# Master's Thesis

# Prospective evaluation of hard- and soft-tissue remodeling after ridge preservation using a resorbable membrane with and without primary wound closure.


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## Abstract:

**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 randomized controlled clinical trial investigated hard-, and soft-tissue changes after ridge preservation using the bone graft materials PepGen® and DFDBA and GORE RESOLUT® Adapt Regenerative Membrane. **Material and Methods:** 11 patients were enrolled in this clinical prospective single-blinded randomized controlled trial that compares the "guided membrane exposure" (RPe) method with the "flap advancement method" (RPc) in a split mouth design. On the test side the membrane was left exposed and no primary wound closure was attempted, while on the control side primary wound closure was achieved. The changes of keratinized tissue, bone width and bone fill and postoperative discomfort were assessed over a period of 6 months. In addition, bleeding- (GI), plaque-index (PI) and pocket probing depth (PPD) at the adjacent teeth were evaluated. An analysis was made using a Paired T-Test and the Wilcoxon Signed Rank Test with SPSS. Bone cores were obtained at the time of implant placement 6 months post-surgery. **Results:** Significant intergroup results were found for the loss of keratinized mucosa (Rpe=1.55mm; RPc=3.45mm, p=0.13) and postoperative discomfort (Rpe=2; RPc=3.8, P=0.004). Significant intragroup results were found for bone fill (Rpe=7.2mm; RPc=7.5mm, $p<0.000$), bone width- (RPe=28%; RPc24%, $p<0.009$) and pocket probing depth reduction (Rpe=0.6mm; RPc=0.8mm, $p<0.05$). The test side presented to be more stable in preserving keratinized mucosa and had less discomfort than the control side. A significant decrease in bone width of approximately 25% was found on both sides. The

adjacent teeth on the control and the test side experienced a similar and significant reduction in PPD. Histomorphometric analysis showed presence of great amounts of PepGen® and low amounts of vital bone on both sides. **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.

## Introduction and Literature Review:

### Alveolar bone changes after extraction

Major alveolar bone changes occur after tooth extraction (Schropp *et al.* 2003). If extraction sockets are left undisturbed, they heal uneventfully within 1 month after extraction (Claflin 1936; Amler *et al.* 1960). Amler and Johnson (1960) showed with their biopsies the different stages of wound healing in human extraction sockets. They outlined the sequences from clot formation after extraction to a physiological hard and soft tissue contour after 50 days. Evian *et al.* (1982) concluded from their histological study of human extraction sockets that between the fourth and the 8th week after extraction, the cellular components and the connective tissue in the extraction socket proliferate. Between the 8th and the 12th week the bone undergoes maturation and forms a trabecular pattern. Bone resorption of varying amounts is a phenomenon, which always occurs after extraction (Lekovic *et al.* 1997; Lekovic *et al.* 1998; Iasella *et al.* 2003). The resorption may lead to esthetic and functional disadvantages, which may even compromise future implant placement. Functional forces such as bruxism, complete

denture wear, and heavy bite forces have been implicated as contributing factors for accelerated bone loss together with different systemic conditions such as osteoporosis (Maeda and Wood 1989; Devlin and Ferguson 1991). It has been described in the literature, that more bone is resorbed on the buccal than on the lingual site (Pietrokovski and Massler 1967). The main reason for the increased amount of bone resorption on the buccal aspect is the thinner bony plate on this site of the alveolar bone. This thin bony plate also facilitates the occurrence of buccal dehiscence defects when teeth are still present. Dehiscences have been described to be the most commonly encountered problem in implant dentistry (Oh *et al.* 2003). The occurrence of a dehiscence is equivalent to the occurrence of a three-wall bone defect. The less bony walls that are present, the less space maintenance for a blot clot is provided by the extraction socket itself. Subsequently the use of membranes has been found to increase regeneration in dehiscence defects (Jovanovic *et al.* 1995; Douthitt *et al.* 2001).

**The significance of ridge preservation today:**

The majority of bone loss occurs in the first month after extraction (Lam 1960). The amount of bone loss in the first three years after tooth extraction varies around 40-60% (Boyne 1995; Christensen 1996). The technique to preserve the alveolar ridge volume by incorporating foreign materials into a human extraction socket was initially described in the mid 80s. These materials initially consisted of hydroxyapatite (HA) and were either root form dental implants or bone graft particles (Cranin and Shpuntoff 1984; Quinn and Kent 1984; Veldhuis *et al.* 1984; Quinn *et al.* 1985; Bell 1986; Kwon *et al.* 1986). The clinician aims to preserve or gain a sufficient width and height of bone when teeth are

removed. By evaluating the width of the ridge, Schropp *et al.* (2003) have found a reduction of the width by approximately 50% from 12 to 5.9 mm. Two thirds of the bone loss occurred during the first three months of healing. The percentage of bone-width reduction has been found to be larger in the molar regions than in the premolar regions, and in the mandible compared to the maxilla. Between the three and the six months evaluation, only minor bone changes were observed. Equally, only minor bone changes were observed between the six and the twelve months evaluation. The maximum bone loss evaluated close to the adjacent teeth of the extraction socket after 12 months was found to be 1.2 mm. A mean vertical loss of 1 mm could be determined in this study.

**Classifications and clinical considerations:**

Bone regeneration requires space making for a blood clot that will need to be stabilized and then be reorganized and replaced with bone (Tinti and Parma-Benfenati 2003). Barrier membranes make space for a blood clot, preserve it and exclude soft tissue ingrowth. Bone graft materials maintain space and promote bone growth by their osteoconductive activity (Brugnami *et al.* 1999). Tinti and Parma-Benfenati (2003) assumed that the envelope of bone determines the treatment and the predictability of regenerative procedures in bone defects like extraction sockets. They differentiated between two classes of extraction sockets:

Class 1 extraction sockets are those where the surrounding bone envelope is intact,

Class 2 extraction sockets are those where the envelope is not intact.

Guided bone regeneration is the common term that can be used to describe clinical approaches, which preserve the alveolar ridge bone volume. Christensen (1996) proposed the following indications for ridge preservation procedures:

1. Prevent collapse of the alveolar bone and soft tissue, which causes an unacceptable aesthetic situation in the anterior maxilla and mandible
2. Prevent collapse of the alveolar ridge, which might result in bone irregularities, and would cause an unacceptable fitting of the future prosthesis
3. After tooth extraction, to provide adequate bone volume for subsequent implant placement

Hermann and Buser (1996) presented basic guidelines that should be considered to achieve a predictable result with GBR procedures.

1. Use of an appropriate membrane
2. Achievement of primary soft tissue healing
3. Creation and maintenance of a membrane-protected space
4. Close adaptation and stabilization of the membrane to surrounding bone
5. Sufficiently long healing period

**Alveolar changes after ridge preservation :**

Ashman (2000) found that ridge preservation after extraction prevents the 40-60 % of the bone loss. Tiefengraber *et al.* (2002) found, in a prospective split-mouth study with a low number of evaluated patients, that much more horizontal bone width could be preserved after extraction, when only a Gore-Tex membrane was placed over the extraction socket.

After approximately 6 weeks the GBR site lost 1.1 mm in the buccal-lingual dimension, while the control site had lost 3.2 mm in the horizontal dimension. The radiologic examination of the vertical bone loss did not show any difference in this particular study (Tiefengraber *et al.* 2002). It has been shown that even though ridge preservation is performed in premolar and anterior extraction sites a horizontal loss of bone between 8-13% can be observed (Zubillaga *et al.* 2003; Iasella *et al.* 2003). Thus, taking into consideration that up to 50% of the width is resorbed after extraction (Schropp *et al.* 2003), ridge preservation can be assumed to preserve the 40% difference that would be lost without ridge preservation.

Gross (2002) stated in an essay that grafting of an extraction socket results in greater patient comfort due to decreased bleeding, lower susceptibility to infection, and more predictable healing. Tiefengraber (2002) and Block *et al.* (2002) indicated from their prospective studies, that GBR procedures in extraction sockets heal without any complications. When an extraction socket is preserved, intra- and postoperative complications are less. This finding has been emphasized since the very beginning of ridge preservation procedures when HA was still the most commonly used graft material (Bell 1986).

**Flap manipulation after extraction:**

When teeth are extracted, the tissue can either be left untouched or the flap can be advanced to achieve partial or complete coverage of the extraction socket. Rehrman (1936) was the first who elevated a buccal full-thickness flap and made two trapezoid vertical releasing incisions mesial and distal to the extraction socket. Tension was eased

by releasing the periosteum and subsequently the buccal flap was used to achieve primary closure of the extraction site. He indicated the necessity of this technique when extraction sockets had a connection to the maxillary sinus due to the occurrence of a perforation to the maxillary sinus after extraction.

Flap elevation and osseous surgery with or without periosteal releasing incisions has been described as a factor that may trigger postoperative bone resorption (Wood *et al.* 1972; Melcher 1976; Moghaddas and Stahl 1980). Marginal recession of adjacent teeth, defective papilla and loss of keratinized mucosa have been described to be the result of flap manipulation to achieve partial or complete coverage of an extraction socket (Landsberg 1997; Bartee 2001). Block *et al.* (2002) made periosteal releasing incisions, and achieved primary closure of the grafted extraction socket without using a membrane. Bartee (2001) described a technique using a nonresorbable PTFE membrane for ridge preservation without manipulating the soft tissue to prevent loss of the papilla, the vestibule and the keratinized mucosa. He left the membrane exposed and did not manipulate the soft tissue. By doing this, he found that he was able to predictably preserve the before mentioned structures and the bone volume.

**Tissue thickness and GBR:**

The tissue thickness after ridge preservation was also observed and compared to no-ridge preservation in a controlled clinical trial (Iasella *et al.* 2003). It was found that ridge preservation with the use of FDBA and a collagen membrane lead to thinner tissue compared to no ridge preservation. The technique used to measure soft tissue thickness

has recently been published to evaluate tissue thickness with an ultrasonic device (Eger and Mueller 1996; Mueller and Eger 2002).

**The significance of keratinized mucosa around implants:**

The presence of thick keratinized mucosa is preferable when implants are placed in the aesthetic zone. Nevertheless the absence of keratinized mucosa does not correlate with higher implant failure rates or increased recession in the presence of good oral hygiene (Wennström 1987). Esposito *et al.* (1998) stated in a review metaanalysis, that patients do benefit from the presence of keratinized mucosa because it facilitates plaque removal and decreases trauma on the tissue, a risk factor for inflammation. Mericske-Stern (1994) also emphasized the importance of keratinized mucosa in cases of traumatizing oral hygiene. Warrer and Buser (1995) examined the significance of keratinized mucosa around implants in the presence of plaque-induced periimplantitis in a monkey study. In this study, less bone loss was observed in the presence of keratinized mucosa.

**The use of soft tissue grafts to seal extraction sockets:**

Soft tissue grafts have been used to seal extraction sockets. Literature shows the successful use of pedicle or connective tissue grafts, to seal implants that were placed immediately after extraction into fresh extraction sockets (Edel 1995; Chen and Dahlin 1996; Bianchi and Sanfilippo 2004). On the other hand, connective tissue has been described as an unpredictable therapy for sealing extraction sockets, due to the unpredictable blood supply below the autogenous graft (Tal 1999). Tal (1999) found in a prospective study evaluating 42 maxillary anterior extraction sockets in 24 patients that

more than 50 % of the connective tissue grafts placed over extraction sockets are either partially vital or even non-vital.

## Materials used for ridge preservation:

### 1. Resorbable and non-resorbable membranes:

Prerequisites for an ideal barrier membrane include biocompatibility, cell occlusivity, tissue integration, space making effect, and clinical manageability (Oh *et al.* 2003). Membranes which have been used in GBR procedures are the following: PTFE, ePTFE, collagen, freeze-dried dura mater allografts, polyglactin 910, polylactic acid, polyglycolic acid, polyorthoester, polyurethane, polyhydroxybutyrate, calcium sulfate, micro titanium mesh, and titanium foils (Hammerle and Jung 2003). Studies in guided bone regeneration procedures have examined the use of resorbable membranes (Lekovic *et al.* 1998; Ito *et al.* 1998; Oh *et al.* 2003), or non-resorbable membranes (Hurzeler *et al.* 1994; Ito *et al.* 1998; Strietzel 2001).

ePTFE membranes seem to be of advantage when space maintenance is of special importance. This was confirmed in a study by Hurzeler *et al.* (1997) in which a bioresorbable barrier (GORE RESOLUT® Adapt Regenerative Membrane) made of polyl (D,L-lactid – cotrimethylencarbonate) in a 70/30 ratio was compared to nonresorbable ePTFE barrier for vertical bone regeneration around implants in a monkey study. The titanium reinforced membrane showed significantly superior results, which may be contributed to its superior ability of space maintenance and blood clot stabilization. Spacemaking property is one of the fundamental requirements of a GBR barrier (Hardwick *et al.* 1994). Mellonig *et al.* (1998) compared the amount of

regenerated bone in dehiscence type defects around implants treated either with a bioabsorbable barrier composed of a copolymer of lactide and glycolide or with a non resorbable e-PTFE membrane. It was concluded from this animal study that the superior results of the e-PTFE membrane are due to its superior space maintenance and blood clot stabilization. It can be concluded that these membranes are favorable in cases where large augmentations vertically or horizontally are necessary.

In cases of early ePTFE membrane removal, Lang *et al.* (1994) found only 42-60% of regenerated bone. Lang *et al.* (1994) stated additionally, that an early exposure of a membrane to the oral environment does not per se preclude the possibility for regeneration, to a certain degree, but rather represents a risk factor for infection. Larger defects were found to regenerate worse than smaller defects.

Ridge preservation does not facilitate the same amount of space maintenance as vertical or large horizontal ridge augmentation does. The socket walls function as space maintainers without the need of additional space maintenance by the membrane itself.

Bioresorbable membranes offer several different advantages (Hammerle and Jung 2003). Bioresorbable membranes improve tissue healing, incorporation of the membrane in the host's tissue and a quick resorption in less often occurring cases of exposure and subsequently also decreased likelihood of infection (Hammerle and Jung 2003).

**2. The significance of membrane exposure:**

Many previous studies indicated that wound dehiscence, early membrane removal, or membrane exposure influences guided bone regeneration procedures negatively (Kohal *et al.* 1998; Wang and Carroll 2001; Hammerle and Jung 2003; Oh *et al.* 2003). Membrane

exposure seems to occur more often when non-resorbable membranes are used. Histologically exposed ePTFE membranes were shown to be contaminated by microorganism, such as neutrophils, degenerated collagen fibrils and necrotic cell components (Noppe *et al.* 1990). In a metaanalysis it was found that ePTFE membrane exposure during healing had a major negative effect on GBR, but only a minimal effect on GTR around natural teeth (Machtei 2001). Nevertheless there are studies that indicate a significant worse treatment outcome when ePTFE membranes become exposed in GTR procedures around natural teeth (Ling *et al.* 2002).

In a study that examined the amount of bacteria on exposed membranes in guided tissue regeneration, it was found that the amount of attachment level gain is significantly lower in cases when the amount of bacteria on their surface exceeds $10^6$ (Ling *et al.* 2002). This fact stresses the idea that GTR works almost equally effective when membrane exposure is seen around natural teeth with low amounts of bacteria on the membrane, but there might be more cases in the GTR membrane exposure group, which show no, or lower attachment level gains. This fact might be due to the cases, where the level of bacteria on the membrane surface exceeds a certain pathologic number. This principle may also be applied to guided bone regeneration procedures, but the correlation remains unstudied so far. Bartee (2001) left a nonresorbable PTFE membranes exposed and encountered a very low number of infections in his descriptive publication. He contributed this to the low porosity (<0.2 μm) of the dense membrane. The low porosity enables the clinician to leave the membrane exposed without extraordinary amounts of bacteria colonizing on the surface and subsequently a low risk of bone graft loss due to infection underneath. He

also emphasized the necessity of the postoperative recall to clean the exposed membrane. The patient should be advised to clean the membrane with a Q-tip.

One prospective study with a low number of patients indicates that exposure of a resorbable membrane does not appear to have a deleterious effect in ridge preservation procedures (Zubillaga *et al.* 2003). Hammerle and Jung (2003) stated in a review on bone regeneration by means of barrier membranes, that bioresorbable membranes render similar success rates when compared to non-resorbable membranes for the treatment of horizontal defects

**3. GORE RESOLUT and GORE RESOLUT® Adapt Regenerative Membrane:**

The biocompatibility of the first polyglycolide:trimethylene carbonate (PGA:TMC) device, the Maxon suture (manufactured by Davis & Geck), was approved by the FDA in 1986 and previously described by Katz *et al.* (1985). It was composed of polyglycolid-co-trimethylencarbonate in a 67.5/32.5 (PGA:TMC) ratio. The material is associated with a very minimal inflammatory response, encapsulation by fibrous connective tissue and finally the break down of its components (FDA April 1986). The use of PGA (GORE RESOLUT) for guided bone regeneration (Hurzeler *et al.* 1997; Mellonig *et al.* 1998), guided tissue regeneration (Hurzeler *et al.* 1997) and for the treatment of class II furcation defects (Hurzeler *et al*. 1997) has been documented in previous studies.

It has been stated that the GORE RESOLUT Membrane is completely resorbed after 5 months. The polymeric components of this barrier are broken down by hydrolysis and are eliminated from the body by the Kreb's cycle as carbon dioxide and water (Hurzeler *et al.* 1997). The use of this material for ridge preservation procedures, especially by leaving

the membrane exposed remains unstudied. In the present study the new GORE RESOLUT® Adapt Regenerative Membrane is used. It is composed of 67% PGA and 33% TMC. According to the manufacturers statement, it performs as a barrier for 8-10 weeks, and is completely resorbed after 5-6 month.

**4. Anorganic bovine-derived hydroxyapatite matrix (ABM) and cell binding peptide P-15 (PepGen P-15) and demineralized freezed dried bone allograft (DFDBA):**

Bartee (2001) described the three different types of grafting materials that are currently used (Bartee 2001). Long-term nonresorbable ridge preservation materials such as hydroxyapatite are not suitable for implant sites, but they allow the preservation of the alveolar ridge volume for dental prosthesis. Transitional bone graft materials such as anorganic bovine bone matrix (ABM, f.i. BioOss or PepGen®), resorbable calcium phosphate ceramics, and macroporous bioactive glass, allow the placement of endosseous implants into the bone grafted site, even in the presence of some unresorbed particles. At least 6 month should be waited after extraction before implant placement. Intact ABM particles were still found after 44-60 month postimplantation (Skoglund *et al.* 1997; Schlegel and Donath 1998). Short-term resorbable materials get readily resorbed and consist mainly of collagen (DFDBA). These materials allow dental implant placement after 3-6 month.

Transitional and short-term bone grafting materials allow ridge preservation and subsequent dental implant placement. Since transitional bone grafting materials seem to have a better space maintenance ability, they may preserve the volume of bone better than short-term bone grafting materials. Short-term bone grafting materials allow early

revascularization and resorption of the graft material. Subsequently their advantage seems to be that a shorter time frame is involved until implant placement. The combination of both materials may combine both advantages, a good space maintenance and an earlier revascularization and resorption of the grafted bone. GBR with DFDBA as bone graft material has been shown to be a successful procedure for implant site development in the presence of human extraction sockets (Brugnami *et al.* 1999; Block *et al.* 2002). Block *et al.* (2002) also concluded that human demineralized bone could preserve or recreate an extraction sites bone bulk in preparation for implant placement without adjunctive grafting procedures in a short-term prospective study.

## Methods described to investigate hard and soft tissue changes:

Most of the clinical parameters that will be evaluated in the present study do not differ from a standardized clinical evaluation. These parameters, like the pocket probing depth (PPD) or the width of keratinized mucosa, will not be explained again, since they are the basis of periodontal evaluation worldwide. Clinical approaches described to measure bone remodeling following tooth extractions include taking impressions (Hurzeler *et al.* 1994; Lekovic *et al.* 1997), the radiographic substraction method (Lehmann *et al.* 2000; Schropp *et al.* 2003), CT-Scans and the use of acrylic stents (Iasella *et al.* 2003; Zubillaga *et al.* 2003). Mueller and Eger (1996) invented an ultrasonic device to examine tissue thickness.

## Summary:

Ridge preservation is an extensively studied and well-understood treatment to prevent bone loss after extraction. Many different materials have been used in the past with similar success. It was formerly believed that primary closure of an extraction socket is crucial in guided bone regeneration procedures. Evidence from the last few years indicates that the exposure of resorbable membranes may be equally beneficial if not more beneficial to preserve keratinized mucosa on the buccal aspect of extraction sockets. The existence of keratinized mucosa is considered to be beneficial around implants. This fact is of major significance if patients have thin tissue, have poor oral hygiene or brush with lots of force. The present study investigates a method to preserve not only the hard tissue but also the keratinized soft tissue around extraction sockets.

## Purpose:

The purpose of the present study is to prospectively evaluate the efficacy of a technique in which a copolymer membrane (GORE RESOLUT® Adapt Regenerative Membrane) is intentionally left exposed (RPe) in a ridge preservation procedure.

In addition, this investigation compares the hard- and soft tissue changes between the Rpe technique and a conventional technique where a flap was released to cover the membrane (RPc) and primary closure was achieved.

Last, the bone will be histomorphometrically examined after 6 month to assess the turnover of the bone graft materials used.

## Hypothesis:

The primary hypothesis is that more keratinized mucosa can be preserved on the buccal aspect when ridge preservation is performed by intentionally leaving the PGA membrane (GORE RESOLUT® Adapt Regenerative Membrane) exposed. Secondarily, the volume of bone is the same irrespective of the method.

## Specific Aims

The following assessments were made on the RPe (test) and the RPc (control) site:

1. The vertical bone fill.
2. Horizontal ridge width that was lost.
3. Changes in keratinized mucosa.
4. Patient comfort.
5. Histomorphometry of the bone graft materials.
6. Gingivitis (GI), plaque index (PI) and pocket probing depth (PPD) at the adjacent teeth.

This study was conducted to explore differences between two different surgical methods. The primary outcome variable is keratinized tissue, the secondary outcome variable is bone width. Additional outcomes such as probing depth and plaque index (see patient screening sheet) will be explored as well.

## Material and Methods:

### Experimental design, sample size and patient recruitment:

11 patients, referred to the Postdoctoral Clinic in the Department of Periodontology Tufts University School of Dental Medicine, were recruited for this controlled clinical trial. The study was conducted in a split-mouth design. The sites for either procedure were chosen randomly.

### Subject Characteristics:

Inclusion criteria:

1. Presence of either 2 premolars or 2 molars that are treatment planned for extraction in the same jaw but in opposite quadrants.
2. Presence of at least 3 intact bone walls and at least half of the fourth bone wall.
3. 18 years of age and older.

Exclusion criteria:

1. Presence of acute periodontal and periapical infection.
2. Presence of systemic diseases that have been proven to affect wound healing.
3. Diabetes.
4. Stage 1 and stage 2 hypertension ( ≥ 140 systolic; ≥ 90 diastolic).
5. Intake of long-term (>3 month) non-steroidal or antibiotic drug therapy.
6. Presence of allergies to one of the used materials for the procedure.
7. Failure to sign an informed consent form.
8. Pregnancy.
9. Current smoking.

**Patient screening, examine if the patient fitted the inclusion/exclusion criteria; informed consent was obtained:**

The purpose of the patient screening visit was to examine whether the patient is suitable for the study. Dr. Engler-Hamm introduced the patient to the study protocol and explained the surgical procedure to the patient. Subsequently, the patient was tested whether he/she fits the inclusion/exclusion criteria. For this purpose the area of the teeth that were treatment planed for extraction were anesthetized, and a bone sounding was performed to determine the presence/absence of the 4 surrounding bone walls of both future extraction sites. If the patient fitted the inclusion/exclusion criteria the Informed Consent Form and the RAF (Research Authorization Form) was obtained. The patient was given the opportunity to ask questions. A comprehensive periodontal evaluation was done and the necessity for a debridement (cleaning) or a deep scaling and root planing (deep cleaning) prior to the surgical treatment was determined. If no recent (last 2 years) x-rays existed, the patient was referred to the radiology department for a full mouth of x-rays (FMX) and a panoramic x-ray.

**Phase I therapy:**

The stent was tried into the patient's mouth. Photographs of the teeth only, not the face, were taken. Oral hygiene instructions were given to the patient. If large amounts of plaque were present a supragingival debridement (cleaning) was performed. If the patient presented with generalized chronic periodontitis a scaling and root planing under local anesthesia was performed to establish overall periodontal health and prevent infection of the bone graft. In the absence of large amounts of plaque, calculus and periodontitis no debridement or deep scaling was performed.

**Phase 2 therapy:**

**Extraction:**

The patient was anaesthetized primarily with a block anesthesia (Marcaine, 1:200 000 Epinephrine). The tooth was extracted atraumatically with periotome instruments and extraction forceps. After the extraction the socket was debrided. The socket was not compressed.

**Surgical procedure test side (RPe):**

For graft material, a mixture (1:1) of DFDBA and PepGen (Dentsply/Friadent/Ceramed, 12860 West Cedar Drive, Lakewood, Colorado, 80228) was used. The extraction socket was filled and slightly condensed with the bone graft material. The flap around the extraction socket was not elevated, but slightly undermined to allow placement of the resorbable membrane on top of the bone walls that surround the socket. The bioabsorbable PGA:TMC copolymer membrane (W.L. Gore & Associates, Inc., Flagstaff, AZ 86004) was trimmed according to the shape and size of the extraction site. Subsequently the membrane was placed on top of the extraction socket approximately 3-4 mm over the defect edge underneath the undermined tissue. Non-resorbable sutures made of PTFE (W.L. Gore & Associates, Inc., Flagstaff, AZ 86004) were used to suture the membrane in place. A criss cross PTFE x-suture 5-0 was used over the extraction site to stabilize the membrane additionally. After the surgery a periapical radiograph was taken of each site to ensure proper seating of the bone graft in the extraction site.

**Surgical procedure control site (RPc):**

The same materials as described for the RPe site were used for the RPc site. The extraction socket was filled and slightly condensed with the bone graft material. An intrasulcular incision and two vertical releasing incisions were made to release the buccal flap of the patient. The buccal tissue was elevated in a full thickness, split-thickness manner. The PGA:TMC copolymer membrane was trimmed according to the shape and size of the extraction site. Subsequently the membrane was placed on top of the extraction socket approximately 3-4 mm over the border of the bony walls that surround the extraction socket underneath the surrounding mucosal tissue. The membrane was stabilized with a 5-0 GORE suture. First, the membrane was sutured to the buccal mucosal tissue in the area of the releasing incisions, second, sutures were used to advance the buccal released tissue to achieve primary closure. Subsequently the patient's released buccal flap was used to cover the extraction site on top of the membrane. The flap was sutured with a PTFE suture and primary closure on top of the membrane was achieved. After the surgery a periapical radiograph was taken to ensure proper seating of the bone graft in the extraction site.

**Postoperative instructions and medications:**

The patient was instructed not to brush or floss in the area of the surgical procedures for 4 weeks. For two weeks the patient was medicated with the antibiotic doxycycline (100 mg once a day for 14 days). In order to decrease postoperative swelling the patient was medicated with Medrol Dos Pak (corticosteroid) postoperatively. In addition, the patient was advised to rinse with chlorhexidine (0,12%/1oz) twice daily for the same period of

time. The patient received ibuprofen (600mg tid prn for three days) to manage postsurgical discomfort and inflammation.

**Follow up and reevaluation:**

The follow up was scheduled after one week and after three weeks. At the one-week follow-up the patients were asked to judge the level of comfort for both sides individually on a scale from 1 to 6, 6 being the worst pain they can imagine. At 6 months, the patient was seen again to obtain the final measurements. For this last visit the patient was anesthetized with local anesthesia for the soft and hard tissue reevaluation. The stent was placed in the mouth and the hard- and soft-tissue measurements were repeated and subsequently compared to the baseline measurements.

**Obtain histology at the time of implant placement:**

Patient's planned for implant therapy in the test and/or control site received implant therapy after the six month reevaluation (6 month postoperatively). Before implant placement an osteotomy was performed to allow implant insertion. This osteotomy was performed with a trephine to obtain a bone core of the hard- and soft-tissue at the implant site. The amount of hard- and soft-tissue that will be removed did not exceed the amount of tissue that needed to be removed to allow implant placement. The tissues were not cultured or sent to a tissue bank. The tissues were processed to obtain histology and determine the amount of residual bone graft and membrane particles at the implant site. The company (W.L. Gore & Associates, Inc., Flagstaff, AZ 86004) performed the histological processing and the histomorphometric analysis. The company (histologist) was blinded in regards to what method was performed on the specimens that are submitted for histomorphometric analysis.

**Specific measurements, methods and techniques used throughout the study:**

**1. Manufacture of the reference stent:**

Two impressions were taken from the patient's jaw that had the two teeth extracted. The impressions were pored and the undercut areas were blocked out with wax to allow application of the acrylic stent suck down device. The stent was strengthened with acrylic resin whenever necessary. The stent only covered the coronal aspect of the teeth that were present. Around the teeth that were extracted, the stent extended the marginal gingiva/mucosa on the buccal and the lingual site beyond the mucogingival junction. The stent was manufactured in a way, that it had a distance of a few millimeters from the tissue to allow proper adaptation and removal of the stent without interferences.

**2. Baseline measurements:**

The plaque index and the bleeding index and the width of keratinized mucosa were evaluated for the teeth adjacent to the extraction sites if present. The fabricated stent had two landmarks on it providing reference points for different measurements. The initial baseline measurements were performed immediately after extraction before bone grafting. At the extraction site, the width of keratinized mucosa on the buccal site was evaluated, and a groove was made in the stent as a reference for the 6-month reevaluation. The absolute width of the bone was determined approximately 4 mm apical to the marginal gingiva. At this height, a groove was made inside the stent that allowed sounding through the hole and the tissue down to the bone with a needle like instrument (#40 reamer). From the sounding point, the bone width will be evaluated with a standardized bone-mapping instrument (IFMI, Ridge Mapping instrument, G. Hartzell & Son, Concord, CA, USA).

Tab 1. Gingivitis-Index (GI)

| Grade | Gingivitis-Index Löe (1967) |
|---|---|
| 0 | No inflammation, discoloration or bleeding. |
| 1 | Slight inflammation, slight discoloration, slight surface texture change, no bleeding. |
| 2 | Moderate inflammation, redness, edema, bleeding on probing. |
| 3 | Excessive inflammation, severe redness and edema, tendency for spontaneous bleeding. |

Tab 2. Plaque-Index (PI)

| Grade | Plaque-Index Sillness and Löe (1964) |
|---|---|
| 0 | No plaque. |
| 1 | Slight plaque close to the gingival margin, only detectable with a probe. |
| 2 | Plaque inside the sulcus along the marginal gingiva, not interdentally. |
| 3 | Plaque at the marginal gingiva and interdentally. |

In summary, the following measurements were taken:

1. Horizontal ridge width (BW).
2. With of keratinized mucosa (KM).
3. Gingivitis-Index (GI), Plaque-Index (PI), Pocket Probing Depth (PPD).
4. Bone fill (Bf).

**3. Bone fill measurement (Bf):**

From the occlusal view, the stent had three perforations (holes): one in the center, and two mesial and distal to the extraction site. The perforations enabled a reamer (40 file Dentsply, Maillefer, Tulsa OK) to penetrate the stent and measure the distance from the occlusal aspect of the stent (reference point) to the most apical point of the extraction site and the mesial and distal bone height adjacent to the extraction site.

**4. Bone width (BW) measurements and keratinized mucosa (KM):**

Buccally and lingually the stent had two grooves. The first one was at the height of the keratinized mucosa of the patient. This groove was applied to the stent in the patient's mouth as a reference for the most apical extend of the keratinized mucosa. The second reference was on the midbuccal and midlingual approximately 3-4 mm below the marginal gingiva to allow bone width measurements. In the case of close proximity of both holes, only the hole at the height of the keratinized mucosa was used as a reference for the baseline of the keratinized mucosa and as a reference for the bone mapping procedure.

**5. Statistical analysis:**

A sample size of 10 in this split mouth study was able to detect a difference of 1.5 mm of keratinized tissue (primary outcome variable) assuming a maximal standard deviation (of the difference) of 2 mm based on similar studies (Engler-Hamm *et al.* 2003) using a paired t-test with 80% power and 0.05 level of significance. Categorical variables were analyzed using the Wilcoxon Signed Rank Test. This sample size also permitted the testing of the secondary hypotheses (bone loss) even with a Bonferroni Correction (nQuery Release 4.0).

The data was analyzed using paired t-test to assess the effects of treatment on the primary outcome (keratinized tissue) and secondary outcome (bone loss). Additional outcome variables were examined using exploratory data analysis in order to generate hypothesis for future work. Spearman Correlations were performed. Each parameter (e.g. bone width) was evaluated twice per side (initial and 6 months reevaulation). The difference between the initial and the 6-months reevaluation was calculated and compared to the control/test side.

Example:

| | Side A (test) | Side B (control) |
|---|---|---|
| Inital | 7mm | 8mm |
| 6 months | 6mm | 5mm |
| Difference | 1mm | 3mm |

In this example the Student T-Test would be performed comparing the difference of 1mm to the difference of 3mm.

The influence of demographic variables (see page 31) was explored using subset analysis, although the study was not powered to formally test these as a hypothesis. The analysis was performed using SPSS with a level of significance of 0.05. Data management analysis was the responsibility of Dr. Daniel Engler-Hamm supported by Dr. Paul Stark (TUSDM), who also generated the randomization plan using the design module of SYSTAT.

# Results:

## 1. Demographic data:

In the following data presentation it will be differentiated between a patient and a case. Patients are all individuals that are enrolled; cases are all procedures that are completed. The reason for this differentiation is, that one patient had 2 ridge preservation procedures (4 extractions). This patient will be regarded as 2 cases.

At the time of the Master's Thesis Defense, nine patients (10 cases) will have undergone the ridge preservation procedure. Another 2 patients are enrolled in the study and will be evaluated soon after the thesis defense. Subsequently the total number of patients enrolled in this prospective clinical trial is 11.

At the time of defense, 7 patients (8 cases) had implants placed and bone cores obtained. After completion of the study, 8 patients (9 cases) will have had bone cores obtained. As a result, from the total of 11 patients (12 cases) 3 patients (3 cases) will not have had bone cores removed, because they were not treatment planned for implants at the site of extraction.

Seven of the 9 patients are women, 2 are men. The age range was between 20-55, with a mean of 38.4 years (SD 14.7).

Tab 3. Demographic Data

| | |
|---|---|
| Patient (cases) | 9 (10) |
| Gender (M vs. F)<br>Age | 2 M 7 F<br>38.4 (mean) ±14.7 |
| Premolar (P)<br>Molar (M) | 2 P (total 4)<br>8 M (total 16) |
| Biopsys/patient<br>Biopsys total | 7<br>14 |

## 2. Clinical parameters

**Gingivitis Index (GI), Plaque Index (PI) and Pocket Probing Depth (PPD)**

The initial data was compared in regards to bleeding, and it was found that 8 out of 10 (80%) of the test sides and 7 out of 10 (70%) of the control sites had a Gingivitis Index (GI) of 1 at the initial evaluation visit. When the GI was compared to the 6-month GI value neither the test, (p=0.705) nor on the control side (p= 0.480) showed a statistical significant difference.

When the plaque index (PI) was correlated with the GI a statistically significant association was found (Pearson Correlation = 1.0, p=0.000). By analyzing the PI as a single parameter, no difference was found between the initial and the 6-month test (p=0.705) and control values (p=0.276).

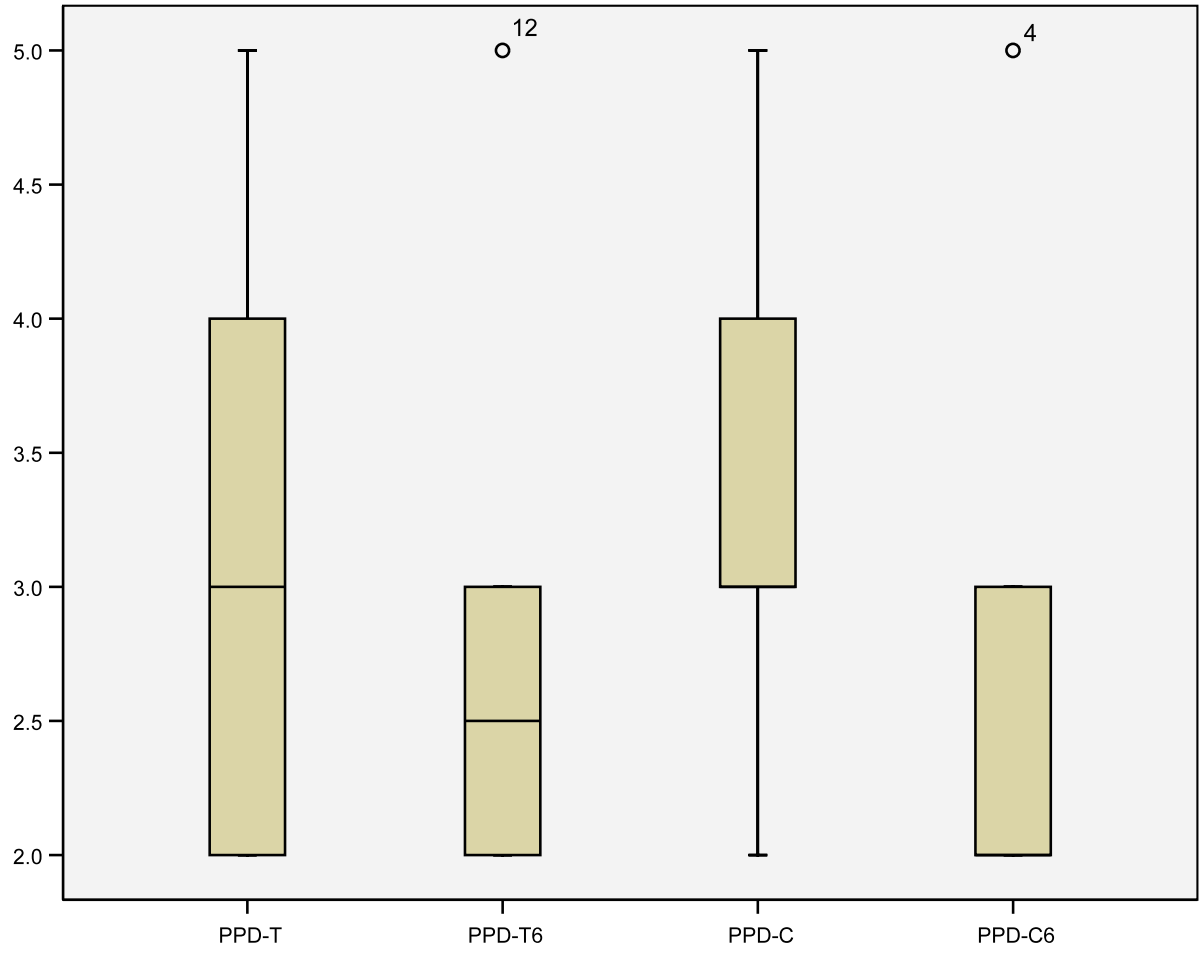
The mean pocket probing depth (PPD) at the adjacent tooth was 3.3 mm at the test and 3.4 at the control before the initiation of therapy. The comparison to the PPD at 6 months showed a significant difference. A significant decrease in PPD for the test (p=0.024) and the control side (p=0.022) was observed. On the test side the mean PPD decreased from 3.3mm to 2.7mm. On the control side a drop from 3.4mm initially, to 2.6mm was found.

Tab 4. Pocket Probing Depth

| | Test | Control | Difference |
|---|---|---|---|
| Initial | 3.3 | 3.4 | 0.1 |
| 6-month | 2.7 | 2.6 | 0.1 |
| Difference | 0.6 ¶ | 0.8 ¶ | |

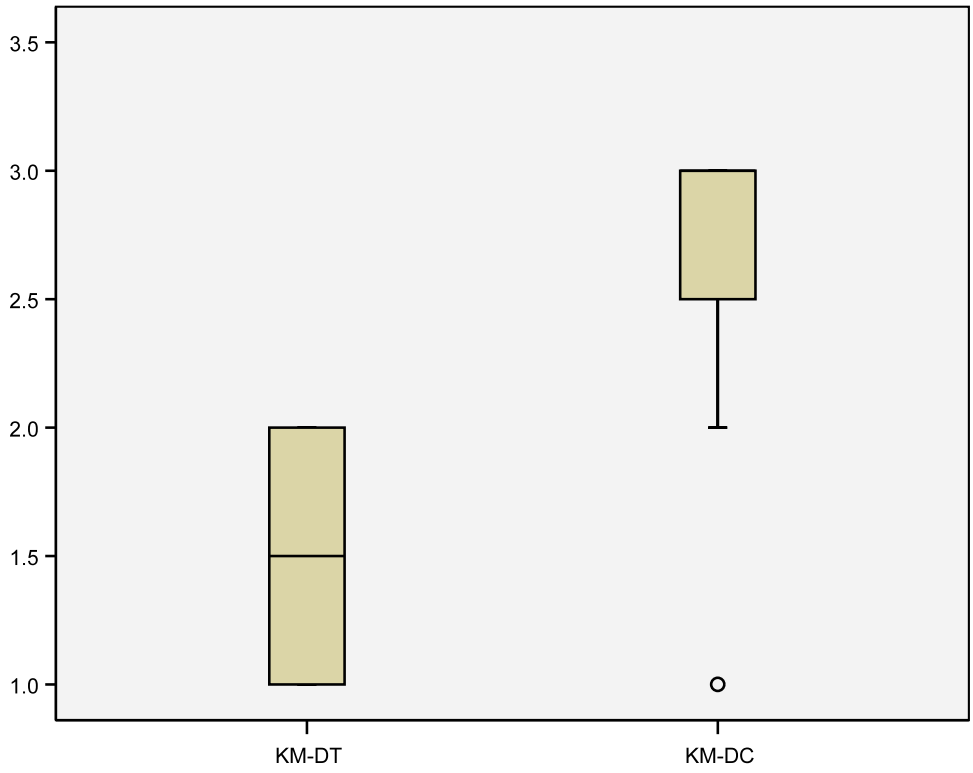
¶ statistically significant

Figure 1. Comparison of the mean reduction in pocket probing depth (PPD)



**Keratinized Tissue**

Figure 2. Comparison of the mean loss of keratinized mucosa measured facially

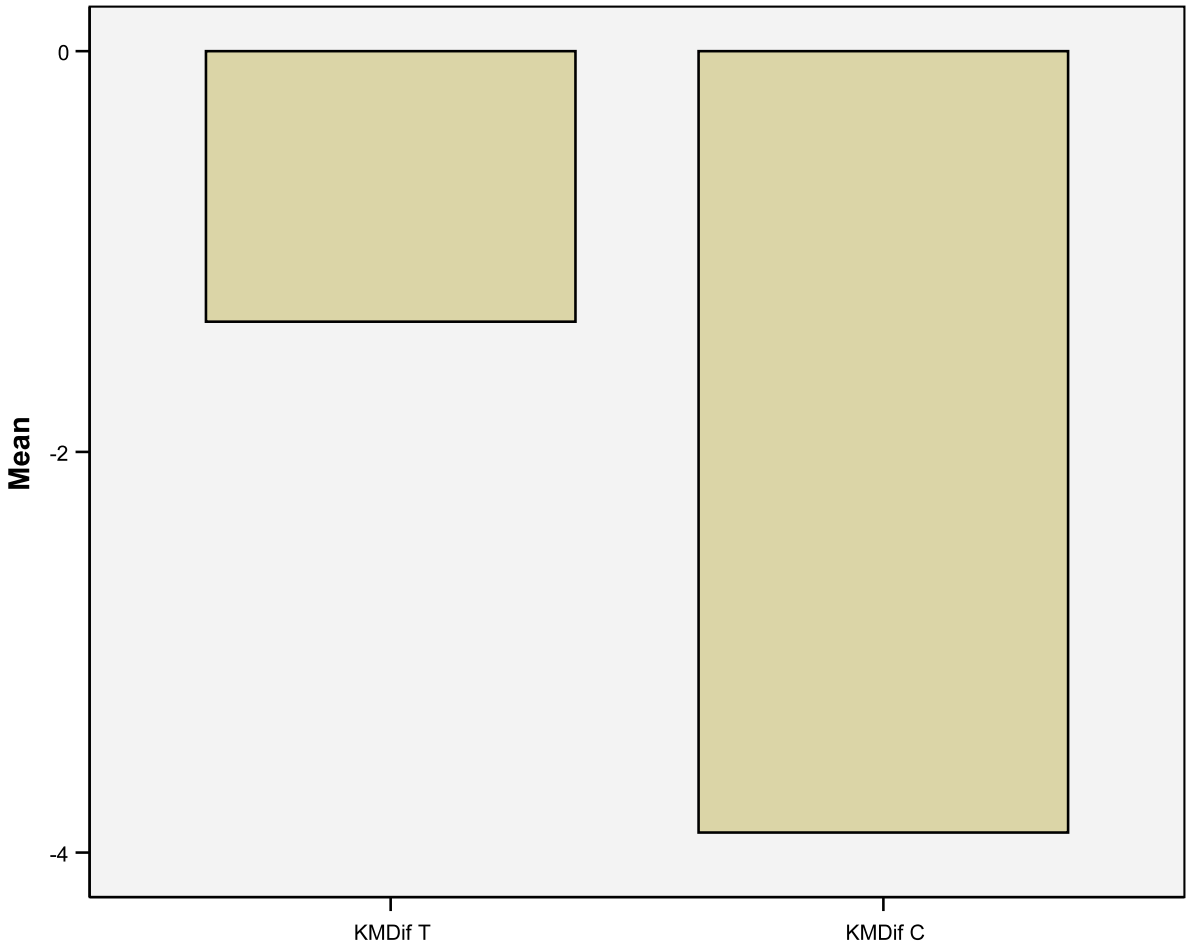



The difference of keratinized mucosa present on the facial aspect of the ridge between the test and the control side was compared. The difference between the test and the control was found to be significant. On the test side, a mean difference of 1.55 mm (SD 0.43) was observed, while the control showed a difference of 3.45mm (SD 1.97) (Fig. 2). The test side preserved the keratinized mucosa on the buccal aspect significantly better than the control side (p=0.013).

A second assessment of keratinized mucosa was the overall loss of keratinized mucosa. This loss was determined to be equal to the number of millimeters that the keratinized mucosa shifted from the buccal to the lingual. This loss took into account how much the mucogingival junction shifted from the buccal aspect to the occluso-lingual. Here the

mean loss on the test side was -1.35mm (SD= 0.94) while the mean loss on the control side was -3.9mm (SD=2.71) (Fig. 3). When the test side was compared to the control side a statistically significant difference was found (p=0.005).

Figure 3. Comparison of the mean difference in keratinized mucosa measured facially and occlusally

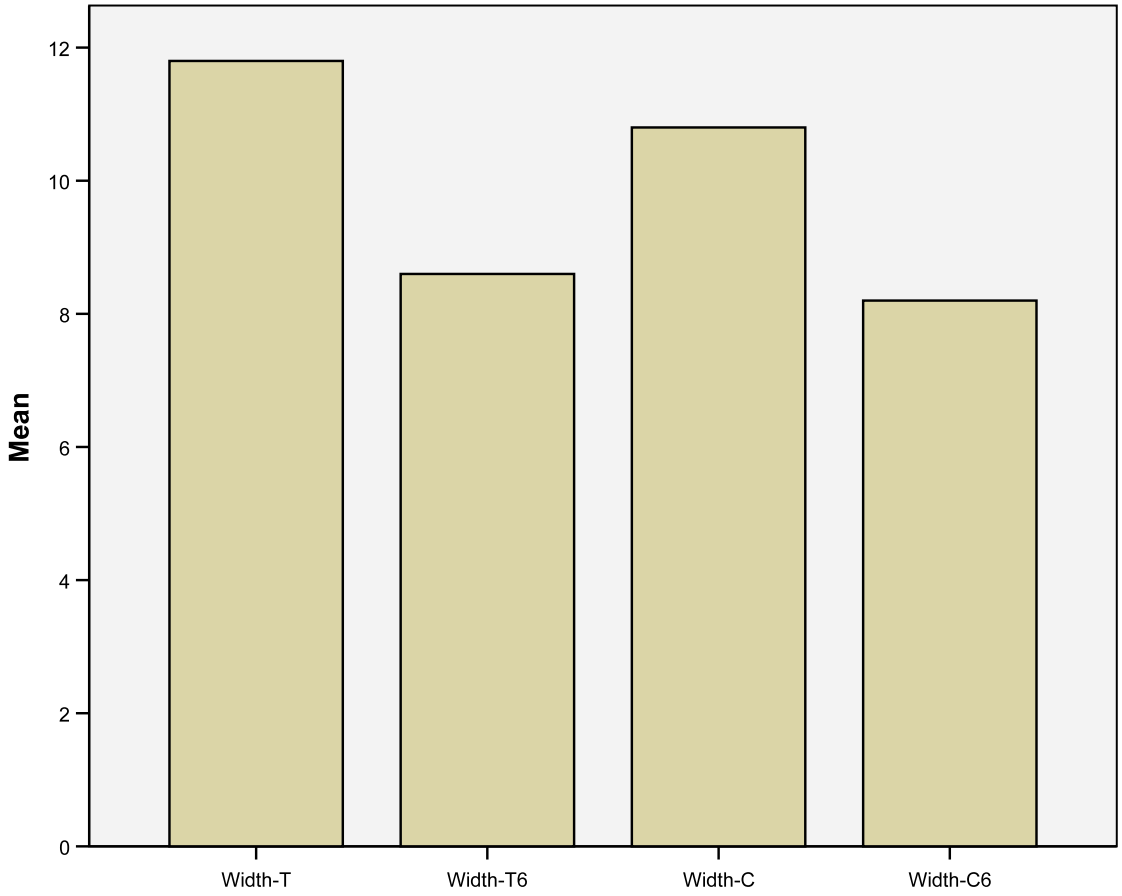



To confirm the finding of the 2 different measurements of keratinized mucosa, the difference of KM on the facial aspect and the overall loss of KM was correlated. A statistically significant association was found (p=0.000).

**Bone Width**

On both sides, the test and the control, a significant reduction of bone width was observed (p=0.000; p=0.009). The mean width was reduced on the test side from 11.8mm to 8.6mm. On the control side the mean width decreased from 10.8mm to 8.2mm (Fig. 4).

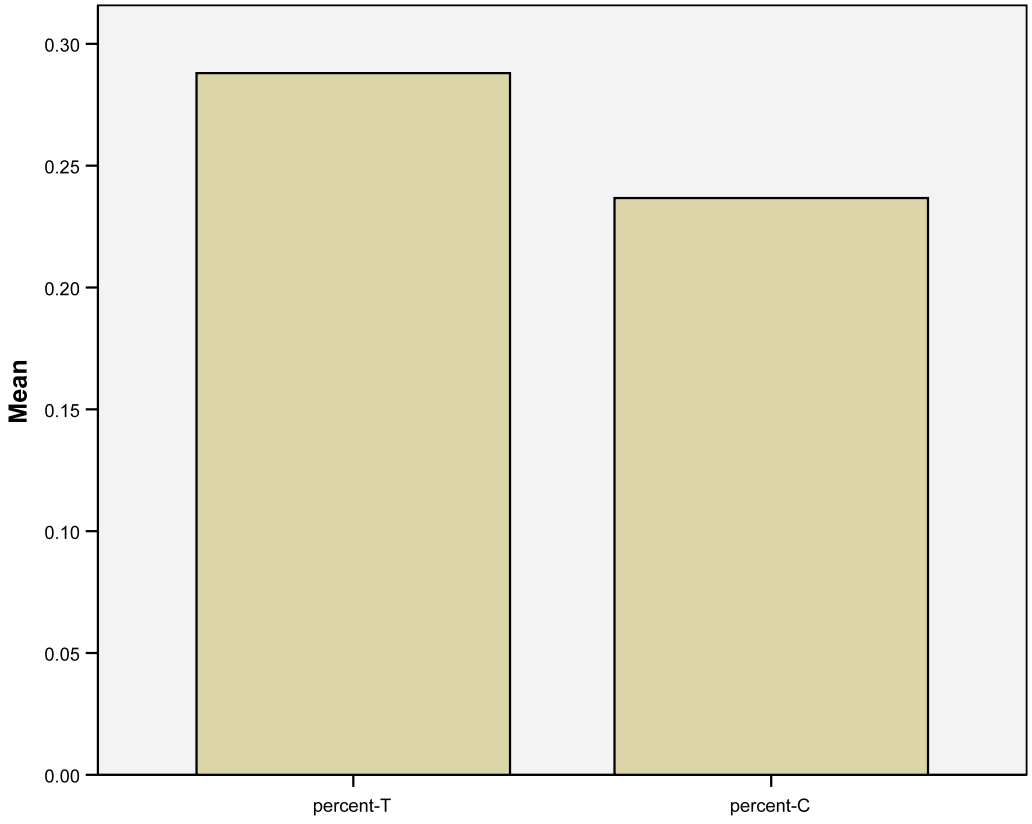
Figure 4. Comparison of the mean bone width in mm




When the difference of bone width on the test side was compared to the control side, no statistically significant difference was found (p=0.181).

Nevertheless the test side had a tendency to show greater bone resorption than the control side. The test side lost a mean of 28% (SD= 15%) of bone, while the control side lost a mean of 24% (SD=23%) within the 6 months time frame (Fig. 5).

Figure 5 Comparison of the amount of bone loss in percent (0.3 = 30%)



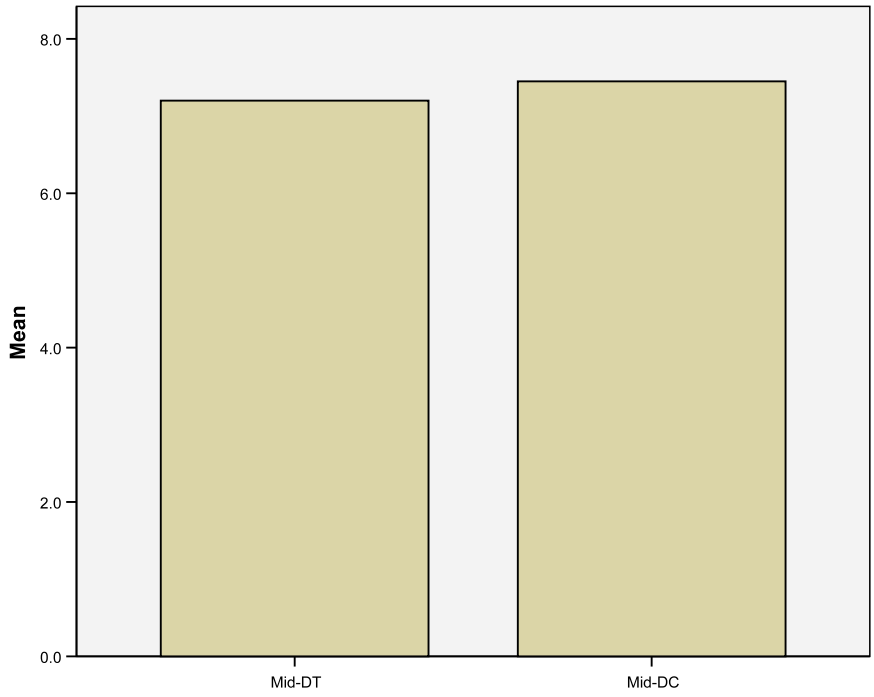

**Bone-fill**

The difference of the vertical bone height measured from the reference stent to the deepest point of the extraction socket was calculated. This difference of bone can also be regarded as the bone fill. The difference between the initial measurement and the final measurement was found to be significant on the test and the control side ($p<0.000$).

Tab.5 Comparison of the mean bone height in mm before and at 6 month

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Bone Height-Test | 10 | 17.0 | 25.5 | 21.400 | 2.8848 |
| Bone Height-Test 6month | 10 | 12 | 19 | 14.20 | 2.519 |
| Bone Height-Control | 10 | 17.5 | 28.0 | 21.450 | 3.2098 |
| Bone Height-Control 6 month | 10 | 7 | 17 | 13.60 | 3.017 |
| Valid N (listwise) | 10 | | | | |

Figure 6. Comparison of the bone-fill from the deepest point of the extraction socket in mm

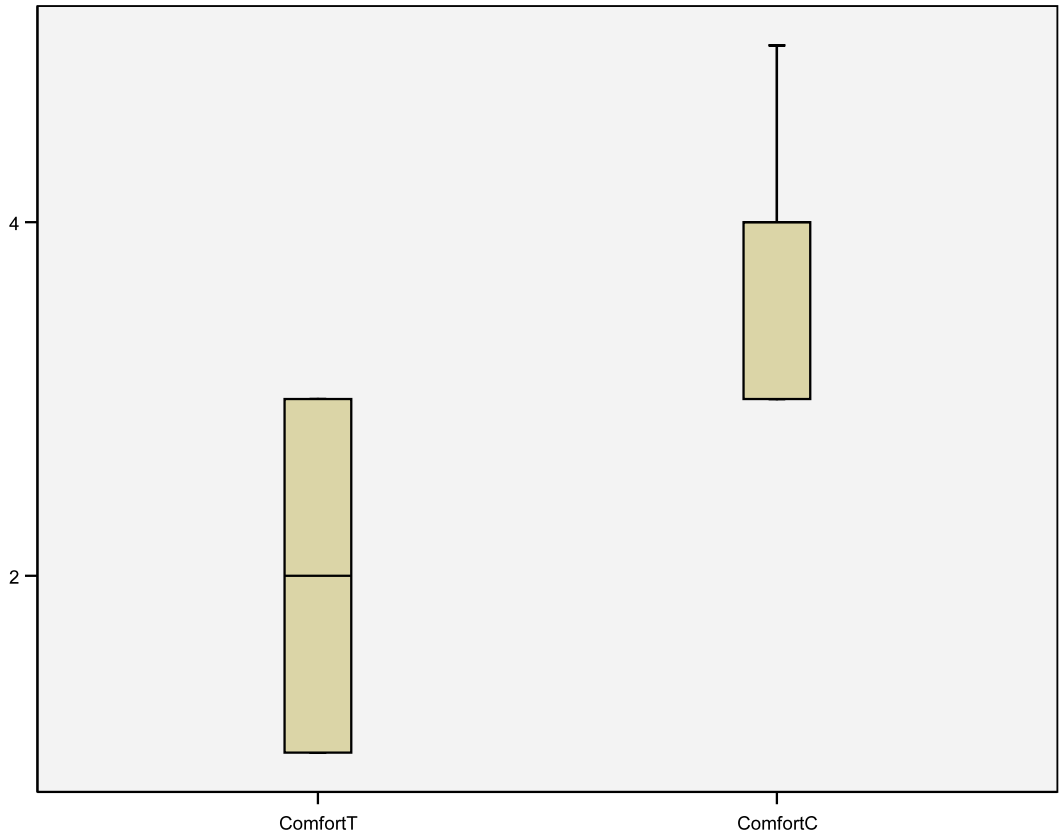



When the bone fill was compared between the test (mean= 7.2mm) and the control side (mean 7.5mm), no statistically significant difference was found (p = 0.789) (Fig. 6).

**Comfort**

The level of comfort was compared. The test side was found to have significantly less discomfort than the control side (p = 0.004). On the scale from 1-6, the mean for the test side was 2.0 (SD=0.81), the mean for the control side was 3.8 (SD=0.78) (Tab. 10).

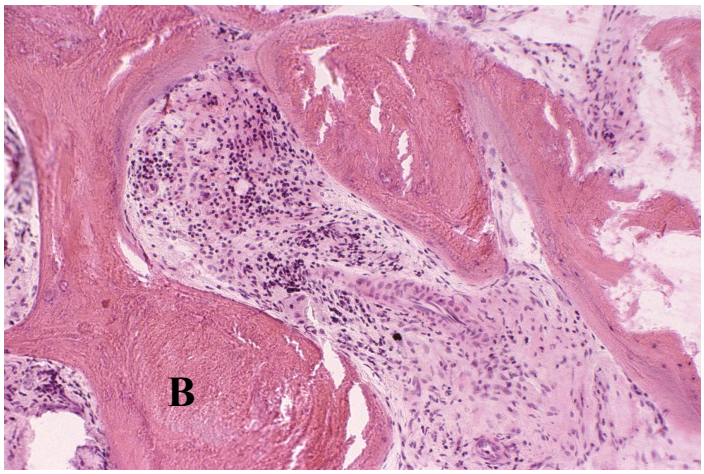
Figure 7. Comparison of the mean comfort level between the test and the control



**3. Histology**

Sixteen bone cores were obtained from 7 patients (8 cases). Owing to inadequate biopsy size, histomorphometric analysis was done in 5 specimens. The limited histomorphometry available precluded statistical analysis of the data. Thus only histological results are described.

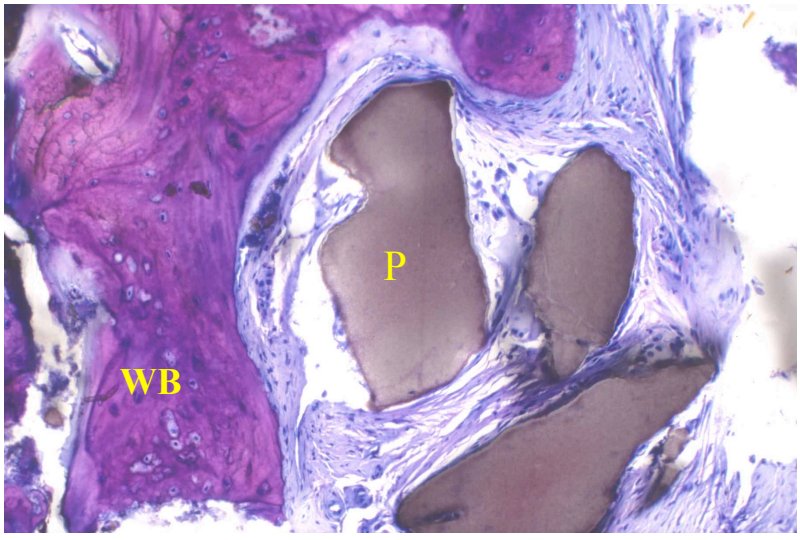
Pic. 1




Patient 1 #2

Much of the bone (B) appears non viable. Marrow spaces appear vascular and filled with lymphocytes and fibroblasts. H&E 10x

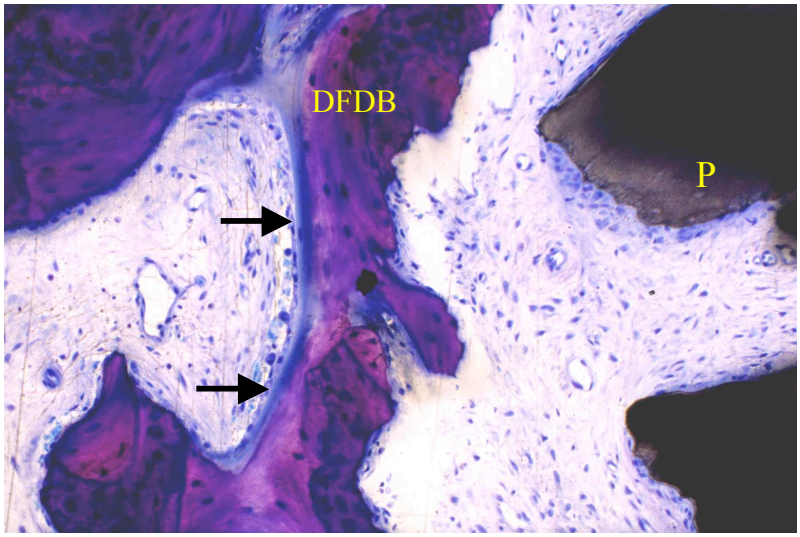
Pic. 2




Patient 1 Tooth #14

Large PepGen (P) particles are observed in the marrow space adjacent to woven bone (WB). The osteocytes are viable indicating new remodeled bone. Paragon stain 25x

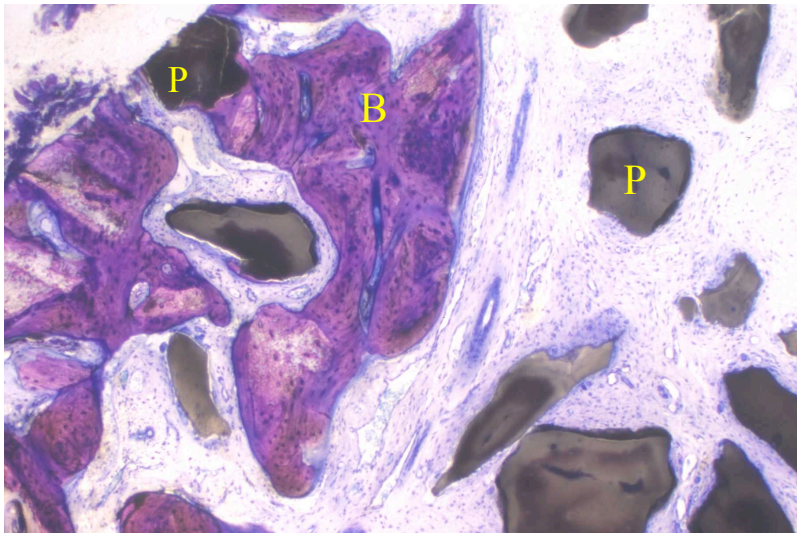
Pic. 3




Pic. 4

Patient 2 Tooth #18

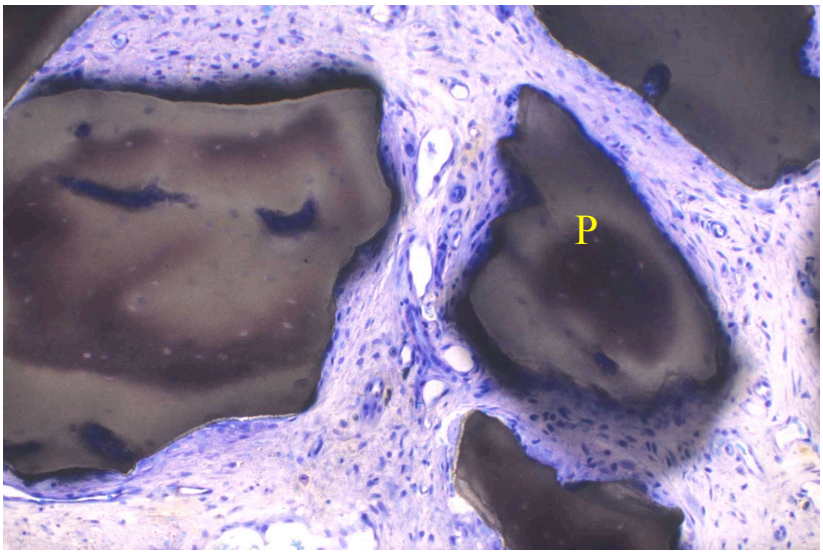
An osteoid seam (arrow) lined by osteoblasts is present along remodeled DFD bone. The marrow space is vascular and two adjacent PepGen particles (P) are visible. Paragon 25x




Pic. 5

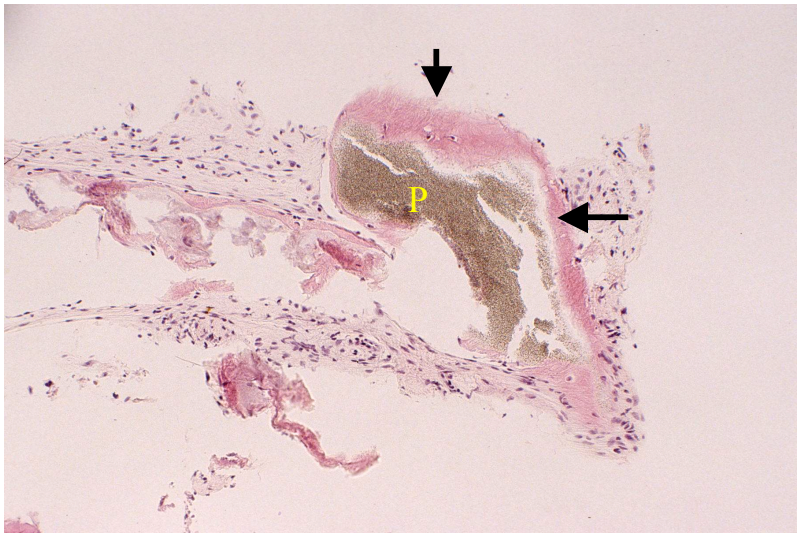
Patient 2 Tooth #30

The bone (B) shows evidence of remodeling with viable osteocytes. PepGen particles (P) are scattered within the bone and marrow space. Paragon 10x




Patient 2 Tooth #30

Numerous large and small PepGen particles (P) are surrounded by vascularized soft tissue. Paragon 25x




Patient 3 Tooth #2

Within the soft tissue, a PepGen particle (arrow) is surrounded by bone (yellow arrow). H&E 25x

By examining the histological sections closely, the majority of sections have a high turnover of DFDBA. New bone formation can be observed and different stages of bone maturation are present. The sections show osteoblasts adjacent to areas of new bone formation (osteoid), osteocytes, woven bone, mature bone and old bone. In addition, large PepGen-P15 particles are present in the majority of the sections. These particles tend to be surrounded by fibrous connective tissue, and do not show great signs of remodeling. As presented in Tab. 11 a large variation in the percentage of old bone, new bone and PepGen P-15 could be observed. The percentage of PepGen in the specimens varied between 0-40%. The amount of vital bone (old bone + new bone) was found to vary between 25% and 70%.

Tab. 6 Data of the histomorphometric analysis

| Patient | Age | Sex | Tooth | | New Bone | Old Bone | Soft Tissue/ Marrow Space | PepGen |
|---|---|---|---|---|---|---|---|---|
| 1 M.H. | 47 | F | 2 | Test | 10 | 60 | 30 | 0 |
| | | | 14 | Control | 15 | 25 | 20 | 40 |
| 2 K.N. | 54 | M | 19 | Control | 10 | 15 | 60 | 15 |
| | | | 31 | Test | 20 | 25 | 35 | 20 |
| 3 N.H. | 25 | M | 2 | Test | 5 | 20 | 70 | 5 |
| | | | 14 | Control | 5 | 60 | 30 | 5 |
| 4 K.L. | 20 | F | 5 | Control | 9 | 37 | 52 | 2 |
| | | | 12 | Test | 20 | 34 | 46 | 0 |
| 5 C.MK. | 47 | F | 29 | Test | 20 | 50 | 23 | 7 |
| | | | ¶ | | - | - | - | - |
| 6 J.S. | 21 | F | ¶ | | - | - | - | - |
| | | | ¶ | | - | - | - | - |
| 7 S.K. | 52 | F | ¶ | | - | - | - | - |
| | | | ¶ | | - | - | - | - |

¶ Histomorphometry was not possible

# Discussion

Ridge preservation is an extensively studied and well-understood treatment to prevent bone loss after extraction. Many different materials have been used in the past with similar success. It was formerly believed that primary closure of an extraction socket is crucial in guided bone regeneration procedures. Evidence from the last few years indicates that the exposure of resorbable membranes may be equally beneficial if not more beneficial to preserve keratinized mucosa on the buccal aspect of extraction sockets. The existence of keratinized mucosa is considered to be beneficial around implants. This fact is of major significance if patients have thin tissue, have poor oral hygiene or brush with lots of force. The present study investigated a method to preserve not only the hard tissue but also the soft tissue around extraction sockets.

Before the initiation of therapy, an overall healthy periodontal status with minimal bleeding and equivalent low plaque scores was obtained. 75% of all extraction sites had a GI of 1 at the day of extraction. There was also no significant difference between the initial and the 6-month follow up GI-value, which confirms that the patients were able to maintain periodontal health within the 6-month time frame of the present study. The authors observed a significant reduction in pocket probing depth (PPD) on the adjacent teeth. On the test side, the mean PPD decreased from 3.3mm to 2.7mm ($p=0.024$), while on the control side, a reduction of 3.4mm initially, to 2.6mm was found ($p=0.022$). Schropp *et al.* (2003) also observed a significant decrease of PPD of about 1mm after extraction without grafting. The lesser decrease of the PPD in the present investigation may be because this study, in contrast to the before mentioned one, performed ridge preservation after extraction.

Since PPD reduction is associated with an improvement in periodontal health, the extractions lead to a healthier periodontal status at the adjacent teeth in the present study. This finding has been confirmed in other studies (Lekovic *et al.* 1998, Schropp *et al.* 2003).

Previous studies focused on the vertical bone loss that is associated with extractions. It has been shown that the vertical changes of the alveolus are much less than the horizontal changes (Schropp *et al.* 2003). In the present study a mean bone fill of 7.2mm was found on the test and 7.5mm on the control side. Lekovic *et al.* (1998) also measured the bone fill and found a similar fill of approximately 6.9mm (Lekovic *et al.* 1998). The greater bone fill in the present study in contrast to the other investigation may be associated with the fact that a bone graft material was used. Lekovic *et al.* (1998) used only a barrier membrane. In addition, the ridge preservation procedure in the present study was performed in a way that the bone-graft slightly exceeded the buccal and lingual bone walls. It can be speculated that the use of a bone graft material leads to an increase in bone fill compared to using a membrane only.

It has been stated, that there is "inconclusive evidence that ridge preservation maintains the original socket dimensions" (Becker 2005). Nevertheless, there are several studies that indicate that there are ridge width alterations even though a ridge preservation procedure is performed (Lekovic *et al.* 1998, Yukna *et al.* 2003, Iasella *et al.* 2003). Yukna *et al.* (2003) measured the amount of ridge width at the time of immediate implant placement and concurrent intra- and extrasocket grafting with a synthetic bone graft and found, that the mean ridge width decreased approximately 8% after 6 months. In the present study the mean width was reduced approximately 24-28% (see Tab. 7).

Tab. 7

| Study | Materials | Time to reevaluation | Intrasocket Augmentation | Extrasocket Augmentation | Molar Other | Immediate Implant | Width Reduction |
|---|---|---|---|---|---|---|---|
| Present Study | B+M Test | 6 month | Yes | No | M+O | No | 24% 2.6mm |
| | B+M Control | 6 month | Yes | No | M+O | No | 28% 3.2mm |
| Yukna (2003) | B | 6 month | Yes | Yes | ? | Yes | 8% 0.8mm |
| Iasella (2003) | B+M | 4-6 month | Yes | No | O | No | 13% 1.2mm |
| | No | 4-6 month | No | No | O | No | 29% 2.6mm |
| Zubillaga (2003) | B+M | 4 month | Yes | Yes | ? | No | 8% 0.54mm |
| | B+M | 4 month | Yes | No | ? | No | 18% 1.8mm |
| Boticelli (2004) | No | 4 month | No | No | O | Yes | 56% |
| Schropp (2003) | No | 12 month | No | No | M+O | No | 50% 6.1mm |
| Lekovic (1998) | M | 6 month | Yes | No | O | No | 1.3 mm |
| | No | 6 month | No | No | O | No | 4.6 mm |
| Nemcovsky (1996) | B | 6 month | Yes | No | O | No | 0.6mm |

Index for Table 7:
B=Bone; M=Membrane; T=Test; C=Control; No=No bone, no membrane; Molar=Molars included; Other=Incisors, Canines, Premolars included.

The lesser amount of bone reduction in Yukna's study may be due to the use of an implant and the amount of extrasocket graft material that was used. Adding more volume by using an implant and extrasocket bone graft material may be the treatment of choice when it is attempted to preserve the ridge as close to 1:1. Iasella *et al.* (2003) found a decrease of ridge width of 13% in grafted sites, while non-grafted sites lost 29% of bone

width. Lekovic *et al.* (1998) found a mean decrease of 1.12mm in anterior and premolar sites that were grafted with a bioabsorbable membrane only. The greater reduction in width in the present study may be because of the inclusion of molar teeth. 80% of the teeth that were extracted in the present study were molars. The larger socket and the greater distance the osteogenesis has to occur in large extraction sockets may have impaired the amount of regeneration. Thus, molars show more postsurgical resorption. Schropp *et al.* (2003), found that the alveolus around premolar and molar extraction sites that are not grafted shows a 50% reduction of the width. Molar teeth had a tendency to show more bone resorption than premolars (Schropp *et al.* 2003). Since the present study showed a mean decrease in bone width of approximately 25%, it can be concluded, that in comparison to the results of Schropp *et al.* (2003) the procedures performed in the present investigation lead to mean decrease in width reduction of approximately 25%. On the other hand, the greater reduction in bone width in the present study may also be a result of one or more of the materials that were used. Though, to the best of the authors knowledge it can't be explained which mechanism could have lead to an increased resorption since the materials and procedures used in this study are commonly used and have been investigated previously (see Tab. 7 and 8). Thus, the authors believe, that the greater reduction of bone width is a result of the size of the extraction socket, not of one or more of the materials that were used.

It has been stated that for a successful guided bone regeneration (GBR) procedure, membranes have to stay in place for 6-8 weeks (Minsk 2005). The GORE RESOLUT® Adapt Regenerative Membrane is stated to preserve its integrity up to 8-10 weeks if it is not exposed to oral saliva. The researchers of the present study observed that the

membrane on the test side looses integrity and is completely dissolved after approximately 3-4 weeks. Within this study, it has been observed that the stability of the membrane is crucial to allow connective tissue to crawl over the membrane. If the exposed membrane is lose, or has wrinkles, the connective tissue does not seem to be able to crawl over the membrane and early maturation of connective tissue over the membrane is inhibited. Instead, the connective tissue seemed to find its way in some cases underneath the membrane, and the membrane dissolved after 3-4 weeks as a result of its exposure to the saliva in the oral cavity. In these cases, the membrane often dissolved before new connective tissue formation occurred on top of the socket. No clinical measurements were performed that could prove this observation. The size of the extraction socket also appeared to make a difference. The larger the socket, the longer seemed to be the time frame that was necessary for the connective tissue to cover the socket. No analysis was made for these clinical observations.

The histomorphometric data shows, that there does not seem to be a difference between the test and the control side in regards to the amount of vital bone or residual bone graft particles. This finding may be a result of two different reasons. Either the finding is true, and there is no difference irrespective of the method, or one or more of the materials used in the present investigation are inadequate for showing that a difference between the test and the control side truly exists. The latter one could be undermined because of the poor turnover capacity of especially PepGen-P15. Though several studies were done on the use of PepGen P-15 that showed promising results (Yukna *et al.* 1998; Yukna *et al.* 2000; Kuebler *et al.* 2004), many other studies were able to present different quantities of residual particles of PepGen P-15 in the obtained biopsies in guided tissue regeneration

(GTR), sinus-lift and ridge preservation procedures (Krauser *et al.* 2000, Yukna *et al.* 2002, Hahn *et al.* 2003, Degidi *et al.* 2004; Degidi *et al.* 2005). Depending on the procedure, and the mixture how the PepGen-P15 was applied in these studies, between 0-28% of residual graft particles can be found after several month of healing (see Tab.8). This finding is consistent with the data of the present analysis. The present study results also show large quantities of PepGen-P15 particles in all specimens. The amount of PepGen P-15 was analyzed histomorphometrically and it was found to vary between 0-40%. The amount of vital bone varied between 25-70% in different specimens. It has been shown in other studies that a similar wide range of vital bone can be found after ridge preservation procedures using different materials (Froum *et al.* 2002). If this bone graft material would actually inhibit bone regeneration to a certain degree, it could be speculated that the graft material could have also masked the ability to show a difference between the test and the control sides with regards to the amount of vital bone present after 6 months, if there really is one. It has been shown in other studies that the use of synthetic and bovine derived hydroxyapatite has disadvantages because of the poor turnover capacity of the graft material (Takeshita *et al.* 1997, Vance *et al.* 2004). In fact, it has been shown, that many hydroxyapatite particles are surrounded and encapsulated by fibrous connective tissue (Rosen and McFarland 1990, Takeshita *et al.* 1997). *In vitro*, it has been documented that one type of hydroxyapatite is different to another (Kuebler et al. 2004), but there seems to be a consistent finding in the literature that some graft materials are in close contact with fibrous connective tissue (see Tab. 8). This finding can be confirmed in the present study. By analyzing the histologic specimens it was found, that large quantities of PepGen-P15 particles are surrounded by fibrous connective tissue,

not bone (see pictures in Results). Though some literature shows direct PepGen P-15 to bone contact (Barbosa *et al.* 2002; Degidi *et al.* 2005), another case-study indicated, that up to 68% of fibrous tissue and bone marrow is present adjacent to PepGen P-15 particles in a previous extraction site that was grafted (Hahn *et al.* 2003). Even though large soft tissue quantities seems to be a consistent finding, one study stated the following: " PepGen P-15 particulate showed a solid, compact core with good osteoid bridging among PepGen P-15 particles (Hahn *et al.* 2003)." An animal study that researched the osteogenic potential of PepGen P-15 and PepGen P-15 flow in comparison to defects that were left to heal without grafting (control) came to a similar conclusion: "Both PepGen P-15 and PepGenP-15 flow enhanced new bone formation (….) Meanwhile control defects showed very little newly formed bone." These observations can't be supported with the present randomized controlled trial (RCT). In contrast, the present researchers had difficulties to obtain "solid" and intact bone cores from the patients. This difficulty is probably a result of the soft bone quality and the high quantity of fibrous tissue and bone marrow, which was histologically confirmed. Thus, the histologist was not able to perform histomorphometric analysis in many obtained specimens. In general, transitional bone graft materials like PepGen P-15 have been described to result in an increase in bone density (Bartee 2001). The denser bone is probably a result of large quantities of residual transitional bone graft materials within the osteotomy site. On the other hand, if an increase in soft tissue and trabecular bone is encountered, the bone quality may also be unfavorable. The latter one seemed to be the more prevalent finding in the present study. Vance *et al.* (2004) showed that differences in materials used in ridge preservation procedures could lead to a 35% difference in vital bone. Vance *et al.* (2004) compared

the used of a bovine derived xenograft with membrane to the use of a putty composed of DFDBA and calciumsulfate graft material used in a 1:1 ratio. The sites grafted with the xenograft had 26% vital bone, while the sites grafted with mixture of DFDBA putty and calciumsulfate demonstrated 61% vital bone. If Vance *et al.*'s (2004) results are compared to the present study results it would explain why the present amount of vital bone (old bone plus new bone) was somewhere between 25-70%. Since a combination of bovine derived hydroxyapatite (PepGen P-15) and DFDBA was used it would make sense that the results of the present study would be somewhere in the middle between Vance *et al.*'s two treatment modality results. This is because, PepGen P-15 seems to have a very slow substitution rate like many other transitional or even long-term bone graft materials. Thus, this material can be found in higher quantities just like the before mentioned xenograft. Subsequently, by staying in place longer, it also decreases the amount of new "vital" bone formation. In contrast, DFDBA has been shown to be substituted to a great extend after 6 months, and no fibrous encapsulation can be observed histologically (Brugnami *et al.* 1999). This finding leads to the conclusion that DFDBA increases the amount of vital bone, as it did in the before mentioned study (Mellonig *et al.* 1981). Gelbert *et al.* (2005) compared the amount of vital bone after 4-5 month in sinus lift procedures after grafting with A. PepGen P-15 and DFDBA, B: PepGen P-15 and PepGen P-15 flow, and C: PepGen P-15 and PRP (platelet rich plasma) (Gelbart *et al.* 2005). The results showed for A: 24%, B: 28% and C: 15% vital bone. The authors concluded that "optimal bone formation requires a spacer (…) to allow sufficient vascular penetration" and subsequent new bone formation (Gelbart *et al.* 2005). In conclusion, using PepGen P-15 alone, as investigated in case "C" leads to a decrease in new bone

formation. Adding a “space-maker”, like DFDBA, increases new bone formation. Thus, the low quantity of vital bone in some specimens of the present study is more likely to be attributed to the PepGen P-15, not the DFDBA.

Tab. 8 page 54

Θ PepGen P-15 Flow contains PepGen P-15 particles suspended in a biocompatible hydrogel, composed of ethylcellulose and glycerol.
¶ PRP= Platelet Rich Plasma

| Study | Histomophometry | Histology after | Procedure | Materials Used |
|---|---|---|---|---|
| Present Study | 25%-70% vital bone<br>0-40% PepGen P-15<br>20-70% Bone marrow/soft tissue | 6 months | Ridge Preservation | PepGen P-15 DFDBA+Membrane |
| Gelbart (2005) | 24% vital bone<br>23% residual graft<br>53% bone marrow | 4-5 month | Sinus-Lift | PepGen P-15+DFDBA+autogenous bone (15%) |
| | 28% vital bone<br>17% residual graft<br>55% bone marrow | 4-5 month | Sinus-Lift | PepGen P-15+PepGen P-15 (flow)Θ+ autogenous bone (15%) |
| | 15% vital bone<br>22% residual graft<br>63% bone marrow | 4-5 month | Sinus-Lift | PepGen P-15+PRP¶+autogenous bone (15%) |
| Vance (2004) | 26% vital bone | 4 months | Ridge Preseration | Bovine Xenograft+Membrane |
| | 61% vital bone | 4 months | Ridge Preservation | DFDBA putty+calcium sulfate |
| Guarnieri (2004) | 59% vital bone | 3 months | Ridge Preservation | Calcium sulfate |
| Iasella (2003) | 28% vital bone<br>37% non-vital b.<br>26% trabecular b.<br>9% amorphous | 4-6 month | Ridge Preservation | FDBA+Membrane |
| | 54% vital bone<br>34% trabecular<br>12% amorphous | 4-6 month | Control | No Graft (Control) |
| Krauser (2000) | 14% vital bone | 4 month | Sinus-Lift | PepGen P-15 (particulate) |
| Degidi (2004) | 32% new bone<br>38% bone marrow<br>28% residual graft | 6 month | Sinus-Lift | PepGen P-15 and autogenous bone |
| Froum (2002) | 34.7% vital bone<br>51% soft tissue | 6-8 month | Ridge Preservation | DFDBA |
| | 32.4% vital bone<br>67% soft tissue | 6-8 month | Control | No Graft (Control) |
| Hahn (2003) | 10% vital bone<br>68% bone marrow and fibrous tissue<br>21% non-resorbed graft | 3 month | Ridge Preservation | PepGen P-15 (particulate) |
| | 18% vital bone<br>82% bone marrow and fibrous tissue<br>0% non-resorbed graft | 3 month | Ridge Preservation | PepGen P-15 (Flow) Θ |

Cardaropoli *et al.* (2003) investigated the natural healing of extraction sockets in beagle dogs when they are left to heal naturally. After 6 months the histomorphometric analysis revealed that the extraction sites had 87% bone marrow and 13% mineralized bone in the midportion of the previous extraction site (Cardaropoli *et al.* 2003). After extraction, a constant decrease of the soft tissue content was observed until it completely vanished on day 60. From that point on, the amount of bone marrow increased, and the amount of mineralized bone decreased. It can be speculated, that ridge preservation as applied in the present study, significantly delays this healing process. As a result, the quantity of vital bone, bone graft material and soft tissue is different. The biopsies of the present investigation show presence of 20-70% soft tissue (bone marrow) within the specimens. This finding is either a result of connective tissue infiltration within the bone graft or a combination of granulation tissue and provisional bone matrix that can be observed in the initial phases of healing (7-30 days) of extraction sockets (Cardaropoli *et al.* 2003). The difference between the two is that provisional bone matrix will eventually turn into bone, while fibrous connective tissue won't. A study by Froum *et al.* (2002) also evaluated the amount of soft tissue in previous extraction sites grafted with A: nothing, B: bioactive glass and C: DFDBA. The soft tissue percentage was found to be in A: 67%, B: 35% and C: 51% after 6-8 month of healing (Froum *et al.* 2002). An earlier study by Becker *et al.* (1996) found equally high percentages of "non-vital bone graft particles within fibrous connective tissue" in extraction sites grafted with FDBA and DFDBA (Becker *et al.*

1996). Becker *et al.* (1998) even suggested in a similar but different study, that due to this finding these materials "are not recommended for enhancement of vital bone to implant contact" (Becker *et al.* 1998). This suggestion can not be completely substantiated with the evidence we presently have. It seems logical to stress the importance of high quantities of vital bone to increase the percentage of bone to implant contact. Though, it may be sufficient for the placement of an implant to have for example >25% of vital bone after 6 month. In an animal study that compared the use A: DFDBA+non-resorbable membrane, B: HA+non-resorbable membrane, C: non-resorbable membrane around implants placed into enlarged extraction sockets the following was found. After 6 months, group A showed 35%, B presented with 29% and C had 27% of bone to implant contact. Interestingly, the torque necessary to remove the implant was > 45Ncm and did not differ between the groups (Kohal *et al.* 1998). This finding leads to the conclusion that fairly low amounts of vital bone in contact with an implant may be sufficient to provide implant survival. Nevertheless the authors believe that the amount of vital bone should not stay at that level but show an additional increase. An increase of vital bone can only occur, if the soft tissue within the specimens is provisional bone matrix and eventually turns into osteoid, woven and lamellar bone. Long-term data needs to evaluate if the amount of vital bone over a longer observation period increases, or if ridge preservation and some bone graft materials are associated with long-lasting low amounts of vital bone because they inherit fibrous connective tissue within the graft particles on a cellular level. When Table 8 is carefully analyzed, it can be found that some studies point out that soft tissue exists within the specimens, other studies don't mention the presence of soft tissue. Instead, the latter ones suggest an increase in bone marrow. Presence of

"bone marrow" and absence of "soft tissue" in these studies mask the existence of fibrous connective tissue. Interestingly, some of the studies that describe the presence of "bone marrow" only, acknowledge the company that produces the bone graft material that is researched (Degidi *et al.* 2004; Degidi *et al.* 2005; Gelbart *et al.* 2005).
To date, the present investigation and others (see table 8) show evidence that soft tissue within the specimens exists. There also seems to be a correlation between a decrease in bone marrow/soft tissue and an increase in vital bone (see table 12). If this finding can be confirmed in future research, bone graft materials or procedures that decrease the presence of bone marrow and soft tissue and increase the quantity of vital bone should get promoted.

In addition, histomorphometric analysis is difficult and none of the studies describe in detail how the biopsies were standardized, and how the amount of vital bone was calculated. The periodontal literature seems to lack materials and methods on how adequate bone cores for histomorphometric analysis have to be, and how the analysis has to be performed to obtain results that allow the comparison between different studies. In the future, standards should be established to allow inter-study data analysis and conclude the superiority of one method or material to another.

Another reason that could have lead to the low quantities of vital bone is, that the membrane has inadequate barrier function. If this was the case, both results would appear equally impaired and this could also explain the present study results. Since membranes composed of glycolide and lactide have been shown to provide adequate barrier function

in ridge preservation procedures (Lekovic *et al.* 1998, Zubillaga *et al.* 2003) and the manufacturer states the membrane has adequate barrier function for 8-10 weeks, it is rather unlikely that the low amount of vital bone is a result of the barrier membrane. Also, taking the results of the before mentioned studies into consideration that showed similar results.

Since the results of the histomorphometric analysis are the same on the test and the control side, the researchers believe that the low amount of vital bone are a result of the bone graft material that was used. No matter if the results are attributable to the bone graft material or the membrane, the belief that bone grafts need to be covered for 6-8 weeks needs to be reinvestigated (Minsk 2005), and maybe, a much shorter time frame is adequate if guided bone preservation is performed in contained defects.

Certainly, there is evidence that the exposure of non-resorbable membranes decreases success of GBR procedures (Lang et al. 1994; Hammerle and Jung 2003). In addition, it has been shown that the exposure of a bioabsorbable collagen membrane can lead to significantly reduced bone fill and bone to implant contact in grafted dehiscence-type defects (Oh *et al.* 2003). Since there does not appear to be a difference between the test and the control side in regards to the bone width preservation and the amount of vital bone in the present study, the "guided membrane exposure" method using a membrane made of 67% PGA (polyglycolic acid) and 33% TMC (trimethylene carbonate) shows different and better results compared to studies that concluded a poorer outcome of exposed membranes in GBR procedures (Machtei 2001). The differences in the results

can be explained in two ways. Either, the enhanced results are due to the different morphology of the defect, or because of the different bioabsorbable membrane that was used. Since space maintenance becomes less important in contained defects, parts of the superior results in the present study can be explained by the more contained morphology of the defect (Hammerle and Jung 2003; Chen *et al.* 2004). The other explanation is the difference in behavior of collagen compared to PGA membranes when they get exposed. The new generation of the latter one may be able to maintain a barrier function for a longer period of time. This would explain why the exposure of the membrane does not seem to make a difference. In contrast to PGA membranes, collagen quickly denaturates when it is exposed. Future studies should look at the different behavior of collagen versus PGA membranes and compare their efficacy when these membranes are intentionally left exposed.

Especially since the amount of keratinized mucosa could be preserved much better on the facial aspect of the test side, and the patient's experienced less discomfort on the test side, the membrane exposure method may actually proof to be superior. Douglass (2005) already described the advantages of leaving a PGA-membrane exposed. He stated that, "the new generation of membranes enable the clinician to leave the membrane exposed, be less invasive and preserve an optimal gingival form" (Douglass 2005). To the best of the authors' knowledge there has been no study that actually proves this hypothesis. Lekovic *et al.* (1998) also used a bioabsorbable membrane and encountered several exposures during the course of healing. Lekovic *et al.* (1998) concluded that, "glycolide and lactide polymers are well tolerated by the gingival tissues"(Lekovic *et al.* 1998).

Zubillaga *et al.* (2003) used a similar membrane and experienced that even though primary closure was achieved at the time of ridge preservation, 45% of the membranes became exposed afterwards (Zubillaga *et al.* 2003). None of the sites experienced an infection or were related to clinical complications. This finding can be supported with the present study. Intentional membrane exposure and postoperative non-intentional membrane exposure could not be related to complications within the healing period in the present investigation. By examining the keratinized tissue, the postoperative comfort-level and the bone width, the authors found that the "guided membrane exposure" method is able to:

1. Preserve the gingival esthetic contours significantly better. Thus the method reduces the necessity of 2$^{nd}$ stage grafting procedures.
2. Significantly reduce the invasiveness (postoperative discomfort).
3. Achieve the same amount of vital bone and the same amount of preserved bone width.

Simion *et al.* (1997) examined the effect of membrane exposure *in vitro* and found bacterial penetration (Simion *et al.* 1997). As a result, it has been hypothesized, that membrane exposure may lead to infections and may also lower the quantity of regeneration (Simion *et al.* 1997). This hypothesis cannot be supported with the results of this study. Since there was no difference in regards to bone width preservation and the percentages of vital bone between the test and the control, membrane exposure, as applied in the present investigation, does not seem to affect ridge preservation procedures negatively.

## Conclusions

The guided membrane exposure method is advantageous whenever there is a need to decrease horizontal bone resorption, enhance postoperative comfort and preserve gingival esthetic and functional contours. This study is the first randomized controlled clinical trial that presents evidence that there is no difference in vital bone formation and the amount of preserved bone width, whether a membrane made of PGA and TMC is intentionally left exposed or primary closure over the membrane is achieved. Subsequently, the belief that membrane exposure leads to a decreased quality and quantity of bone has to be rejected based on these findings.

The combination graft PepGen P-15 and DFDBA (1:1) leads to 25-70% of vital bone and 0-40% of residual PepGen P-15 particles. Due to the high percentage of residual PepGen P-15 particles and their proximity to soft tissue, the use of this material cannot be recommended for ridge preservation procedures. The use of DFDBA can be supported because of its great substitution rate.

Intra-socket ridge preservation leads to a significant decrease in horizontal ridge width of approximately 25% in molar extraction sites. In order to further minimize the resorption, it seems advantageous to suggest extra-socket ridge preservation, whenever the bone width is indicated to be preserved in a 1:1 ratio. Since extra-socket ridge preservation will likely be associated with flap advancement to achieve primary closure, a significant decrease in the amount of keratinized tissue in conjunction with greater postoperative discomfort, as shown in the present investigation, can be anticipated. Future research

should investigate the difference between intra- and extra-socket ridge preservation in regards to the bone width and the gingival esthetic contour.

## Study Criticism

Studies that involve appliances (stents) as devices to perform measurements are common (Iasella *et al.* 2003, Zubillaga *et al.* 2003). Nevertheless, stents are associated with different inaccuracies of the measurements. These inaccuracies evolve from malfitting of a stent e.g. if patients have restorative work done between the initial and the final measurement. Also, measurement errors occur due to shifting and super-eruption of teeth within a 6-month time frame. Different positioning of the instruments that are used to measure certain parameters is also associated with measurement errors. The researchers tried to minimize these measurement errors by always having the same 2 examiners present at each evaluation. Some measurements were taken repetitively until both examiners agreed on the result value.

The study can be also criticized because of the low number of subjects that are enrolled. It has to be considered, that the study power was initially calculated for the primary outcome variable. Since the difference between test and control is very large, only a low number of patients is needed to show that there is a statistically significant difference. We found that the test group had a mean difference of 1.55 for the primary outcome variable keratinized mucosa, and the control group had a mean difference of 3.45. Based on the standard deviations (0.44 and 1.98, respectively) we found that the data of the present thesis has 94% power with a sample of 10 cases. To achieve 80% power we would only need 8 patients (nQuery Advisor). Thus, 10 cases are sufficient to show the difference in regards to keratinized mucosa.

Obviously, the study was not designed to detect a statistically significant difference between other parameters like bone width. It was calculated that with the present mean difference and standard deviations 58 cases would have been necessary to find out if there really is a significant difference in regards to bone width between the test and control side. Due to the difficulty to recruit patients and the high costs of the materials, it appears impossible to increase the "n" to such a high number of cases. The difficulty to recruit patients mainly arose from the very narrow inclusion criteria. The fact that only intact extraction sites qualified for this investigation excluded most patients that were initially screened.

Another criticism can be applied towards the large variation of bone cores that were obtained. These differences in harvesting bone cores occurred because of 2 reasons. One, the different size of premolars and molars may have lead to a different turnover in bone graft materials. Thus, molars may have more graft particles inside the grafted socket, while premolars are more likely to have greater amounts of vital bone inside the previous extraction site. In addition, if less vital bone is seen and more trabecular and connective tissue is found instead, it becomes difficult to obtain solid bone cores because of the class III or IV characteristic of the bone quality. This being said, the results of the histomorphometric analysis were impaired because of the inadequate biopsies that were often obtained.

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# Appendix:

## Patient screening sheet:

Patients Name: Record Number:

Age: Sex: Date:

| | | |
|---|---|---|
| Two hopeless upper or lower molars or premolars are going to be extracted in the same jaw in opposite quadrants | Yes ○ | No ○ |
| Long term antibiotic or antisteroidal tx | Yes ○ | No ○ |
| Allergies to any of the used substances | Yes ○ | No ○ |
| Acute infection (pus, pain) | Yes ○ | No ○ |
| Current smoking | Yes ○ | No ○ |
| Systemic diseases | Yes ○ | No ○ |
| Pregnancy | Yes ○ | No ○ |
| Diabetes | Yes ○ | No ○ |
| Hypertension Stage 1 or 2 (140≥systolic or/and ≥ 90 diastolic) | Yes ○ | No ○ |
| 3 bone walls present and at least 50% of the fourth wall | Yes ○ | No ○ |

I, …… agree to participate in this study, which observes differences in bone and gum changes after extraction,

with the use of DFDBA, PepGen and GORE RESOLUT® Adapt Regenerative Membrane.

……Patients Signature

Version 08/04/04

| Lower or Upper | | 25 | 24 | 23 | 22 | **1. Visit** | 21 | 20 | 19 | 18 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| keratinized mucosa buccal | | | | | | keratinized mucosa buccal | | | | | |
| stent-bone buccal | | | | | | stent-bone buccal | | | | | |
| stent-bone lingual | | | | | | stent-bone lingual | | | | | |
| stent-bone distal | | | | | | stent-bone distal | | | | | |
| stent-bone mesial | | | | | | stent-bone mesial | | | | | |
| stent-bone center | | | | | | stent-bone center | | | | | |
| horizontal width of the bleeding points | | | | | | horizontal width of the bleeding points | | | | | |
| socket width/ | | | | | | socket width/ | | | | | |
| length | | | | | | length | | | | | |
| number of walls | | | | | | number of walls | | | | | |
| Buccal dehiscence in mm | | | | | | Buccal dehiscence in mm | | | | | |
| GI | | | | | | GI | | | | | |
| PI | | | | | | PI | | | | | |
| PPD | | | | | | PPD | | | | | |
| Follow up: Discomfort A-F | | | | | | discomfort A-F | | | | | |
| | 26 | 25 | 24 | 23 | 22 | | 21 | 20 | 19 | 18 | 17 |

Version 10/25/04 **nth follow up reevaluation:**

Patients Name:……..

Record Number:…….

Date:……...

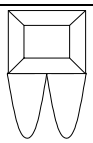
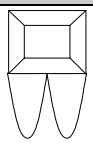
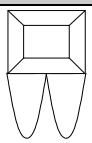
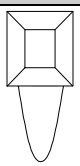
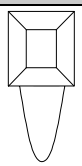
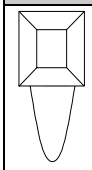
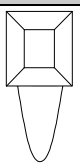
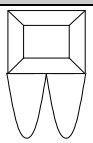
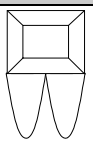
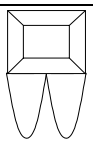
| | 26 | 25 | 24 | 23 | 22 | | 21 | 20 | 19 | 18 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Lower or Upper |  |  |  |  |  | **2. Visit** |  |  |  |  |  |
| keratinized mucosa buccal | | | | | | keratinized mucosa buccal | | | | | |
| keratinized mucosa occlusal | | | | | | keratinized mucosa occlusal | | | | | |
| stent-bone buccal | | | | | | stent-bone buccal | | | | | |
| stent-bone lingual | | | | | | stent-bone lingual | | | | | |
| stent-bone distal | | | | | | stent-bone distal | | | | | |
| stent-bone mesial | | | | | | stent-bone mesial | | | | | |
| stent-bone center | | | | | | stent-bone center | | | | | |
| horizontal width of the bleeding points | | | | | | horizontal width of the bleeding points | | | | | |
| number of walls | | | | | | number of walls | | | | | |
| Buccal dehiscence in mm | | | | | | Buccal dehiscence in mm | | | | | |
| GI | | | | | | GI | | | | | |
| PI | | | | | | PI | | | | | |
| PPD | | | | | | PPD | | | | | |
| Follow up: Discomfort A-F | | | | | | discomfort A-F | | | | | |
| | 26 | 25 | 24 | 23 | 22 | | 21 | 20 | 19 | 18 | 17 |

**Table of random assignments:**

Version 10/25/04 ıt

| | |
|---|---|
| | Treatment A |
| 2 | Treatment A |
| 3 | Treatment B |
| 4 | Treatment B |
| 5 | Treatment A |
| 6 | Treatment B |
| 7 | Treatment B |
| 8 | Treatment A |
| 9 | Treatment B |
| 10 | Treatment A |
| 11 | Treatment A |
| 12 | Treatment B |
| 13 | Treatment B |
| 14 | Treatment A |
| 15 | Treatment A |
| 16 | Treatment B |
| 17 | Treatment A |
| 18 | Treatment A |
| 19 | Treatment B |

Treatment A refers to the right side of the patient being the test side. Treatment B refers to the left side of the patient being the test side.

# Dataset for n=10

| | KM-T | KM-C | KM-T6 | KM-C6 | KM-DT | KM-DC | KMDif T | KMDif C | Width-T | Width-C | Width-T6 | Width-C6 | Width-DT | percent-T | Width-DC | percent-C | Distal-T- |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gilboy | 2 | 2 | 1 | 0 | 1 | 2 | -1 | -5 | 14 | 12 | 12 | 12 | 2 | 0.14 | 0 | 0.00 | 18 |
| McKee | 3.5 | 3 | 2 | 0 | 1.5 | 3 | -2 | -4 | 8 | 9 | 5 | 5 | 3 | 0.38 | 4 | 0.44 | 11 |
| Mckee | 3.5 | 3.5 | 2 | 1 | 1.5 | 2.5 | -2 | -3 | 10 | 10 | 4 | 3 | 6 | 0.60 | 7 | 0.70 | 8 |
| Hoa | 12 | 7 | 10.5 | 0 | 1.5 | 7 | -2 | -3 | 18 | 15 | 14 | 12 | 4 | 0.22 | 3 | 0.20 | 13 |
| Gonzalez | 3 | 3 | 2 | 0 | 1 | 3 | -2 | -8 | 11 | 10.5 | 8 | 10 | 3 | 0.27 | 0.5 | 0.05 | 23 |
| Santorelli | 7 | 9 | 6 | 2 | 2 | 7 | -1 | -5.5 | 13.5 | 12 | 10.5 | 6 | 3 | 0.22 | 4.5 | 0.38 | 12 |
| Lavigne | 5 | 4 | 3 | 1 | 2 | 3 | 0.5 | 2.5 | 10.5 | 10 | 8 | 7 | 2.5 | 0.24 | 3 | 0.30 | 12.5 |
| Han | 4 | 3 | 3 | 0 | 1 | 3 | 0 | -3 | 9 | 8 | 5 | 6 | 4 | 0.44 | 2 | 0.25 | 11 |
| Kelly | 3 | 1 | 1 | 0 | 2 | 1 | -2 | -5 | 12 | 9.5 | 8.5 | 9 | 3.5 | 0.29 | 0.5 | 0.05 | 13.5 |
| Robash | | | | | | | | | | | | | | | | | |
| Trimble | | | | | | | | | | | | | | | | | |
| Norton | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 |

| Distal-T6 | Distal-C6 | Z-T | Z -T6 | Z-C | Z-C6 | Z-Diff T | Z-Diff C | Mid-T | Mid-C | Mid-T6 | Mid-C6 | Mid-DT | Mid-DC | Mesial-T | Mesial-C | Mesial-T6 | Mesial-C6 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 17 | 15 | 5 | 8 | | | 3 | | 25.5 | 20.5 | 19 | 15 | 6.5 | 5.5 | 23 | 12 | 15 | 13 |
| 10.5 | 13.5 | | | | | | | 21 | 23 | 11.5 | 13.5 | 9.5 | 9.5 | 13 | 10 | 10 | 13 |
| 7.5 | 8.5 | | | | | | | 17 | 19.5 | 11.5 | 13 | 5.5 | 6.5 | 11 | 6 | 9.5 | 7 |
| 16 | 12 | | | | | | | 24 | 24 | 16 | 16 | 8 | 8 | 24 | 12 | 15.5 | 16 |
| 17 | 14 | | | | | | | 23 | 18 | 15.5 | 15.5 | 7.5 | 2.5 | 23 | 18 | 16 | 15.5 |
| 13 | 11.5 | | | | | | | 20.5 | 17.5 | 13 | 7 | 7.5 | 10.5 | 11 | 10 | 13.5 | 10.5 |
| 15 | 12 | | | | | | | 20 | 23 | 14 | 11 | 6 | 12 | 10 | 11 | 14 | 12 |
| 13.5 | 15.5 | | | | | | | 20 | 22 | 13.5 | 17 | 6.5 | 5 | 12 | 12 | 12 | 15.5 |
| 13 | 10 | | | | | | | 18 | 19 | 11.5 | 12 | 6.5 | 7 | 13 | 10.5 | 11 | 12 |
| | | | | | | | | | | | | | | | | | |
| 15.5 | 13 | | | | | | | 25 | 28 | 16.5 | 16 | 8.5 | 8 | 27 | 20 | 15.5 | 16 |

| Right=Test=1, Left=Test=0 | Bleeding-t | Bleeding-C | Bleeding-6t | Bleeding-C6 | Bleeding-Dif T | Bleeding-Dif C | Plaque-T | Plaque-C | Plaque-T6 | Plaque-C6 | Plaque-Dif T | Plaque-Dif C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1 | 1 | 1 | 2 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 |
| 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 |
| 1 | 2 | 2 | 1 | 1 | -1 | -1 | 1 | 1 | 1 | 1 | 0 | 0 |
| 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 2 | 1 | 2 |
| 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 |
| 0 | 1 | 0 | 3 | 3 | 2 | 3 | 1 | 1 | 3 | 3 | 2 | 2 |
| 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 |
| 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 |
| | | | | | | | | | | | | |
| 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 |

| ComfortC | Age | Sex1=male | 50% absece wall 1=yes | Socket LengthT | Socket Length C | Socket Width T | Socket Width C | Post-Op In | Molar=1, Premolar=0 |
|---|---|---|---|---|---|---|---|---|---|
| 5 | 52 | 0 | 0 | 9 | 12 | 11 | 11 | 0 | 1 |
| 3 | 47 | 0 | 0 | 5 | 5 | 7 | 6 | 0 | 0 |
| 3 | 47 | 0 | 0 | 11 | 10 | 9 | 8 | 0 | 1 |
| 4 | 35 | 1 | 0 | 12 | 10 | 10 | 11 | 0 | 1 |
| 5 | 22 | 0 | 0 | 10 | 11 | 10 | 7 | 0 | 1 |
| 4 | 21 | 0 | 0 | 13 | 14 | 10.5 | 9 | 0 | 1 |
| 3 | 20 | 0 | 0 | 6 | 5.5 | 7 | 8 | 0 | 0 |
| 3 | 47 | 0 | 1 | 10 | 10 | 7 | 7 | 1 | 1 |
| 4 | | 0 | 0 | 10 | 6 | 10 | 7 | 0 | 1 |
| | | | | | | | | 0 | |
| | | | | | | | | 1 | |
| 4 | 55 | 1 | 0 | 10 | 10 | 9 | 9 | 0 | 1 |

| PPD-T | PPD-C | PPD-T6 | PPD-C6 | PPD-Dif T | PPD-Dif C |
|---|---|---|---|---|---|
| 5 | 4 | 3 | 3 | 2 | 1 |
| 2 | 2 | 2 | 2 | 0 | 0 |
| 2 | 2 | 2 | 2 | 0 | 0 |
| 4 | 5 | 3 | 5 | -1 | 0 |
| 3 | 3 | 2 | 2 | -1 | -1 |
| 3 | 3 | 2 | 2 | -1 | -1 |
| 3 | 3 | 3 | 3 | 0 | 0 |
| 4 | 4 | 3 | 3 | -1 | -1 |
| 2 | 3 | 2 | 2 | 0 | 1 |
| | | | | | |
| 5 | 5 | 5 | 2 | 0 | -3 |

# Consent Form to Participate in a Research Study

## Purpose of the Research

You are invited to participate in a research study evaluating and comparing the effectiveness of two standard dental surgical procedures. You are being invited to take part because you have two teeth on opposite sites of the same jaw that are in such bad condition than another dentist has recommended they be extracted. The two standard surgical procedures are: 1. Ridge Preservation and sealing of the extraction site with a resorbable membrane, or 2. ridge preservation and sealing of the extraction site with a resorbable membrane and the your gum tissue.

A ridge preservation procedure is a surgery, in which a tooth in a very bad condition is removed and the surrounding bone is preserved by packing a bone substitute (bone graft) into the extraction socket. Preserving the bone is important, because dentures need bone to be stable.

The extraction site can be closed either with a membrane and your tissue (gum) or only with the membrane. It is believed that both techniques are equally good for preserving bone. The membrane that is used is similar to human tissue structure. It becomes a part of the body and does not need a second surgery for the removal. The purpose of this study is to see if there are differences in gum and bone changes when these two techniques are used. It is believed that the gum level doesn't change as much when the gums are not used to seal the extraction site. Nevertheless the amount of preserved bone should be the same whether your gum is used, or is not used, to seal the extraction site. A maximum of 20 patients will be enrolled at Tufts University School of Dental Medicine.

Taking part in this research study is totally your choice. Please read all of the information carefully. Ask Dr. Griffin, or his representative, to explain any words, terms, or sections that are unclear to you. If you choose not to take part in this research study, it will not change your dental care. The relationship you have with your doctor or Tufts University will also not change. You may change your mind about being in this study and stop being in this study at any time for any reason

## Inclusion criteria:

Subject must:

Have two last teeth that are scheduled (treatment planed) for extraction in the same jaw but at opposite sides. Treatment plan for extraction means that another dentist said that these teeth cannot be saved, because of their bad condition.

Have the majority of bone remaining around the teeth that are scheduled for extraction.

Gum infection leads to bone loss around teeth. If your teeth have lost a lot of bone, you will be excluded from the present study.

Be 18 years of age and older.

## Exclusion criteria:

Subjects will be excluded who:

Have an acute infection around the teeth that are scheduled for extraction.

Have systemic diseases that have been proven to affect wound healing such as certain immune defense dysfunction diseases (HIV) or certain tissue diseases.

Are diabetics.

High blood pressure ( ≥ 140/ 90).

Take long-term (>3 month) anti-inflammatory or antibiotic drug therapy.

Have allergies to one of the materials used for the procedure.

Fail to sign an informed consent form.

Are pregnant.

Are current smokers.

Are unable to keep follow up appointments.

## Study Procedures

A ridge preservation procedure has been recommended for you because it will preserve the bone at the extraction sites. The preserved bone will make any fixed or removable prosthesis that you may have in the future more stable. If you take part in this research study, you agree to have the two different surgical techniques to seal the extraction sites applied on you.

On one side of the mouth the extraction site will be covered with a resorbable membrane, on the other site of the mouth the extraction site will be covered with a membrane and your gum tissue.

By chance it will be decided which side of the jaw will undergo one or the other treatment. During the period of the study, the following procedures will be done in four steps over a period of seven months. The procedures for the present study are listed below:

| | |
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| **Pre-Treatment (2 visits)** | **Visit 1. Screening and obtaining of informed consent**<br>1. Introduce you to the study and check whether you are suitable for the present study. For this visit you will be asked several questions. The area of the teeth that are going to be extracted will be locally numbed with an anesthetic similar to novocaine (Xylocaine). After your teeth are numb, the amount of bone that is present/lost will be evaluated with a periodontal probe (needle like instrument). Separate consent will be obtained for this evaluation.<br>2. Obtain your informed consent and review the research authorization form (RAF).<br>3. Review and update your medical and dental history.<br>4. Review your home care.<br>5. A standard gum (periodontal) examination will be done on you. Your teeth and gums will be measured and examined.<br>**6.** Take a full mouth of x-rays and, if necessary, a panoramic x-ray. A panoramic x-ray shows a larger area of the mouth. It is necessary to have one if a nerve or a wisdom tooth is close to the teeth that will be extracted. A full mouth of x-rays is necessary if no full mouth of x-rays exists, or the existing x-rays are older than two years. Two impressions will be taken with a natural material called alginate. After processing the impressions a cast will be obtained that will look like a duplicates of your teeth. This duplicate will be used to make a stent. A stent is similar to a mouth guard and will be used for several measurements before and extraction. |
| | Visit: 2 Inflammation Control (Phase 1 therapy)<br>Inflammation control decreases the amount of gum bleeding if present.<br>1. Try fitting of the stent. A stent is similar to a mouth guard and will be used as a reference for measuring bone and gum changes.<br>2. Photographs will be taken. These are photographs of your gums on which the surgery will be performed. These photographs will not include your face. You can not be identified by the pictures.<br>3. A regular cleaning is done if large amounts of staining exist above or below the gumline. A deep cleaning is more a thorough treatment (which will be performed, if inflamed tissue and pockets around the teeth indicate it is necessary. This antibacterial treatment may be necessary before extraction, to allow proper |

| | |
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| | wound healing after extraction. A local anesthetic agent will be given to numb your gums if necessary to make you comfortable before the deep cleaning is performed. |
| **Treatment (1 visit)** | Visit: 3 Baseline measurements and extraction of teeth<br>1. The area of the teeth that are going to be extracted will be locally numbed with an anesthetic similar to novocaine (Xylocaine). This will make your gums numb so that you do not feel any pain during the surgery.<br>2. Photographs will be taken. These are photographs of your gums on which the surgery will be performed. These photographs will not include your face. You can not be identified by the pictures.<br>3. On one side of the jaw the tooth will be extracted very carefully, to preserve as much bone as possible. The extraction site will be cleaned from infected tissue.<br>4. The stent will be placed in your mouth after extraction. Gum measurements and the volume of bone will be evaluated with two needle like instruments using the stent as a reference guide. The stent will have marks and grooves that allow an accurate evaluation of your gum and bone in the area of the tooth that needs to get extracted. An x-ray will be taken. The x-ray will confirm the extraction of the entire tooth.<br>5. After that, the bone substitute (bone graft) will be applied into the extraction site. A resorbable membrane will be used to close the extraction site, so that the bone graft material is not lost. The membrane will be held in place with a couple of resorbable stitches.<br>Next, the tooth on the other side will be extracted and the same ridge preservation procedure will be performed. The difference in the surgical procedure is, that on the second side your gum tissue will be used to cover the socket on top of the membrane as well.<br>After the surgery, another x-ray will be taken of each site to ensure proper sealing of the bone graft in the extraction site. An antibiotic (doxycycline 100mg once a day for 14 days) will be prescribed to prevent infection of the bone graft. In addition, an antimicrobial mouthrinse will also be prescribed for the same periode of time (chlorhexidine 0.12%, rinse twice a day with one oz). Two different pain medications will be also prescribed to manage any discomfort if present (Iboprofen 600mg three times a day for three days and Percocet 5mg every 6 hours for three days as needed). |
| Follow-up after surgery | Visit: 4 Routine postsurgical follow up<br>Routine postsurgical follow up visit one week after surgery<br>This will include:<br>• A visual examination. |

| | |
|---|---|
| (2 visits) | • One of the doctors will review and, if needed, change some instructions to help the healing process according to your clinical situation.<br>• Selective clinical measurements and photographs will be taken. Sutures (stiches) will be removed. You will not be identified by the photographs. |
| | Visit: 5 Routine postsurgical follow up<br><br>Routine postsurgical follow up three weeks after surgery<br>This will include:<br>• A visual examination.<br>• One of the doctors will review and, if needed, change some instructions to help the healing process according to your clinical situation.<br>• Selective clinical measurements and photographs will be taken. You will not be identified by the photographs. |

| | |
|---|---|
| **Bone and tissue evaluation six month after extraction (1 visit)** | Visit: 6 six month after surgery<br>1. Application of local anesthesia on your gums to make measurements of your bone and your gums. This will make your gums numb so that you do not feel any pain during the bone evaluation.<br>2. The stent will be placed in your mouth, and the volume of bone will be evaluated using the stent as a reference guide.<br>3. The same bone and gum measurements as on the visit of the surgery will be made.<br>4. Photographs will be taken. You will not be identified in the photographs. |
| Only for patient's who are treatment planed for implant therapy: **Obtaining a biopsy at the time of implant placement (1 visit)** | Visit: 6 six to eight month after surgery at the time of implant placement<br>Implant therapy is not part of the research. Implant therapy means the replacement of a tooth by an implant. An implant is an artificial root on which a tooth or a denture is placed.<br>There will be some patient's that are treatment planed for implants in the extraction sites. A biopsy will be needed before placing the implant. These patient's will be followed as described below:<br>1. The surgical protocol for implant therapy will be followed as usual.<br>2. Photographs will be taken. You will not be identified in the photographs.<br>3. A biopsy will be obtained from the previous extraction sites that will receive implant therapy. The biopsy will show how much of the self-resolving materials that were used are still present after 6 month (bone and membrane). A biopsy is the removal of gum and bone tissue to allow looking at the tissue under a microscope. The amount of tissue that will be removed will not exceed the amount of tissue that needs to be removed anyway to allow placement of the implant into your jaw. |

## Potential Risks

One general risk of a surgical procedure is the risk of infection. The risk of infection is considered to be similar whether teeth are extracted with or without bone grafting. In addition, the risk of infection in the present study is considered to be very minor since an antimicrobial mouthrinse is prescribed together with an antibiotic for 14 days. In case an infection occurs, the bone grafted extraction site would have to be cleaned out, and the procedure would have to be redone in a later stage of treatment. To clean out the infected extraction site your gums need to be locally numbed. Metallic hand instruments are used to scrape the bone substitute (bone graft) out of the extraction site. Besides the risk of infection there are other side effects, including bleeding and dry socket. Dry socket is a painful extraction site of a tooth. It is a bacterial infection that usually happens when patients smoke excessively after an extraction. It has not been described to happen in ridge preservation procedures so far. Bleeding is very unlikely to occur since the extraction sites are sealed.

## Benefits

The benefit in participating in this study is, that you will not lose the 50% of bone width patients usually lose after extraction, because the empty space in your bone will be filled up (grafted) with bone graft material. As a result, future placement of any kind of fixed or removable denture in the extracted area may be more stable, better-looking and even longer lasting. The results of this study may increase periodontal knowledge with regards to which of the two techniques is more beneficial.

## Alternatives

The alternative to this procedure is to have extraction without preserving your bone volume. The Principal Investigator will talk with you about this option before you sign this consent form.

## Costs

There are no costs for the participation in this study. The bone grafting procedure would normally cost more than $500 per side. This procedure would usually be billed to you or your dental insurance. Since you participate in this study it will not be billed to you or your insurance.

## Voluntary participation

If you leave the study (for example, if you are not able to keep the appointments), you are free to do so without penalties. Your decision to not participate or to discontinue will involve no penalty or loss of benefits to which you are otherwise entitled.

## Necessary follow up care and self care

For your own safety (prevent infection) and the success of the surgery it is important that you attend the postsurgical follow up visits and that you take the prescribed medications. It is important for you to continue seeing your dentist, and if necessary, your specialists.

The success of your dental and periodontal health depends on your home care hygiene regimen and long-term evaluations and treatment from your doctors.

## Confidentiality

All patients will be protected in the following manner. Your confidentiality and the security of your information and identification will be handled in the following manner:

- Student and employee information will be kept confidential. Data from the study about any student or employee will be coded and placed in locked area. The study data will only be used for the purpose of the study. Students and employees participation in this study will not affect their employment, career path, educational plans, or social relationship within the hospital/school community.
- Dental records and case reports will be coded and will not have your name on them.
- All subjects will be assigned an ID code made up of your three initials and a two digit-number. This information will be placed in a locked area. The study data will only be used for the purpose of this study.
- Pictures will be taken of your teeth and gums only. No picture will be taken of your face.
- Data will be stored in a locked cabinet.
- Data collected is accessible to Dr. Griffin and Dr. Engler-Hamm only.

## Payment

You will not be paid for your participation in this study.

## New Information

New things might be learned during this study that you should know about. The investigators will tell you about new information that may affect your willingness to stay in this study.

## Payment for Research Related Injury

You will not be paid for research related injuries.

## Contact Information

Feel free to contact Dr. Engler-Hamm at 617 292 3871 or Dr. Griffin at 617 636 6530. If you have questions regarding about your rights as a research study subject, call the Tufts –New England Medical Center and Tufts University Health Sciences Institutional Review Board at 617 636 7512. The Health Sciences Institutional Review Board is a group of doctors, nurses and non-medical people, who review human research studies for safety and protection of human subjects.

## Participant's Statement

I have read this consent form and have discussed with Dr. Griffin or Dr. Engler-Hamm the procedure as described above. I have been given the opportunity to ask any questions, which have been answered to my satisfaction. I understand that any questions that I might have will be answered verbally, if I prefer, with a written statement.

I understand that I will be informed of any significant new findings discovered during the course of this study that might influence my continued participation.

I understand that my participation is voluntary and that I may refuse to participate or may withdraw consent and discontinue participation in the study at any time without prejudice to my present or future care at the Tufts University School of Dental Medicine.

I understand that in the event that I become ill or injured as a result of participating in this research study, medical care will be provided for me. However, such care will not be provided free of charge, even if the injury or illness is a direct result of this study. I understand that no funds to provide financial compensation for research related injury or illness are available.

If I have any questions concerning my rights as a research subject in this study, I may contact the Tufts – New England Medical Center Institutional Review Board at (617) 636-7512.

I have been fully informed of the above-described study with its risks and benefits, and I hereby consent to the procedure set forth above. I will be given a signed copy of this consent form.

I understand that as a participant in this study my identity and my medical records and data relating to the research study will be kept confidential, except as required by law, and except for inspections by the U.S. Food and Drug Administration which regulates investigational drug studies, and the study sponsor.

_____ _____

Date Participant

I have fully explained to_____the nature and purpose of the above-described study and the risks that are involved in its performance. I have answered all questions to the best of my ability.

_____

Principal Investigator or Representative

_____ _____

**Date** **Witness**

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