevicular fluid (GCF) were measured using High Performance Liquid Chromatography (HPLC). Localization of cannabinoid receptors (CB1 and CB2) in periodontal tissue were carried out by means of immunohistochemistry. Expression of cannabinoid receptors in human gingival fibroblasts (HGFs) were examined with the RT-PCR and Western blot analysis. The productions of pro-inflammatory cytokines from HGFs were measured by ELISA. Gel shift assay was employed to detect NF- $\kappa$ B activation.

**Results:** We found that GCF contained a detectable level of AEA. CB1 and CB2 were expressed by HGFs, and markedly up-regulated under pathological conditions. AEA significantly reduced the production of pro-inflammatory mediators (IL-6, IL-8 and MCP-1) induced by *Porphyromonas gingivalis* (Pg) LPS in HGFs, and this effect was attenuated by AM251 and SR144528, selective antagonists of CB1 and CB2, respectively. Moreover, AEA completely blocked Pg LPS-triggered NF- $\kappa$ B activation.

**Discussion/Conclusion:** Our results imply that AEA may down-regulate inflammatory reactions in periodontitis through NF- $\kappa$ B pathway inhibition.

277

### The possible relationship between insulin-like growth factor-I and central giant cell granuloma: a preliminary report

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Central giant cell granuloma (CGCG) is an intraosseous non-neoplastic lesion consisting of fibrous tissue that contains aggregations of multinucleated giant cells, fibroblast proliferation, foci of haemorrhage and trabeculae of woven bone. Although many studies are presented about CGCG, the relationship between CGCG and the systemic polypeptides is still unclear. The aim of the present report is to analyse the possible relationship between insulin-like growth factor (IGF) and CGCG. Patients diagnosed as CGCG in maxillary or mandibular sites participated in the study. A laboratory investigation of complete blood count, systemic hormone levels and systemic biochemical profile was analysed prior to surgery, and at 3 and 6 months post-surgery. A 3 to 5-fold-of increase in systemic IGF-I level prior to surgery was noted in all cases. After immunohistochemical staining, a significant IGF-I staining was obtained at intra-cytoplasmic areas of multiple multinucleated giant cells at baseline specimens. Following surgical therapies, systemic evaluation of IGF-I demonstrated a trend of reduction at 3 months that resulted with normal limits at 6 months post-surgery. The relationship between neoplastic tumours and IGF-I is clear. However, to the authors' knowledge, this is the first report demonstrating the possible relationship between CGCG, a non-neoplastic lesion, and IGF-I. It is speculative that circulating IGF-I levels may influence the etiology and progression of CGCG.

278

### The impact of clinical inflammation on peri-implant sulcus fluid and gingival crevice fluid myeloperoxidase and nitrite levels

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As enzymes and free radicals play important roles in inflammatory process, the aim of the present study was to analyse the impact of the presence/severity of clinical inflammation on peri-implant sulcus fluid (PISF) and gingival crevice fluid (GCF) myeloperoxidase and nitrite levels. Forty-two implants and 67 natural teeth were included. Clinical parameters were recorded, and PISF and GCF myeloperoxidase and nitrite levels were spectrophotometrically determined at each sampling site. Differences between non-inflamed and inflamed implant and tooth sites, and correlations between clinical parameters, and nitrite and myeloperoxidase levels were statistically analysed. Inflamed teeth and implant sites both exhibited higher fluid volume, myeloperoxidase and nitrite levels than non-inflamed sites ( $P < 0.05$ ). The number of sites with detectable levels of nitrite was generally higher than sites with detectable levels of myeloperoxidase. Despite the higher GCF myeloperoxidase levels at all sites and higher nitrite levels at non-

inflamed sites, differences between PISF and GCF were not significant. Correlations were more prominent between gingival inflammation, bleeding and myeloperoxidase levels than nitrite levels ( $P < 0.05$ ). PISF nitric oxide metabolites and myeloperoxidase may serve as measures of inflammatory response at peri-implant sites. When nitrite and myeloperoxidase levels are concerned, the inflammatory process around implants and natural teeth seem to present similarities.

279

### Neutrophil functions before and after periodontal treatment in generalized aggressive periodontitis

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The objective of this study was to evaluate clinical parameters, phagocytosis and oxidative burst functions of neutrophils in generalized aggressive periodontitis (GAP) and chronic periodontitis (CP) before and after initial periodontal therapy. 10 GAP, 10 CP patients and 10 healthy individuals were included. At 0. and 49 days peripheral blood analyses including phagocytosis and oxidative burst functions have been performed by flow cytometric method. At the same days plaque index, bleeding on probing, probing depth and relative attachment level were measured. Significant clinical improvements were observed in two patient groups ( $P < 0.01$ ) compared to their respective baseline values only with the exception of relative attachment level changes in CP. Intergroup comparisons revealed no significant differences. Regarding to the immunological evaluations % phagocytosis and oxidative burst functions demonstrated no significant differences between the three groups. The differences between % phagocytosis and oxidative burst functions in intergroup comparison did not reveal statistical significance. The mean fluorescence intensity considered as the degree of phagocytosis was found to be much higher in GAP patients than in CP and healthy group before the initial periodontal treatment but reached almost similar levels after the treatment. It can be concluded that the investigated neutrophil functions of GAP can be considered as in expected normal ranges.

280

### The role of dietary habits in the aetiology of recurrent aphthous ulcers -a preliminary study

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Recurrent aphthous ulcer (RAU) is one of the most common oral mucosal disorders characterized with painful, recurrent ulcers on unattached mucosa. The aetiology of RAU remains unsolved but factors such as stress, trauma, genetic predisposition, haematological deficiencies, gastrointestinal disorders, immunologic abnormalities, hormonal disturbances, viral factors and food hypersensitivity have all been implicated. There are some contradictory reports about the relationship between RAU and food sensitivity. In this preliminary study, we aimed to investigate some of the suspected foods in a group of RAU patients by questionnaire. Thirty patients suffering with RAU were aged between 19–55 years old and were all healthy otherwise. They were compared with 17 non-RAU subject aged between 22–45 years old. Results are discussed under the light of some of the previously reported studies. Some allergenic foods like; wheat flavours, chocolate, nuts, milk, tomatoes were investigated and allergenic food consumption was found to be statistically higher in RAU group when compared with the control group ( $P < 0.05$ ). Elimination diets due to the suspected foodstuffs may have an importance treatment role in some of RAU patients who are haematologically and immunologically

healthy. Dietary treatment is advised only if the definite allergenic factor is known and may be a useful treatment method in RAU patients who are resistant to other treatment modalities.

281

### IL-1A, IL-1B and TNF-A gene polymorphisms in Turkish lap patients

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IL-1 and TNF- $\alpha$  are a potent immunomodulators and proinflammatory cytokines implicated in the pathogenesis of autoimmune and infectious diseases. As in other inflammatory diseases of infectious origin, periodontitis could be linked to altered proinflammatory cytokine expression. Localized aggressive periodontitis (LAP) is a genetic disorder in which inflammatory pathways mediate disease. The aim of this study was to investigate the association of the polymorphisms in IL-1 $\alpha$ <sup>+4845</sup>, IL-1 $\beta$ <sup>+3954</sup> and TNF  $\alpha$ <sup>-308</sup> genes with LAP patients in Turkish population. The LAP group consisted of 31 patients, and the control group included 31 healthy subjects. All subjects were genotyped for IL-1 $\alpha$ <sup>+4845</sup>, IL-1 $\beta$ <sup>+3954</sup> and TNF  $\alpha$ <sup>-308</sup> loci by standard PCR amplification followed by restriction enzyme digestion and gel electrophoresis. A higher frequency of heterozygous of the IL-1 $\alpha$ <sup>+4845</sup> was found in LAP (65%) than controls (35%). While allele 1 of the IL-1 $\beta$ <sup>+3954</sup> was the most frequent genotype in LAP (62%), there was no allele 1 of the IL-1 $\beta$ <sup>+3954</sup> in the controls. For both of these traits, the differences were statistically significant ( $P < 0.05$ ). The frequency of the allele 1 of the TNF- $\alpha$  was similar in LAP and controls; the difference was not statistically significant. These findings suggest that both IL-1 polymorphisms are associated with LAP in Turkish population and provide risk factors for LAP. Knowledge of IL genotype is valuable in risk management, treatment planning and prediction of tooth survival.

282

### Differences in the composition of the subgingival microflora among Hispanic American populations

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To compare the subgingival microbiota of distinct patient populations demonstrating Periodontitis, in Colombia, Chile and Spain. **Patients and methods:** Patients suffering chronic Periodontitis were selected. Microbiological pooled samples were taken at four sites per patient and analysed by culture. Identical training in anaerobic culturing procedures was provided for the three centers. Clinical parameters were recorded at the sampled sites. Results were statistically analysed by means of ANOVA or Kruskal-Wallis, and Chi square tests.

**Results:** Samples from 37 Chilean, 41 Colombian and 26 Spanish patients were analysed. No differences were found in age or gender distribution, but a significant lower proportion of smokers was found in Colombia ( $P < 0.05$ ). The mean probing depth was significantly higher for Colombian patients ( $P < 0.001$ ). The mean anaerobic total counts were significantly higher in Chile ( $P < 0.05$ ). No significant differences were found for the frequency of detection of *P. gingivalis* and *A. actinomycetemcomitans*, but important differences were detected for *T. forsythia*, *P. intermedia*, *M. micros*, *F. nucleatum* or enteric rods. Regarding the proportions of flora in positive sites, significant differences were found for most pathogens, being of special relevance those of *P. gingivalis* ( $P < 0.001$ ) and of *T. forsythia* ( $P = 0.04$ ).

**Conclusions:** Important differences were detected in the subgingival microflora of three distinct populations from different geographical origins.

283

### b-FGF regulates MMP and TIMP mRNA expressions of PDL cells

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Basic-Fibroblast Growth Factor has been suggested as a valuable molecule to consider in designing therapies to regenerate periodontal tissues. In this study, the effects of b-FGF on periodontal ligament cells behaviour were examined. Specifically we evaluated b-FGF effects on (a) matrix metalloproteinase(MMP)-1,2,3 and tissue inhibitor of matrix metalloproteinase(TIMP)-1,2 mRNA expression; (b) mineralization; and (c) cell morphology. PDL cells were obtained from premolar teeth extracted for orthodontic reasons. Cells were cultured with DMEM media containing: (1) 5% Fetal Bovine Serum, (2) 5% FBS + ascorbic acid (AA, 50 mg/mL), (3) 5% FBS + b-FGF (10 ng/mL) + AA. Cells within each group were evaluated on day 14 and 21 for gene expression profile using semi quantitative RT-PCR for MMP-1,2,3 and TIMP-1,2 and on day 21 for mineralization by von Kossa staining. Results from this study demonstrated that while b-FGF stimulated markedly MMP-1 and MMP-3 mRNA expression and inhibited TIMP-2 mRNA expression, no differences were noted in MMP-2 and TIMP-1 transcripts at both time points when compared to control groups. b-FGF blocked the mineralization activity of PDL cells. Inhibition of mineralization by b-FGF is correlated with an increase in the levels of MMP-1,3 expressions and a decrease in TIMP-2 transcripts. These results suggested that b-FGF may block PDL cell mediated biomineralization, and regulate MMP and TIMP allowing for a balance between hard and soft tissues required for complete regeneration.

284

### Effects of diode laser irradiation on IGF-1, receptor of IGF-1, and basic FGF in gingival fibroblast and osteoblastic cells

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Laser enhanced biostimulation produces metabolic changes within the cells. Among many physiological effects of low level laser (LLL) irradiation, it is important to recognize that LLL may affect immune cells, which secrete various growth factors for fibroblasts and osteoblasts. Therefore, the aim of the present study was to examine the effects of LLL irradiation on the secretion of insulin like growth factor-1 (IGF-1), receptor of IGF-1, and basic fibroblast growth factor (bFGF) from fibroblasts and osteoblasts. Laser irradiation was carried out with a diode laser. The study consisted of two experimental groups (24 and 48 hours irradiated) and a control group (non-irradiated) for both fibroblast and osteoblast cells. The IGF-1, receptor of IGF-1, and bFGF analysis of experimental and control groups were performed. The irradiated fibroblast and osteoblast cells revealed considerably higher growth factors than the control. The differences were significant in both 24 and 48 hours irradiation groups compared to control. LLL therapy causes increases in IGF-1, receptor of IGF-1, and bFGF in fibroblast and osteoblast cultures. LLL irradiation causes an increase in the secretion of growth factors that play important role in periodontal wound healing and regeneration. This may reveal the effects of LLL on biostimulation of gingival fibroblasts and osteoblasts.

285

**Effects of low-power CO<sub>2</sub> laser in growth and proliferation of cultured human pdl fibroblast in vitro.**

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The aim of this study is to evaluate the effect of low-power CO<sub>2</sub> laser in growth and proliferation of cultured human PDL fibroblast in vitro and to find out the most effective degree of energy and power density where cell proliferation and differentiation to osteoblastic cells highly takes place after laser irradiation. The experimental groups were divided into 4 groups by applying different irradiating distances from cultured cell to laser tip; 2Cm(Focal spot = 0.74mm), 3Cm(Focal spot = 1.04mm) and different irradiating time; 1 second, 3 seconds. And they were applied to laser at a power of 0.5W with 50Hz under continuous mode. The control group was not applied to laser. MTT and ALP activity test were performed to observe the growth of PDL cells and cell differentiation to osteoblastic cells. On the 5th day after laser irradiation, statistically significant increase of cells were found in all groups. But, no significant differences were found among groups. Statistically significant increase of ALP activity was seen on the 3rd, 5th, 7th, and 10th day after laser irradiation compared to the baseline. In this study, there was a weak effect of low-power CO<sub>2</sub> laser on cell proliferation, but there was a better effect on cell differentiation. 2cm, 1sec irradiation was found to be the most effective condition for PDL cell differentiation in this study and 3cm, 3sec was found to be efficient.

286

**Effectiveness of an anaesthetic gel (Dynexan®) on pain sensivity and early wound healing following non-surgical periodontal therapy**

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The purpose of the present study was to evaluate clinically the effect of an anaesthetic gel on pain sensivity and early wound healing following nonsurgical periodontal therapy. A total of 40 patients with chronic periodontitis were enrolled in this randomized, split-mouth, double-blind, placebo-controlled trial. Each subject had 3 sites in each of 2 contra-lateral jaw quadrants with a probing pocket depth (PPD) of  $\geq 5$  mm and bleeding on probing (BOP+). All teeth received scaling and root planning without local anesthesia followed by irrigation with sterile saline and assessment of pain sensivity using a standardized Visual Analogue Scale (VAS). After treatment, the patients randomly received the active or placebo gel into the periodontal pockets and overall pain was again assessed after 10, 20 and 30 min. The VAS showed a statistically significant ( $P < 0.0001$ ) reduction in reported pain, favouring the active gel over the placebo at all 3 different points in time. After 30 minutes the median VAS score was 0.3 in the Dynexan® group as opposed to 4.4 in the placebo-treated group ( $P = 0.0001$ ). In terms of wound healing no differences were found between the test and control sites after 1 week. The results of the study showed that the anaesthetic gel was statistically more effective than the placebo in reducing pain following non-surgical periodontal therapy. However, in terms of early wound healing no significant differences were seen between the two treatment sites.

287

**Histomorphometric study on the effect of interimplant distances on papilla formation and crestal resorption in implants with a morse cone connection**

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The aim was to evaluate the effect that different interimplant distances, after prosthetic restoration, will have on papilla formation and crestal resorption in sub and non-submerged implants. 56 Ankylos implants were placed, 1.5 mm subcrestally, in seven dogs. They were placed so that two bridges, with three interimplant contacts, with 1 (group 1), 2 (group 2) and 3 mm (group 3) distances could be constructed on each side. The sides and the position of the groups were randomly selected. After 12 weeks, the implants received metallic prostheses with 5 mm between the contact point and the bone crest. After 8 weeks, the distances between the contact point and the papilla was measured. Histomorphometry of the distance from the top of the implant to the first interimplant bone-implant contact (CP-IP) was made. The clinical measurement of CP-P for submerged (sub) and non-submerged (nonsub) implants was for group 1:  $3.57 \pm 1.17$  and  $3.10 \pm 0.82$  mm, for group 2 it was:  $3.57 \pm 0.78$  and  $3.16 \pm 0.87$  mm and for group 3 it was  $3.35 \pm 0.55$  and  $3.07 \pm 0.93$  mm respectively. The CP-IP for sub and nonsub group 1 was:  $0.3 \pm 0.17$  and  $0.32 \pm 0.1$ , for group 2 it was  $0.21 \pm 0.09$  and  $0.19 \pm 0.07$  and for group 3 it was  $0.24 \pm 0.15$  and  $0.3 \pm 0.13$  mm respectively. Interimplant distances of 1 to 3 mm, submerged or not, did not significantly affect papilla formation or crestal resorption. The contact point to bone crest distance should be less than 5 mm if interimplant papilla formation is the objective.

288

**The levels of plasma lipids in younger patients with aggressive periodontitis**

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Periodontitis a disease which comprises bacterial infection and local chronic inflammation. Epidemiological studies suggest a relationship between periodontitis and some systemic diseases, diabetes mellitus, coronary artery diseases, etc. Studies presented periodontal disease associated with an increased risk of cardiovascular disease, which in term is associated with hyperlipidemia, amongst other factors. The aim of this study was to measure levels of plasma lipids in younger patients with aggressive periodontitis. **Materials and methods:** We have measured levels of plasma lipids in 26 subjects with aggressive periodontitis (CPITN score IV pocket depth 6 mm and more), mean age  $35.4 \pm 3$  years and 26 age and sex matched control subjects. Both groups were healthy without any systemic diseases. Some of the subjects and control had a smoking habit (10 and 6 respec).

**Results:** Total cholesterol (CHL) and triglycerides (TG) were significantly higher in case group 9% ( $P < 0.03$ ) and 42% ( $P < 0.001$ ), respectively, when compared to controls. HDL and LDL cholesterol were slightly higher in case subj. but with no significant difference. Pathologic values of TG and CHL were significantly higher in patients.

**Conclusion:** The results indicate that hyperlipidemia may be associated with AP in younger healthy subjects, but it remains unclear whether hyperlipidemia comes due to periodontitis or whether periodontal and cardiovascular diseases share hyperlipidemia as a risk factor.

289

### Periodontitis in essential hypertensive patients: a preliminary investigation

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**Aim:** The study was designed to search the relationship between control of essential hypertension (EH) and severity of periodontal disease.

**Materials and methods:** Patients over 34 years of age using antihypertensive drugs for EH ( $n = 56$ ) were enrolled. Patients with diabetes and coronary artery disease were excluded. Patients were considered uncontrolled hypertensive if systolic blood pressure was greater than or equal to 140 mmHg and/or diastolic reading was 90 mmHg or higher. Cardiovascular risk (CVR) factors including body mass index, hsCRP, haptoglobin, alpha<sub>1</sub>-antitrypsin, lipid levels were recorded. Periodontal examination included probing depth (PD), bleeding on probing, plaque index, tooth loss. Association of periodontitis with EH, after adjusting for age, gender, smoking, education and CVR factors was analysed by logistic regression analysis.

**Results:** Forty five percent of 56 hypertensive patients had moderate/severe periodontitis and 20% of them had uncontrolled EH. Compared to patients with no/mild periodontitis ( $< 4$  of non-adjacent teeth with PD  $> 4$  mm), patients with moderate/severe periodontitis ( $> 3$  of non-adjacent teeth with PD  $> 4$  mm) were less likely to have uncontrolled EH, but this difference wasn't statistically significant. After adjusting demographic characteristics and CVR factors, association of EH and periodontitis was statistically insignificant.

**Conclusion:** The control of EH and severity of periodontitis doesn't seem to be related in Turkish population.

290

### Calcium sulphate versus bioabsorbable membranes in periodontal defects: a split-mouth study. long-term results

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**Abstract:** The aim of this study was to evaluate the long-term results obtained with calcium sulphate, and to compare them with the results obtained using bio absorbable membranes.

**Materials and methods:** A split-mouth design was used in 30 patients. Thirty 3-wall periodontal defects were treated using calcium sulphate + autologous bone graft (test) and compared with 30 contralateral defects treated with absorbable membrane + autologous bone graft (control). Before surgery, patients were instructed about oral hygiene and S/RP was completed. All patients were put in periodontal maintenance program. Probing depth (PD), clinical attachment level (CAL) and bleeding on probing (BOP) were recorded at baseline, six months and six years.

**Results:** At baseline, mean PD was  $8.1 \pm 1.06$  mm (control) and  $8.2 \pm 1.24$  mm (test). Mean CAL was  $9.067 \pm 1.36$  mm (control) and  $9.2 \pm 1.24$  mm (test). At 6 months, mean PD was  $3.77 \pm 0.82$  ( $P < 0.001$ ) for control defects and  $3.87 \pm 0.68$  ( $P = 0.001$ ) for test defects. CAL showed a mean of  $5.23 \pm 0.93$  for controls ( $P = 0.001$ ) and  $5.43 \pm 0.75$  for test defects ( $P = 0.001$ ). At 6 years, mean PD was  $4.56 \pm 1.33$  ( $P < 0.001$ ) for control defects and  $3.99 \pm 0.68$  ( $P = 0.001$ ) for test defects. CAL showed a mean of  $6.03 \pm 1.38$  for controls ( $P = 0.001$ ) and  $6.23 \pm 1.36$  for test defects ( $P = 0.001$ ).

**Conclusions:** Both therapies led to both short and long-term improvement of the measured parameters. None of them showed to be superior over the other one.

291

### Serum IgG antibody response to periodontopathogens in three ethnic groups with periodontitis in Malaysia

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**Objectives:** To determine serum IgG response to four periodontopathogens among three ethnic groups in Malaysia and whether increased levels of serum IgG antibody to one species were associated with another species.

**Methods:** Thirty subjects with advanced chronic periodontitis and 30 healthy controls who were ethnically, gender and age-matched were equally selected in numbers from each race. Serum IgG antibody levels to *Pg*, *Tf*, *Pi* and *Aa* were assessed by enzyme-linked immunosorbant assay (ELISA).

**Results:** Mean serum IgG antibody response to *Pi* was found to be significantly higher in the Malays while serum IgG antibody to *Aa* was lowest in the Indian subpopulation. IgG levels to *Tf* and *Aa* in the Chinese population were higher than those in Malays and Indians. Irrespective of the ethnic groups, the diseased subjects had higher mean serum IgG antibody response as compared to the healthy subjects. There was a significant positive correlation between serum IgG antibody response to *Tf* and *Pg* and also between serum IgG antibody response to *Pi* and *Aa*.

**Conclusions:** *Pg*, *Tf*, *Pi* and *Aa* were found in the Malays, Chinese and Indians in the present study. Elevated serum IgG antibody to *Tf* appeared to be positively associated with that of *Pg* and that to *Aa* likewise appeared to be associated with increase in serum IgG to *Pi*. Study supported by Vote F 0131/2003C

292

### Periodontal status of patients with crohn's disease

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**Objectives:** Crohn's disease (CD) has been reported to have periodontal manifestations. However, data on periodontal parameters and microbiology is rare. Recent studies showed an association of single nucleotide polymorphisms (SNPs) in the NOD2/CARD15 gene with CD. These SNPs are involved in immune response towards bacterial products. Our aim was to investigate the periodontal status of patients with CD taking into account periodontal pathogens and the NOD2 SNPs 8, 12 and 13.

**Methods:** The periodontal status of 147 patients with CD was assessed. Detection of periodontopathic bacteria were established by PCR using 16S rRNA probes. NOD2 genotyping was done with the Taqman assay.

**Results:** Mean clinical attachment level (CAL) was  $3.79 \pm 1.01$  mm and mean probing depth (PD)  $3.59 \pm 0.79$  mm. 89.1% of the patients had at least one site with CAL  $> 3.5$  mm and 32.0 % at least one site with CAL  $> 5.5$  mm. There was no significant difference of CAL and PD between the 3 SNP! s. *A. actinomycetemcomitans* was detected in 76.8%, *T. forsythensis* in 64.6% and *C. rectus* in 94.6%. Smoking and medication had no influence on CAL, PD and detection of pathogens.

**Discussion:** Our results suggest that CD patients have an increased prevalence but only moderate severity of periodontal disease. Our data do not support a role of NOD2/CARD15 on periodontal status in CD. However, colonization of periodontal pathogens, in particular *C. rectus*, might be of particular value for periodontal manifestation of CD.

293

**Evolution of salivary chlorhexidine concentrations after oral application of three bioadhesive gels**

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Introduction and aims: Concentration and permanence time of bioadhesive gels of chlorhexidine may vary according to the drug dose and formulation involved. This study investigates the salivary chlorhexidine concentration after the single application of three bioadhesive gels. Material and methods: Forty healthy subjects were included in a randomized, double blind cross-over comparative study. A single oral application of three 0.2% bioadhesive gels (Lácer, Perio-Kin and Bexident Encías). All patient signed an Informed consent. Salivary samples were collected at baseline and 5

minutes and 1, 2, 6, 12, 24, 36 and 48 hours after application. Mean chlorhexidine concentration at each control timepoint, maximum concentration, clearance rate and area under the concentration-time curve were registered. The study was authorized by the Clinical Research Ethics Committee of Virgen de la Arrixaca Hospital (Murcia, Spain). Results: All 40 subjects completed the study. No significant differences were detected among the three gels for any of the study variables. Although the salivary levels of chlorhexidine were high after 5 minutes, they decreased rapidly at the controls made after 1 and 2 hours. Nevertheless, chlorhexidine was still detected in saliva at the last control (48 hours). Conclusions: Forty-eight hours after a single application of three bioadhesive chlorhexidine gels, the antiseptic is still detectable in saliva with no significant differences among the three formulations.