ALVEOLAR RIDGE PRESERVATION
AT DIFFERENT ANATOMICAL LOCATIONS-
CLINICAL AND HISTOLOGICAL EVALUATION OF
TREATMENT OUTCOME

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ABSTRACT

Background: Alveolar ridge preservation (ARP) is a surgical technique designed to prevent naturally occurring post-extraction bone resorption. It is well documented that alveolar bone height and width are reduced following tooth extraction as a result of physiologic bone remodeling. Depending on the type of post-extraction intrabony defect, an immediate or early implant placement itself may preserve the bone height and width. However, if the defect is generally too wide for immediate and/or early implant placement, it is recommended to perform ARP surgery to preserve the bone volume for future implant placement. The purpose of this study was to investigate clinical and histological healing outcomes following ARP performed on molar and premolar sites by using freeze-dried bone allograft (FDBA) together with a collagen membrane. Maxillary and mandibular sextants were compared for clinical and histological parameters.

Methods: Patients who were scheduled to have tooth extraction and implant placement for a molar or premolar tooth were included into this study. Inclusion criteria were single tooth extraction with intact mesial and distal adjacent teeth. Exclusion criteria were smokers, systemic health problems that may affect wound healing and acute infection
that may prevent bone graft placement. An impression was taken prior to tooth extraction and a stent was prepared from clear acrylic. This allowed repeating measurements exactly at the same location. Pre-operative clinical measurements included the amount of keratinized gingiva at implant site and adjacent teeth and, clinical attachment level at tooth scheduled for extraction and at adjacent teeth. The thickness of buccal and lingual plate, the length and diameter of the extracted tooth, buccal-lingual and mesial-distal defect size and, the distance from stent in place-occlusal plate to alveolar crest were measured either with a periodontal probe or a caliper following extraction. FDBA and collagen membrane were placed and flap was sutured. A re-entry surgery was performed following 139 ± 6 days healing period. Clinical measurements were repeated by using the same stent. A bone core was obtained with a 2.5 mm diameter trephine bur and immediately frozen in liquid nitrogen. Frozen bone cores were analyzed with micro-CT scan for bone volume density and bone trabecular connectivity. Following micro-CT scan, cores were fixed in formalin, decalcified in a solution containing formaldehyde and formic acid, embedded in paraffin and sectioned at approximately 4 micron thickness. Slides obtained from the mid-portion of the bone core were stained with trichrome and analyzed under light microscopy.

**Results:** Twenty-one patients were completed the study. Following ARP, ridge height loss change was negligible (a loss of 0.4±0.3 mm in maxilla and a gain of 1.3±0.3 mm in mandible). However, average ridge width loss was 2.4±0.8 mm and 2.5±0.5 mm in maxilla and in mandible, respectively. In maxilla, initial CAL at mesial surface of the extracted tooth had statistically significant effect on post-treatment interproximal bone height (r=0.747; p=0.021 and r=0.682; p=0.043 for mesial and distal interproximal sites, respectively) meaning that if the initial CAL was worst, the final bone height loss at maxillary interproximal areas was more. Also, maxillary root length of the extracted tooth was negatively correlated with post-treatment mid-buccal bone height (r=-0.752; p=0.019) meaning that if the root was long then the final alveolar bone height loss at mid-buccal was less. Similarly, initial palatal bone thickness had statistically significant effect on post-treatment maxillary mesial-distal distance (r=0.797; p=0.01) meaning that
if the initial palatal bone thickness was bigger then it helped to preserve mesial-distal space. Otherwise, approximately 1.2±0.7 mm narrowing in maxillary M-D distance was observed. In mandible, baseline CAL at mid-buccal site of extracted tooth was negatively correlated with post-treatment ridge height at the site (r=-0.562, p=0.046) meaning that if the initial CAL was worst, the final bone height loss measured at this specific mandibular site was less. Also, initial buccal and lingual bone thicknesses and root length of the extracted tooth had statistically significant effect on post-treatment mesial-distal distance (r=0.771; p=0.002, r=0.811; p=0.001 and r=-0.816; p=0.001, respectively) meaning that if the initial lingual and buccal bone thicknesses were larger in size this might help to preserve mesial-distal space. In general, a widening of 1.2±0.5 mm in M-D distance was observed in mandible. Similarly, if the mandibular root length was long, post-treatment M-D distance became narrower. Micro CT scan results revealed no statistically significant differences in trabeculation between grafted maxilla and mandible (P>0.05). However, mineralization per unit volume was higher in newly forming bone than in residual bone graft in mandible (P=0.03) while this difference was not statistically significant in maxilla (P>0.05). Histometric analysis revealed similar distribution of residual biomaterial, new cellular bone and nonmatured tissue in maxilla and in mandible (P>0.05).

**Conclusion:** Within the limits of this study, it appears that in different anatomical locations different factors may determine the clinical treatment outcome following ARP. Histometric and micro-CT treatment outcome evaluations results mostly reveal no statistically significant differences in regenerated /regenerating bone at the grafted site. Further studies are needed to better understand wound healing at the cellular level at grafted alveolar sockets and to determine clinical limitations of ARP.
DEDICATION

Dedicated to my parents Arturo and Soledad and my sisters Greta, Geraldine and Magali.

Gracias a Dios y a ustedes este sueño fue cumplido. Su apoyo y su amor incondicional me acompañó cada día de estos tres excitantes años e hizo que cada esfuerzo valiera la pena.
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Major Field: Dentistry
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CHAPTER I

INTRODUCTION

Tooth replacement with dental implant supported restoration is currently a well-accepted treatment alternative to traditional fixed and removable dental prostheses. Short and long term clinical studies report similar if not higher prognosis for these restorations. The challenge is that even the current dental implant design mimics natural root form, it is cylindrical in shape. This geometry does not allow immediate implant placement following tooth extraction in general; the extraction site would be ovoid and larger than available implant sizes and, there would be not sufficient bone-implant surface contact for more predictable osseointegration, and poorer peri-implant soft and hard tissue contours outcome following healing. There is a significant interest in developing new surgical techniques and biomaterials to preserve alveolar bone loss following tooth extraction. Despite the progress observed throughout the years, the treatment outcome following these surgeries is not always predictable; bone loss still occurs and additional surgeries to correct hard and soft tissue discrepancies may often be needed.¹
HEALING PATTERNS OF ALVEOLAR BONE FOLLOWING TOOTH EXTRACTION

The healing in the early weeks following tooth extraction has been studied histologically in animals and humans. Several histologic and radiographic studies have shown that extraction-wound repair in animals was basically similar in sequence but more rapid than in human beings. Immediately after tooth extraction the socket fills with blood from the severed vessels, which contains proteins and damaged cells. These cells initiate a series of events that will lead to the formation of a fibrin network, which, along with platelets, forms a blood clot or coagulum within the first 24 hours. The coagulum acts as a physical matrix and directs the movement of mesenchymal cells and growth factors. Neutrophils and later macrophages enter the wound site and digest bacteria and tissue debris to sterilize the wound. Growth factors and cytokines are released resulting in the induction and amplification of the migration of mesenchymal cells. Within a few days the coagulum begins to break (fibrinolysis). After 2 to 4 days granulation tissue gradually replaces the coagulum. A vascular network is formed by the end of 1 week and by 2 weeks the marginal portion of the extraction socket is covered with young connective tissue rich in vessels and inflammatory cells. Between 4 and 8 weeks after extraction, osteogenic tissue proliferates and trabecular bone is formed followed by a process of bone maturation. Although bone deposition in the socket will continue for several months, it will not reach the coronal bone level of the neighboring teeth. Trabecular bone formation reaches the edge of extraction socket, whereas osteoclastic bone resorption takes place on the surface of the residual ridge, a combination of which results in a distinct porosity on the crest of the residual ridge alveolar bone.

In the long term, there is a progressive loss of ridge contour as a result of physiologic bone remodeling. While the extent and pattern of resorption is variable among individuals, prosthodontic complications, loss of function and inadequate bone for the placement of dental implants may result.
Studies showed that there is a reduction in the height and width of the bone as a result of a surface resorption in combination with loss of bundle bone originally located within the alveolar bone proper, adjacent to the periodontal ligament containing a great number of Sharpey’s fibers. Bone loss occurs in the vertical and horizontal planes, with the degree of horizontal bone loss typically exceeding the degree of vertical bone loss. In the horizontal plane, bone loss occurs largely at the expense of the facial cortical plate, increasing the risk for facial soft tissue collapse, especially in the presence of a thin periodontal biotype.

Several studies in man have been performed in order to explain the clinical changes in the residual alveolar ridge after tooth extraction. Cryer et al. described the macroscopic appearance of the edentulous ridge after all teeth were extracted and observed that the lower jaw was external to and wider than the upper jaw. Other studies in cadavers have showed that in the maxilla the vertical height of the ridges had decreased and the crest of the edentulous ridge had shifted lingually after tooth extraction. It seemed that the most extensive resorption of the alveolar bone in the mandible occurred in the superior surface of the ridge and on the lingual surface of at least the posterior part of the ridge. This may accounted for the relative smallness of the upper edentulous arch where compared with the edentulous lower ridge.

Schröpp et al. reported that the loss of volume in the horizontal dimension amounts to 5–7 mm within the first 12 months. This corresponds to approximately 50% of the original width of the alveolar bone. Using subtraction radiography, the same group of investigators found that bone formation in the alveoli and loss of height of the alveolar bone crest occurred simultaneously during the first 3 months. The size of the loss was almost unchanged from 3 to 12 months. On the other hand, Lekovic found that post-extraction bone loss is accelerated in the first 6 months with as much as 40% of the alveolar height and 60% of alveolar width. Iasella et al. showed minimal differences on the effect on vertical ridge height or horizontal ridge width changes between the 4- or 6-month healing time. In another human study, changes in the attachment levels at the
teeth adjacent to the extraction site were evaluated. The extracted teeth were upper and lower molars and premolars. A 0.3mm gain in attachment level was found during the 12 first months of healing. The authors also demonstrated that the morphology of the alveolar crest became curved, with the lowest point situated 1.2mm apical to the mesial and distal sites of the extraction socket. Artzi et al \(^{16,17}\) demonstrated that in the posterior mandible, resorption happens primarily in the buccal/labial direction, resulting in a lingual displacement of alveolar crest. Moreover, it has been shown that the rate of reduction of residual alveolar ridges is greater in the mandible (0.4 mm/year) than in maxillary arches (0.1mm/year) \(^{18}\)

Animal studies have also demonstrated the macroscopic results of the bone modeling and remodeling process associated with tooth extraction. Pietrokovsky et al \(^{19}\), in their study in rats, confirmed the findings of human studies. They concluded that the resorption pattern results in a smaller edentulous ridge in the maxilla than in the mandible. An explanation of this may be that in the mandible the crest of the ridge is located at the center of the residual ridge and in the maxilla the crest is located near the palate. In the maxilla, the socket of both rat and man is formed by a thin alveolar plate on the buccal wall in contrast to the thick structure that forms the palatal wall. In the rat, after tooth extraction, the buccal plate was resorbed whereas new bone formation took place near the palatal structure.

Araujo et al \(^{20}\) performed an experimental study about dimensional ridge alterations following tooth extraction in dogs. It was found that marked dimensional alterations occurred during the first 8 weeks following the extraction of mandibular premolars. Nishimura et al \(^{21}\) found that in rats and humans, the rate of resorption is similar. The fastest rate of resorption in rats occurred in the first 2 to 4 weeks after tooth extraction, while the rate of resorption decreases during the following term. In humans, the fastest rate of resorption occurs in the first several months to two years, followed by a decreased rate of resorption \(^{21}\). When the findings in rats where compared to those observed in humans, the results produce similar bone loss curves,
FACTORS AFFECTING THE AMOUNT OF RESIDUAL ALVEOLAR BONE FOLLOWING TOOTH EXTRACTION

The treatment outcome of tooth extraction is mainly limited by the architecture of the existing bony walls. It has been shown in experimental studies that the coronal part of the buccal bone wall often consists of bundle bone\textsuperscript{20, 22}. The bundle bone loses its function after tooth extraction and is resorbed due to osteoclastic activity, resulting in a substantial vertical and horizontal reduction of the buccal crest.\textsuperscript{20}

Surgical trauma from tooth extraction induces microtrauma to surrounding bone, which may accelerate bone remodeling. As the buccal wall of the tooth socket is frequently partially or completely resorbed\textsuperscript{22}, the consequent collapse of the buccal soft tissue leads to marked bucco-oral alterations.

As stated before, the goal of alveolar ridge preservation techniques is to preserve bone, which is why the extraction should be performed in combination with ARP to maintain as much of the alveolar process as possible. Many extractions techniques have been described. Most of them include the elevation of a flap. However, if the elevation of the flap per se can be the cause of further bone loss has become controversial.

In a recent animal study, Fickl et al\textsuperscript{23} demonstrated that during a 3 month period of healing after elevation of mucosal flaps for tooth extraction, soft and hard tissue reduction was 14% more than “flapless” exodontia. Blanco et al\textsuperscript{24} showed the same results in a study in beagle dogs. According to the authors, by avoiding the elevation of a flap, the buccal bone plate can be protected and less resorption will occur. However, Araujo et al\textsuperscript{25} in another experimental animal study indicated that after 6 months of healing, the flapless and flap extraction resulted in similar amounts of reduction of the hard tissue dimension (35%). The authors concluded that flap elevation does not provoke
an additional mechanical trauma with a subsequent inflammatory response large enough to have a long-lasting effect on the final dimensions of the edentulous ridge. Previous studies have shown that flap reflection alone can lead to up to 1mm of bone resorption. However, since the resorptive process is initiated by extraction, and will occur with or without flap reflection, the addition contribution of the flap may be minimal.

Age of the patient has been pointed out as a factor that may affect the outcome of post extraction ridge dimension changes. A previous study have shown that in subjects ≥ 50 years of age there is a tendency to have greater horizontal and vertical resorption following extraction than those <50. The ridge preservation procedure performed in this study offset the loss of vertical ridge height in the <50 subjects, but not in the ≥50 subset. Ridge preservation only partially offset the horizontal ridge width, and the effect was similar for both age groups. However, when comparing extraction sites and preserved sockets, both tended to heal with a higher percentage of vital bone in the ≥50 age group.

Araujo et al. in a study in dogs showed that despite the difference in buccal bone width, the vertical reduction of bony walls is similar. This means that factors other than the thickness of remaining bone must play important roles in bone tissue remodeling.

ALVEOLAR RIDGE RESERVATION TECHNIQUE AND GRAFTING MATERIALS

Alveolar ridge preservation (ARP) is a guided bone regeneration (GBR) application at the time of tooth extraction to control bone resorption. The ARP technique follows the principles of GBR; however, the goal of ARP differs from that of GBR. GBR specifically, is performed when the remaining alveolar ridge is markedly deficient due to developmental defects, advanced periodontal disease, traumatic injury and surgical trauma. On the other hand, ARP is indicated after extractions to preserve original ridge dimensions and contours (hard and soft tissues), when immediate implant placement is not possible. Both techniques allow for bone augmentation, making it
possible to prepare sites for implants in areas otherwise impossible to utilize. However, the outcome of these procedures is not always predictable. The degree of bone loss varies among individual subjects and between anatomic sites. Preservation of the residual alveolar ridge starts with an atraumatic extraction. Most extraction techniques include the use of periotomes. Periotomes are used to perform the extraction of the tooth with minimal bone damage. After local anesthesia is achieved, rupture of the supracrestal attachment apparatus is performed with a sharp instrument such as a 15-C scalpel. Periotomes are then used to sever the subcrestal attachment apparatus. First, the instrument is used to complete rupture of the gingival fibers at the cervical area of the tooth. The blade should be angled at a 20 degrees to ensure that the tip of the periotome is within the crest of the alveolar bone and not out of the ridge. The instrument is then inserted first in the gingival sulcus and then in the periodontal ligament space. The periotome is moved repeatedly in a mesio distal direction, along the circumference of the root. It is possible to reach up to tow thirds of the root length by repeating this movement many times. After finishing this procedure, the tooth remains attached to the alveolus only by the most apical part of the periodontal ligament. Finally, the extraction of the tooth is performed with forceps without having to distort or damage the alveolar bone.

If all the walls of the socket were preserved but immediate implant placement is not indicated, ARP procedure needs to be performed. After cleaning the socket thoroughly, the bone graft material is packed in the socket. The technique includes the use of a resorbable or non resorbable membrane.

The techniques for alveolar ridge preservation were introduced in the 1980s using hydroxyapatite in the form of root-shaped cones. Although the technique was successful in terms of ridge preservation, problems with soft tissue encapsulation and the resultant exfoliation of the cones led to the abandonment of this technique in favor of particulate hydroxyapatite materials. Clinical evaluation of these materials again indicated successful outcomes in terms of bone preservation; however, problems such as particle migration and loss prevented the widespread acceptance of the procedure.

Current methods to prevent ridge resorption include the use of particulate alloplasts, xenografts, autografts, allografts, and membranes manufactured from various
materials, including those that are bioabsorbable or non-resorbable, naturally derived or synthetic.\textsuperscript{15, 18, 32} Most of these materials have been shown to be osteoconductive, providing a scaffold for the osteoblasts to migrate and form bone.

Alloplasts include bioactive glass, synthetic forms of calcium phosphate, and dense and porous hydroxyapatite\textsuperscript{33} and other similar materials\textsuperscript{34, 35} With the use of this synthetic bone graft materials the risk for disease transmission and need for harvesting bone tissue are eliminated. However, these materials are osteoconductive and have no intrinsic potential for osteogenesis or induction\textsuperscript{36}

Xenografts, typically of bovine origin, have successfully been used for alveolar ridge preservation\textsuperscript{22, 37, 38} Although the use of these grafting materials leads to predictable positive outcomes, the long time necessary for these materials to be replaced by mature bone is a possible disadvantage.\textsuperscript{18, 39} Barone et al\textsuperscript{38}, in a 7-month randomized clinical trial, confirmed that using porcine bone in combination with collagen membrane limited bone resorption after tooth extraction compared to extraction alone. Histologically, this study showed significantly higher trabecular bone formation and total mineralized tissue in grafted sites compared to extraction sites. On the other hand, using the same grafting material, Ersanli et al\textsuperscript{40} conducted a study to analyze histologically the healing after bone augmentation in the upper and lower jaw. The authors concluded that the maturation of hard tissue graft material occurs over a longer period of time in the upper jaw compared to the lower jaw. Berglundh & Lindhe\textsuperscript{41} showed that if once embedded into the mature bone matrix the bone graft will be included and partake in the physiological remodeling process. It was hypothesized that the consistency and slow resorption of the grafting material could have been the responsible for the results after ARP.

Autologous bone, when available, is probably the material of choice. However autografts have many limitations. These limitations include donor site morbidity, potential resorption and inadequate volume of graft material. Allografts have the advantage of being available in higher quantities and eliminate the need of a second
surgical site. Their use has been reported for several applications such as sinus augmentation, ridge augmentation and socket preservation.

Mineralized (FDBA) and demineralized freeze-dried bone allografts (DFDBA) are good substitutes for autologous bone. Demineralized freeze-dried bone (DFDBA) and mineralized freeze-dried bone allograft (FDBA) have been used in extraction sites because of their osteoconductive nature and the fact that they will resorb and be replaced within a relatively short period of time.

FDBA has been demonstrated to be a successful osseous grafting material. This material provides an osteoconductive scaffold and resorbs when implanted in mesenchymal tissues. DFDBA provides an osteoconductive surface as well as a source of osteoinductive factors. Blumenthal et al showed the high osteoinductive effect of DFDBA when compared to blood coagulum, bone blended and FDBA. However, some investigators have reported poor clinical results with the use of DFDBA. Because FDBA is still mineralized, it may have better physical characteristics. However, it lacks of an osteoinductive property.

A variety of non-resorbable and bioabsorbable barrier membranes has been used in bone augmentation procedures. The choice of membrane depends on the required duration of membrane function. These devices are biocompatible, function as a space maintainer and exclude the undesired cell ingrowth. Human and animal studies have demonstrated that up to 4mm of vertical augmentation was possible without the use of any grafting material under non-resorbable membranes. However, because of lack of rigidity, most of the bioabsorbable membranes must be used in combination with a graft material for space maintenance.
FACTORS AFFECTING THE OUTCOME OR ALVEOLAR RIDGE PRESERVATION

As stated before, the principles of guided bone regeneration must be followed to achieve good results. These include generating a blood supply, maintaining a stable protected space for bone growth; and achieving tension-free flap closure. Any movement during healing results in disruption of the blood supply and a change in the type of tissue formed in the site from bone to connective tissue.

Zitzman et al. 49 in a study of the factors affecting the success of guided bone regeneration around implants, found that several factors may influence the outcome of GBR. However, the jaw location was the only one that showed a clear evidence of influence in the process. Defect sites around maxillary implants showed significantly more bone fill (96%) compared to those in the mandible (78%).

Other studies 43, 49 pointed to the graft material employed and the micro movement of the bioabsorbable membrane during healing as important factors that could explain the significant loss of augmented bone during the GBR healing procedures.

Membrane stability seems to be a crucial a factor for a positive treatment outcome. No or only minimal bone formation seems to take place exceeding the existing bony walls, as was demonstrated in previous studies. 10, 50 Vertical defects of the buccal wall are the exception to this. In cases in which these defects are entirely limited to the buccal wall, the height of the proximal bone margins seems to be the determining factor for the degree of possible regeneration. This may be due to the fact that the membrane is supported up to this level. However, a prospective clinical study performed in humans, showed that 12 months following extraction, the bone level at the extraction site, rather than the bone level of the adjacent teeth, dictates the level to which the bone crest heals.
Lekovic et al \cite{10} used direct measurements between the alveolar ridge crest and fixed reference points and found a statistically significant reduction in bone loss when using membranes alone or in combination with particulate materials. The average loss of alveolar height and width in sockets that were left to heal with only a membrane covering them was 0.38 mm and 1.32 mm, respectively; considerably less than the average loss in sockets that healed naturally (1.5mm and 4.56 mm respectively); considerably less than the average loss in sockets that healed naturally. In this study, the authors included non-resorbable and bioabsorbable materials with and without particulate augmentation materials.

Another study compared a non-resorbable membrane to a bioabsorbable collagen barrier in 84 defects \cite{51}. The results demonstrated that 44% of the non resorbable membranes had to be removed prematurely. Overall, less bone fill was achieved with the non resorbable membrane/xenograft compared to the collagen membrane/xenograft group (78% vs 92%).

It has been previously shown that use of a membrane in conjunction with a ridge augmentation procedure produced loss of soft tissue thickness, thus diminishing the combined hard and soft tissue ridge width. \cite{52} The loss of soft tissue thickness is most likely due to interference with flap vascularity by the membrane and graft. Since the membrane is interposed between the flap and the bone surface, the vascular supply for the flap comes only from the flap base rather than the dual blood supply from the underlying osseous bed and flap base found in normal (non-grafted) sites.

**FREEZE DRIED BONE ALLOGRAFT FOR ALVEOLAR RIDGE PRESERVATION PROCEDURE**

There is a diversity of opinion regarding which particulate allograft material should be used for a specific clinical procedure. Because bone contains organic and
inorganic material, the cellular reaction to the processed mineralized bone is dependent on the specific method of processing \(^53\).

FDBA and DFDBA are both harvested from cadavers in the same manner; but, DFDBA undergoes the additional step of decalcification \(^54\). The decalcification process will expose bone morphogenetic proteins (BMPs). DFDBA, therefore, is considered to be osteoinductive, as well as osteoconductive. However, the amount of BMP found in DFDBA has been shown to be variable and its clinical significance has been challenged \(^48, 48\).

FDBA has been demonstrated by investigators to be a successful osseous grafting material \(^15, 44, 55\). Becker et al \(^56\) showed that mineralized bone obtained from a bone bank without removal of the organic matrix may not have an adverse antigenic response when implanted. Moreover, animal \(^57\) and human \(^58\) studies report markedly attenuated antigenicity associated with FDBA obtained from cortical tissue. Besides, as cortical bone contains more collagenous matrix than cancellous bone \(^59\), FDBA derived from a cortical source may have a greater osteogenic potential than the allograft derived from cancellous bone.

In a comparative study using FDBA or DFDBA for local ridge augmentation and sinus augmentation, histologic observations showed regeneration of approximately 42% new bone with no statistical difference between the two materials \(^42\). On the other hand, Piatelli et al \(^60\) performed a histological and histochemical study in man to compare bone regeneration with FDBA and DFDBA. The histological results demonstrated that mineralization could be seen only in the DFDBA particles that were near to the host bone. On the other hand, even the particles of FDBA that were farthest from the host bone, were lined by osteoblasts. Active secretion of osteoid was also seen in these samples. Nevertheless, no osteoinduction was observed with FDBA or DFDBA.

Iasella et al \(^15\) studied the effect of ridge preservation with FDBA in preventing post-extraction resorptive changes. Clinical and histologic parameters were evaluated
during a 6-month period. The results showed that there was 1.6 mm more width reduction in the group of extraction alone as compared to the group where FDBA and membrane was used. The authors also found that the maxillary sites lost more width than mandibular sites. Moreover, the extraction group lost height whereas the socket preservation group gained height (mean difference of 2.2 mm). They concluded that the most predictable maintenance of ridge width, height, and position was achieved when a ridge preservation procedure was employed.

Feuille et al \(^ 6\) evaluated the clinical and histologic efficacy of particulate, mineralized, cortical FDBA in conjunction with a membrane for localized ridge augmentation prior to implant placement. They observed a mean ridge width increase of 3.2 mm at the 6-month reentry. There were no differences in height between baseline and reentry. Histologic analysis showed few graft particles encapsulated in fibrous tissue. The new bone appeared viable. The mean percentage of new bone was approximately 47%. These authors concluded that the use of FDBA in bone augmentation procedures can lead to predictable outcomes.

The working hypothesis for this study was:
The anatomical location affects the clinical and histological outcome of healing following ARP.

The following specific aims were structured to test this hypothesis:
1- To evaluate the clinical outcome of ARP at different anatomical locations.
2- To evaluate histologically the amount and quality of new bone formation.

The significance of this study was that the data generated in this study would be helpful to compare treatment outcome following ARP at different anatomical locations and could also be useful in determining possible limitations of this commonly used surgical procedure.
CHAPTER 2

MATERIALS AND METHODS

Patient Selection and Study Design:

Thirty-six patients treatment planned for extraction and placement of a single dental implant at maxillary or mandibular posterior sextant were recruited from the patient pool of the Graduate Periodontology Clinics at the Ohio State University between January 2007 and May 2009. Inclusion criteria were: 1) adult; 2) a single molar or premolar tooth in maxillary or mandibular sextant, scheduled for extraction and implant placement with intact adjacent teeth, 3) systemically healthy, 4) non-smoker; 5) periodontally healthy; 6) able and unwilling to comply with study procedures and visits. Exclusion criteria were: 1) any systemic diseases that may affect healing process; 2) no natural teeth adjacent to single implant sites; unable or unwilling to comply with study procedures and visits. Exit criteria were: 1) voluntary withdrawal; 2) non-compliance with study procedures and visits; 3) development of systemic or oral diseases asked in exclusion criteria. The study protocol and informed consent forms were approved by the Institutional Review Board of the Ohio State University. All participants signed a written
consent prior to entry into the study. The study design was a prospective observational clinical trial. Subjects were recruited and designated to one of two groups based on the anatomical location of the tooth scheduled for extraction.

Sequence of Clinical Data Collection

Appointment 1 (Implant consult)

* Obtained Consent
* Record Probing Depth, Clinical Attachment Level, Gingival Index and Plaque Index of the test tooth and the teeth mesial and distal to it.
* Take two impressions. One for baseline clinical measurements and one to prepare stent.

Appointment 2 (Tooth Extraction)

* Use surgical guide to measure the residual ridge height.
* Use periodontal probe to measure the bucco-lingual and mesio-distal width of the socket and the ridge.
* Use caliper to measure the thickness of the buccal and lingual plates.
* Measure root length and root diameter.
* Document surgery details.

Appointment 3 (Implant placement)

* Take final impression.
* Use surgical guide to measure height of residual ridge.
* Use periodontal probe to measure the bucco-lingual and mesio-distal width of the ridge.
* Obtain bone core.
* Document surgery details.
**Surgical technique:**

An alginate impression was taken prior to tooth extraction and two stone casts were prepared; one for baseline clinical measurements and one to prepare surgical stent. The stent was fabricated with standardized 3” aluminum tubes integrated into a 0.060” thermo-forming material. It was used for both standardization of clinical measurements during extraction and implant placement surgery. Following establishment of local anesthesia, the tooth was atraumatically extracted after reflection of full thickness mucoperiostal flaps. After degranulation of the extraction site, an indication for socket preservation procedure was given based on residual intrabony walls, presence of dehiscence and/or fenestration, and the size of the alveolar socket diameter. The flaps were released, as needed, to achieve primary wound closure. Freeze Dried Bone Allograft and collagen membrane were placed and flaps were sutured with interrupted sutures. Peridontal dressing was not applied. Patients were given prescription for antibiotics for 7 days and 0.12% chlorhexidine rinse twice a day for 2 weeks. At the end of the 4-month study period, mucoperiostal flaps were again reflected in the treated sites. Clinical measurements were repeated at the time of implant placement by using the same stent.

**Clinical Parameters:**

Each subject received complete periodontal examination to determine their periodontal health status. Probing depth, gingival recession, gingival and plaque indices were documented for the surgical quadrant prior to tooth extraction. Clinical attachment level (CAL) was calculated for six surfaces per tooth for the tooth assigned for extraction and, for the mesial and distal adjacent teeth.

A preliminary study was conducted to calibrate the clinician involved in documenting the clinical parameters obtained with the stent in place. Based on this

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i K & S Engineering, Chicago, IL, USA
ii FDBA ©DENTSPLY Tulsa Dental. Tulsa, Oklahoma, USA
iii Biomend Extend. ©2009 Zimmer Dental Inc, Carlsbad, California, USA
preliminary study intra-examiner differences for various clinical measurements were within 1 mm with no statistically significant difference between any of the repeated readings performed by the same clinician (p>0.05; Repeated Measures ANOVA).

Measurements of alveolar ridge width and height (the distance from occlusal plate to alveolar crest) were taken immediately after tooth extraction and prior to implant placement by the same investigator using the stent. Similarly, the distance from buccal bone plate to lingual bone plate and the distance from the distal root surface of mesial adjacent tooth to the mesial root surface of distal adjacent tooth were measured immediately after tooth extraction and prior to implant placement. Similar measurements were repeated outside the oral cavity by using a periodontal probe or caliper and stone casts prepared prior to tooth extraction and implant placement.

**Bone Core Sampling:**

Following an average of 4.5 months healing for graft material maturation (mean was 139 ± 7 days), reentry was performed for implant placement. An indentation mark was placed at the implant site with a round bur by using the surgical stent to determine the location of the implant and to retain the orientation of the bone sample. An internally irrigated trephine bur was used to obtain bone core samples (an average of 2.5 mm in diameter and 6-8 mm in length, cylindrical bone specimen). Samples were immediately frozen in liquid nitrogen and kept at -80°C until micro-CT scanning.

**Micro-CT Analysis:**

For quantitative three dimensional analyses of the bone core samples, cores were thawed and scanned parallel to their vertical axes in a 360 degree full scan. The Inveon® microCT\textsuperscript{iv} scanned at a resolution of 10.11 micron in all three spatial dimensions. A filter thickness of 1.5 mm of Al was used to account for some beam hardening. The Siemens proprietary 3D software package, Inveon Research Workplace, was used to

\textsuperscript{iv} Siemens Medical Solutions, USA, Pennsylvania
gather volumetric and density data. A commercial imaging software was used for structural analysis. A custom software and a special code were used to obtain CT attenuation number and mineralization values. The volume of interest (VOI) was drawn with a slice-based method starting from outside of the core moving dorsally. Microstructural indices were calculated directly from VOI (4.60±1.02 mm^3) for all specimens. Total amount of bone, new bone, grafted material; mineralization per unit and trabeculation was analyzed.

**Histological Analysis:**

Following micro-CT scan, cores were fixed in formalin, decalcified in a solution containing formaldehyde and formic acid, embedded in paraffin and sectioned at approximately 4 micron thickness. Three slides each carrying 6 sections were obtained from the mid-portion of the bone core, were stained with trichrome and analyzed under light microscopy. The apical, mid and coronal portion of each section were documented at 5X magnification with digital pictures. A grid of 10 equally sized and spaced lines dividing each picture into 100 regions was used to measure the surface area occupied by residual graft material, new cellular bone and nonmatured tissue. Similarly, software was used to calculate the same parameters. Data obtained by naked eye and by software were compared for differences.

**Statistical Analysis:**

Computer software was used to conduct the statistical analysis. Between-group differences at baseline and at 4 months re-entry were analyzed by the exact Mann-Whitney U test. Within-group differences for each parameter between baseline and at 4 months re-entry were analyzed by the exact Wilcoxon signed-rank test. The correlation between initial clinical parameters and final alveolar bone ridge height and width.

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vi Axiovision software, © Carl Zeiss MicroImaging, Inc., Thornwood, New York, USA
vSPSS Inc. Chicago, Illinois, USA
measurements was analyzed by using the Spearman correlation analysis. The level of statistical significance was set at $\alpha=0.05$. 
CHAPTER 3

RESULTS

Study Population

Twenty-one patients completed the study. Information related to patient population is presented in Table I. The mean age in maxilla group was 49 ± 4 years while it was 52 ± 5 years in mandible group. Gender distribution was similar between two groups with female subjects establishing most of the study population (16 female and 5 male). Each patient provided one single implant site except one patient in maxilla group had two sites. Most of the maxillary teeth scheduled for extraction were premolars (5 first premolar, 2 second premolar and 2 first molar) while most of the mandibular teeth scheduled for extraction were molars (1 first premolar, 2 second premolar, 9 first molar and 1 second molar). Most of the patients were periodontally healthy with main reason for extraction being non-restorable tooth due to severe decay. Only two cases in mandible group had tooth extraction due to tissue lost to periodontal disease. Based on surgeon’s classification (1 being easy; 4 being extremely difficult), the extraction was difficult in 3 out of 9 maxillary sites and none in mandibular sites (data not shown). Septum was intact following extraction in 5 maxillary and 9 mandibular sites. A dehiscence following extraction was noted in 2 maxillary and 4 mandibular sites while a
fenestration was present in 1 maxillary site (data not shown). Membrane exposure following alveolar ridge preservation procedure was a common finding (8 out of 9 sites in maxilla group and 11 out of 13 sites in mandible) (Table I). The exposure ranged between 1 to 7 mm with a mean 2.2±0.2 mm (data not shown). The mean healing time following alveolar socket preservation was 139±7 days (138 ± 14 days for maxilla and 139 ± 6 days for mandible) (Table I). At four months re-entry, all sites had enough bone volume to place an implant. The mean implant diameter and length were 3.9 ± 0.2 mm and 12.3 ± 0.3 mm for maxillary sites while they were 4.2 ± 0.2 mm and 11 ± 0.3 mm for mandibular sites. One site in maxilla and one site in mandible required additional bone graft placement due to dehiscence occurred during implant placement.

Clinical Treatment Outcome

Initial clinical measurements were performed immediately following tooth extraction. Based on these measurements the mean initial distance from occlusal surface of the stent to residual alveolar crest (S-Ac) in maxilla was 10.2±0.7 mm on the mesial (MS-Ac), 10.2±1.0 mm on midline (TS-Ac) and 10.3±0.5mm on the distal (DS-Ac) aspect of the extraction site. Same initial measurements for mandible were 11.8±0.9 mm, 11.4±0.6 mm and 11.7±0.6 mm, respectively. There was no statistically significant difference between two groups for these initial measurements (Mann-Whitney Test; P>0.05). Similar initial measurements for buccal-palatal (buccal-lingual) [B-P and B-L] and mesial-distal [M-D] distances and, B-P [B-L] and M-D defect sizes were performed. The mean initial B-P and M-D distances for maxillary sites were 11.2±0.6 mm and 10.8±0.7 mm, respectively. The B-L and M-D distances for mandibular sites revealed a mean 11.3±0.7 mm and 12.3±0.8 mm, respectively. There was no statistically significant difference between two groups for these initial measurements (P>0.05). M-D and B-P defect sizes following extraction in maxillary sites were 6.7±0.6 mm and 8.4±0.4 mm, respectively. Same defect sizes in mandibular sites were 9±0.6 mm and 8.7±0.5 mm, respectively. The difference between initial M-D defect size in maxillary and mandibular
sites was statistically significant (P=0.051). The mean root length and root diameter for extracted maxillary teeth were 13.1±0.7 mm and 6.8±0.5 mm, respectively, while these were 13.7±0.5 mm and 8.5±0.4 mm for extracted mandibular teeth. The difference between maxillary and mandibular root diameters was statistically significant (P=0.023). Mean buccal bone thickness at the crest was 1.6±0.2 mm for maxillary sites and 1.4±0.2 mm for mandibular sites. Mean palatal (lingual) bone thickness at the crest was 1.3±0.2 mm for maxillary sites and 2.1±0.4 mm mandibular sites. The differences between groups were not statistically significant (P>0.05).

The data related to clinical measurements obtained at the time of extraction and four months re-entry is presented in figures 1 and 2 (maxilla) and figures 3 and 4 (mandible). Initial and re-entry measurements for maxillary alveolar ridge height were as following: MS-Ac 10.2±0.7 mm versus 11.8±0.9 mm; TS-Ac 10.2±1.0 mm versus 11.4±0.6 mm; DS-Ac 10.3±0.6 mm versus 11.7±0.6 mm. These changes observed in maxillary alveolar ridge height were not statistically significant (Figure 1; Wicoxon Signed Ranks Test, P>0.05). Initial and re-entry measurements for mandibular alveolar ridge height were as following: MS-Ac 11.8±0.9 mm versus 10.3±0.5 mm (P=0.089); TS-Ac 11.4±0.6 mm versus 10.7 mm (P=0.264); DS-Ac 11.7±0.6 mm versus 10.3±0.3 mm (P=0.025) (Figure 3). Thus, the only statistically significant difference was at the distal aspect of the extraction site with an approximately 1.4 mm gain in bone height at 4 months re-entry.

The changes in alveolar ridge width are presented in figure 2 (maxilla) and figure 4 (mandible). Initial and re-entry measurements for maxillary alveolar ridge were as following: B-P distance 11.2±0.6 mm versus 8.8±0.6 mm (P=0.02); M-D distance 10.8±0.7 mm versus 9.4±0.8 mm (P=0.125). Thus, there was statistically significant bone resorption in buccal-palatal distance for maxillary sites (figure 2). Initial and re-entry measurements for mandibular ridge width were as following: B-L distance 11.3±0.7 mm versus 8.8±0.7 mm (P=0.03); M-D distance 12.3±0.8 mm versus 13.5±0.7 mm.
(P=0.027). Thus, there was a statistically significant bone resorption in buccal-lingual distance for mandibular sites (figure 4). But, there was also a statistically significant increase in mesial-distal distance meaning that adjacent teeth probably shifted during healing period. The measurements related to alveolar ridge width were repeated on the casts obtained prior to tooth extraction and at prior to implant placement. The results for maxillary and mandibular sites were similar to actual clinical measurements obtained directly from patient during surgery except that M-D distance difference for mandibular sites was not statistically significant (For maxilla; B-P distance 11.4 ± 0.3 mm versus 9.9±0.5 mm [P=0.016]; M-D distance 9.9±1.0 mm versus 9.3±0.8 mm [P=0.257]); for mandible B-L distance 11.1±0.6 mm versus 8.9±0.5 mm [P=0.001]; M-D distance 12.5±0.7 mm versus 12.6±0.7 mm [P=1.000]. Thus, the possible tooth movement noticed at re-entry may not be real.

**Possible factors affecting treatment outcome**

Initial clinical parameters including the amount of keratinized tissue, probing depth, gingival recession and clinical attachment level (CAL) together with extracted tooth length and diameter and, residual bone thickness at buccal and palatal (lingual) aspect of extraction site were investigated as possible factors affecting clinical treatment outcome. Spearman correlation coefficient and related p values are presented in Table II (maxilla) and Table III (mandible). There was a statistically significant correlation between the amount of initial clinical attachment loss at the buccal mesial of the extracted maxillary tooth and the interproximal distance between occlusal surface of the plate to the crest at re-entry (MS-Ac and DS-Ac at re-entry) (Table II; Spearman Correlation r=0.747, p=0.021 and r=682, p=0.043, respectively). There was also a statistically significant correlation between residual palatal bone thickness and M-D distance at re-entry for maxillary sites (r=0.797, p=0.01). In addition, there was statistically negative correlation between root length and the distance between occlusal surface of the stent to the crest at re-entry (DS-AC at re-entry; r=−0.752, =0.019). There was also a statistically
significant negative correlation between the initial amount of keratinized tissue at the distal-buccal of extraction site and B-P distance at re-entry (r=−.762, p=0.017) (Table II). The main factors affecting mandibular sites were buccal and lingual residual bone thickness which had statistically significant effect on M-D distance at re-entry (r=0.771, p=0.002 and r=0.811, p=0.001, respectively). Root length was also an effective factor in M-D distance at re-entry for mandibular sites and this effect was a negative correlation (r=−0.816, p=0.001). The effect of initial clinical attachment loss at mid-buccal of extracted tooth also negatively affected the distance between occlusal surface of the stent to the crest at the mid-buccal of the surgical site at re-entry (r=−0.562, p=0.046). There was no statistically significant correlation between initial probing depths and any of the clinical treatment outcome measurements for both maxillary and mandibular sites (p>0.05). Similarly, the amount of initial gingival recession did not affect clinical measurements at re-entry (p>0.05).

Micro Computer Tomography Evaluation

Micro CT results revealed no significant difference in trabeculation between maxilla and mandible (p>0.466). However, mineralization per unit volume was higher in newly forming bone than in residual bone graft in mandible (p<0.03), while this difference was not significant within maxilla (p=0.375).

Histometric Analysis

The mean percentage of residual graft material with no new bone formation around it was 5.3±3.3% and 7±3.8% in apical and coronal parts of maxillary bone cores, respectively (Wicoxon signed ranks test, P=0.078). When similar analysis was conducted for percentage of graft and new bone formation around it, very similar percentages were found between apical and coronal sections of maxillary bone cores (26.3±5 and 29.1±4.8; P= 0.820). Percentage of new cellular bone was 11±2.4 for apical sections and 11±3.3% for coronal sections (P=1.000). In addition, there was no statistically significant
difference for the percentage of non-matured tissue for these sections (18.5±4 and 27±5; P=0.098).

For the mandibular sections, the mean percentage of residual graft material with no new bone formation around it was 2.3±0.9 and 2±0.5% in apical and coronal parts of mandibular bone cores, respectively (P= 0.234). Similar analysis for percentage of graft and new bone formation around it revealed 2.8±4.4% in apical section and 23±5% in coronal section (P=0.653). Percentage of new cellular bone was 12% for both apical and coronal parts of the mandibular bone core. Also, there was no statistically significant difference for the percentage of non-matured tissue for these sections in mandible (23±3 and 21±5; P=0.310).

Mann-Whitney test was performed to compare sections obtained from same region in maxilla and mandible. There was no statistically significant difference between maxillary and mandibular bone cores for the four parameters included into this study (P>0.05).
<table>
<thead>
<tr>
<th></th>
<th>MAXILLA</th>
<th>MANDIBLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>GENDER</td>
<td>1 MALE</td>
<td>4 MALE</td>
</tr>
<tr>
<td></td>
<td>7 FEMALE</td>
<td>9 FEMALE</td>
</tr>
<tr>
<td>AGE</td>
<td>49 ± 4 yrs (mean ± s.e)</td>
<td>52 ± 5 yrs (mean ± s.e)</td>
</tr>
<tr>
<td>RACE</td>
<td>1 AFRICAN AMERICAN</td>
<td>2 ASIAN</td>
</tr>
<tr>
<td></td>
<td>8 CAUCASIAN</td>
<td>11 CAUCASIAN</td>
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<tr>
<td>ANATOMICAL LOCATION</td>
<td>7 premolar</td>
<td>3 premolar</td>
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<tr>
<td></td>
<td>2 molar</td>
<td>10 Molar</td>
</tr>
<tr>
<td>REASONS FOR TOOTH EXTRACTION</td>
<td>4 FRACTURE</td>
<td>2 FRACTURE</td>
</tr>
<tr>
<td></td>
<td>1 ENDODONTAL</td>
<td>2 ENDODONTAL</td>
</tr>
<tr>
<td></td>
<td>0 PERIODONTAL</td>
<td>2 PERIODONTAL</td>
</tr>
<tr>
<td></td>
<td>4 NON-RESTORABLE</td>
<td>7 NON-RESTORABLE</td>
</tr>
<tr>
<td>HEALING TIME</td>
<td>138 ± 14 days (mean ± s.e)</td>
<td>139 ± 6 days (mean ± s.e)</td>
</tr>
<tr>
<td>NEED FOR SECOND PROCEDURE</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MEMBRANE EXPOSURE</td>
<td>7</td>
<td>10</td>
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Table I. Demographics
Figure 1- Changes in maxilla alveolar ridge height pre and post ARP.
MS-Ac= Distance between the stent and the mesial aspect of the extraction site.
TS-Ac= Distance between the stent and the mid facial aspect of the extraction site.
DS-Ac= Distance between the stent and the distal aspect of the extraction site.
Figure 2- Changes in maxillary alveolar ridge width pre and post ARP.

B-P= Bucco palatal width.

M-D= Mesio distal width.
Figure 3- Changes in mandibular alveolar ridge height pre and post ARP.

MS-Ac= Distance between the stent and the mesial aspect of extraction site.
TS-Ac= Distance between the stent and the mid facial aspect of the extraction site.
DS-Ac= Distance between the stent and the distal aspect of the extraction site.
**Figure 4-** Changes in mandibular alveolar ridge width pre and post ARP.

B-L= Bucco lingual width.

M-D= Mesio distal width.
<table>
<thead>
<tr>
<th></th>
<th>TEST TOOTH CAL Mesial</th>
<th>TEST TOOTH CAL Mid</th>
<th>TEST TOOTH CAL Distal</th>
<th>ROOT LENGTH</th>
<th>ROOT DIAMETER</th>
<th>BUCCAL BONE THICKNESS</th>
<th>LINGUAL BONE THICKNESS</th>
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</thead>
<tbody>
<tr>
<td><strong>MAXILLA SPEARMAN CORRELATION</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>MS-AC distance at re-entry</td>
<td>0.747 0.021</td>
<td>0.146 0.707</td>
<td>0.471 0.201</td>
<td>-0.250 0.516</td>
<td>0.109 0.780</td>
<td>-0.028 0.942</td>
<td>-0.053 0.892</td>
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<td>TS –AC distance at re-entry</td>
<td>0.350 0.356</td>
<td>0.028 0.942</td>
<td>-0.169 0.664</td>
<td><strong>-0.752 0.019</strong></td>
<td>0.018 0.964</td>
<td>0.083 0.832</td>
<td>-0.010 0.979</td>
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<td>DS-AC distance at re-entry</td>
<td><strong>0.682 0.043</strong></td>
<td>0.247 0.522</td>
<td>0.222 0.566</td>
<td>-0.602 0.086</td>
<td>-0.142 0.716</td>
<td>0.231 0.551</td>
<td>0.134 0.731</td>
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<tr>
<td>B-P distance at re-entry</td>
<td>-0.197 0.611</td>
<td>-0.190 0.625</td>
<td>-0.222 0.566</td>
<td>0.044 0.910</td>
<td>0.611 0.081</td>
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<tr>
<td>M-D distance at re-entry</td>
<td>0.333 0.381</td>
<td>0.155 0.690</td>
<td>0.370 0.327</td>
<td>-0.193 0.619</td>
<td>-0.320 0.401</td>
<td>0.334 0.380</td>
<td><strong>0.797 0.010</strong></td>
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</table>

**Table II- Maxilla Correlations. Pre-operative and operative measurements**

MS-AC distance = Distance between the stent and the mesial aspect of the residual alveolar crest at re-entry.

TS-AC distance = Distance between the stent and the mid facial aspect of the residual alveolar ridge at re-entry.

DS-AC distance = Distance between the stent and the distal aspect of the residual alveolar ridge at re-entry.

B-P= Bucco palatal distance.

M-D= Mesio distal distance.

Test tooth = Extracted tooth.
### Table III - Mandible Correlations. Pre-operative and operative measurements

<table>
<thead>
<tr>
<th>MANDIBLE SPEARMAN CORRELATION</th>
<th>TEST TOOTH CAL Mesial</th>
<th>TEST TOOTH CAL Mid</th>
<th>TEST TOOTH CAL Distal</th>
<th>ROOT LENGTH</th>
<th>ROOT DIA.</th>
<th>BUCCAL BONE THICKNESS</th>
<th>LINGUAL BONE THICKNESS</th>
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<tr>
<td>MS-AC distance at re-entry</td>
<td>0.019</td>
<td>-0.003</td>
<td>-0.237</td>
<td>0.050</td>
<td>0.352</td>
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<td></td>
<td>0.950</td>
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<td>0.239</td>
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<tr>
<td>TS–AC distance at re-entry</td>
<td>-0.366</td>
<td>-0.562</td>
<td>-0.457</td>
<td>-0.015</td>
<td>0.153</td>
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<td></td>
<td>0.218</td>
<td>0.046</td>
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<td>0.961</td>
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<td>DS-AC distance at re-entry</td>
<td>0.049</td>
<td>-0.127</td>
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<td>0.490</td>
<td>-0.112</td>
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<td></td>
<td>0.875</td>
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<td>B-Ldistance at re-entry</td>
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<td>M-D distance At re-entry</td>
<td>0.118</td>
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<td>0.001</td>
<td>0.102</td>
<td>0.002</td>
<td>0.001</td>
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</table>

**MS-AC distance =** Distance between the stent and the mesial aspect of the residual alveolar crest.

**TS-AC distance =** Distance between the stent and the mid facial aspect of the residual alveolar ridge.

**DS-AC distance =** Distance between the stent and the distal aspect of the residual alveolar ridge.

**B-L =** Bucco lingual distance.

**M-D =** Mesio distal distance.

Test tooth = Extracted tooth
Figure 5- Micro Computerized Tomography.
Figure 6. Micro-CT analysis
Figure 7. Histological analysis of bone cores
Figure 8. Maxilla- Histometric analysis of bone cores
Figure 9. Mandible- Histometric analysis of bone cores.
Figure 10. Sample stained with Trichrome
CHAPTER 4

DISCUSSION

The patients in the study were randomly selected based on their need for extraction and preservation of the alveolar ridge prior to implant placement. The subjects were in their fourth or fifth decade. Age, as a factor in the healing of extraction wounds has been previously studied. Amler et al\(^{62, 63}\) in studies about the healing process, demonstrated that after 10 post-extraction days, the tissues of the younger patients (second decade or less) undergo progressive healing and osteoid formation, while the tissues of the older patients (sixth decade or over) remain in a resting (lag) phase. Following approximately three weeks, the older tissues entered into an accelerated growth cycle, and by 30 to 40 days equaled the healing pattern of the younger tissues. Taking into account that the healing period in this study was 4 months, thus, it may not have a significant effect on treatment outcome.

The gender distribution was similar between groups; however, the study population was formed mainly by middle aged females. Since most women go through menopause, in their late 40s or 50s, it can be implied that these patients were menopausal or postmenopausal. It has been widely proved that after menopause, there is collagen loss and reduced capillary blood flow\(^{64-66}\). It has also been hypothesized\(^{67}\) that sex hormones
influence wound healing rates, possibly through their modulating effects on inflammation. Engeland et al. 68 showed that the deficits in mucosal wound healing which have been reported in older women involve post-menopausal changes. They suggested that testosterone might impact upon the proliferative phase of healing which involves immune processes such as re-epithelialization and angiogenesis. August et al 69 showed that the maxilla is particularly more susceptible to resorption and atrophic changes caused by both metabolic and mechanical factors because of its largely trabecular composition. This fact often complicates implant placement in the atrophic maxilla and is compounded by the effects of estrogen withdrawal and loss of mineral density. The mandible consists mainly of cortical bone, even in the broad body region, and therefore would be expected to show less osteopenic changes in postmenopausal women.

Other studies have also demonstrated that the healing rates seemed to improve when women used hormone replace therapy (HRT). 70. However, none of the female subjects in this study reported being on hormonal replacement therapy. It is well accepted that delays in wound closure lead to increased risk of infection and poorer healing outcomes. Therefore, the magnitude of these effects may have clinical implications, especially if combined with other risk factors for slowed healing.

The patients in this study were non-smokers. Tobacco usage was found to negatively affect bone grafting procedures. Smoking impairs the revascularization of the bone in regenerative procedures, 71, 72 mainly due to its effect on vasoconstriction of the artery. 73, 74. The subsequent decrease in blood supply further contributed to the retardation of graft integration. Moreover, the altered oral flora from smoking increased the infection rate by 2 to 3 times in smokers, which adversely influenced the complications of periodontal procedures, including any type of regeneration. Levin et al. 75 stated that nicotine, carbon monoxide, and hydrogen cyanide from smoking are possible risk factors that leads to poor wound healing. This, in turn, may jeopardize bone grafting and implant placement surgeries. Patient with a history of smoking had 50% of complication rate in monocortical onlay graft whereas only 23.1% for the nonsmokers as showed in a study by Li et al. 76. Interestingly, maxillary bone was more susceptible to
adverse reactions of tobacco, reaching a failure rate of 1.6 times as much compared with mandible undergoing the same periodontal procedures in smokers. Furthermore, it has been shown that bone loss in smokers can be 4 times as much as nonsmokers. Such bone loss was mainly due to over expression of interleukin-1, interleukin-6, and tumor necrosis factor (TNF). Therefore, non-smokers were selected in order to avoid a factor that could compromise or interfere with the normal healing of bone graft.

When the initial clinical measurements were analyzed, a statistically significant increase in the mesio-distal distance in the mandible was observed. However, when the measurements were repeated in the final cast this difference was not statistically significant. It can be assumed that mobility of the teeth adjacent to the extraction socket could have increased due to surgical trauma during the extraction. Thus, an accurate measurement of the mesio distal dimension could not be obtained. Moreover, the position of the tooth in the arch may have prevented an ideal access to the area.

The mesiodistal size of the mandibular defects as well as the mandibular root diameters was statistically significantly higher than the maxillary sites. This was expected since the study sample had more premolars in maxilla compared to mandible which had more molars.

The high rate of membrane exposure encountered in our study was considered a possible factor influencing the amount of bone loss. Resorbable membranes were used in all the cases. The membrane is designed to predictably remain intact for at least four weeks, functioning as a barrier during the critical period of wound healing. The average retention for the resorbable membrane used is six to seven weeks. It is then fully absorbed by host enzymes into the surrounding tissue within eight weeks. However, once the membrane is exposed to the oral environment, the membrane resorption rate by salivary enzymes action is faster. The membrane exposures found in this study may have occurred due to the presence of tension in the flaps when suturing. Postoperative membrane exposure may contribute to bacterial infection of the site compromising the healing process. The size of exposures in this study varied. In general, they were small in
size, none showed signs of infection, and none of the membranes have to be removed. Simon et al. 43 suggested that membrane exposures may be detrimental to bone regeneration. However, they showed that this effect was a result of disturbances caused by the periostal reflection required for membrane removal and not because the exposure per se. Since resorbable membranes were used in this study, there was no need for a second surgical procedure for membrane removal. On the other hand, Zubillaga et al. 78 found no significant detrimental effect of membrane exposure with respect to net loss of bone healing.

Another factor that may have an effect on the bone remodeling process after ARP is flap elevation. Flap elevation often results in poor blood supply, more bone loss, delayed wound healing, and compromise soft tissue appearance. Previous study showed that the order of magnitude of bone resorption caused by flap reflection alone is only about 1mm. 26 This study was based on non extraction sites. Nevertheless, since the receptive process is initiated by extraction, and will occur with or without flap reflection, the additional contribution of the flap may be minimal.

In this study, the healing time after ARP and before implant placement was 4.5 months on average. This schedule was assigned following a standard protocol 79 where a 4 to 6 month period is recommended. In a study in humans Hammerle et al. 80 found that after 12 weeks of GBR, specimens were entirely composed of soft tissue while specimens with a regeneration time of 4 months and more were composed of both soft and increasing amounts of mineralized tissue. Lang et al. 81 found that after 3-5 months of GBR, at membrane removal, bone regeneration varied between 3 to 5 months. However, Iasella et al. 15 showed minimal differences on the effect on vertical ridge height or horizontal ridge width changes between the 4- or 6-month healing time. It is generally assumed that, during the 4th to the 6th months after GBR-treatment, no additional regenerated bone volume is formed but rather the regenerated bone matures.

This study confirms previous human and animal reports that post-extraction healing involves loss of ridge width despite ARP. The greater loss is horizontal ridge
width occurs primarily at the expense of the buccal wall\textsuperscript{15, 18, 21, 82}. Following ARP, ridge height loss change was negligible (a loss of 0.37 ± 0.3mm in maxilla and a gain of 1.26 ± 0.3 mm in mandible) in this study. However, average ridge width loss was 2.44±0.71mm and 2.54±0.5mm in maxilla and in mandible, respectively. This is in disagreement with the results of the study by Feuille et al\textsuperscript{61} where it was reported an increase in mean alveolar ridge of 3.2mm when FDBA was used as a grafting material. However, in this study a non resorbable membrane was used as a barrier. The extraction defects in our study varied in morphology and no effort was made to select a predetermined osseous deformity. In the present study it was observed that the location and the size of the socket influenced the possible outcome. This is in accordance with the results of the study by Iasella et al.,\textsuperscript{15} in which a generally higher degree of bone loss in the maxilla was reported. Hoffmann et al\textsuperscript{1} also demonstrated a significant greater buccal-palatal bone loss in the anterior region compared to the posterior region in the maxilla and in the mandible. The relative and absolute bone loss was greater in the maxilla for the single sockets.

In maxilla, initial CAL at mesial surface of the extracted tooth had statistically significant effect on post-treatment interproximal bone height (r=0.747; p=0.021 and r=0.682; p-0.043 for mesial and distal interproximal sites, respectively) meaning that if the initial CAL was worst, the final bone height loss at maxillary interproximal areas was more. This is a clinically expected treatment outcome since alveolar bone height is always more difficult to regenerate. Several techniques are currently being used, including various vertical guided bone regeneration (GBR) procedures\textsuperscript{83-86}, alveolar distraction osteogenesis and onlay bone grafting\textsuperscript{86}. While it has been shown that it is possible to vertically augment bone with different techniques, the number of complications and failures of the augmentation procedure is still too high (well over 20\%)\textsuperscript{87}. Also, maxillary root length of the extracted tooth was negatively correlated with post-treatment mid-buccal bone height (r=−0.752; p: 0.019) meaning that if the root was long then the final alveolar bone height loss at mid-buccal was less. This finding may be interpreted as having a deeper intrabony defect with narrower angle; it has been well documented that Guided Tissue Regeneration Procedures performed to regenerate new
attachment around teeth have more predictable treatment outcomes when the defect has narrow angle with deeper intrabony component. Similarly, initial palatal bone thickness had statistically significant effect on post-treatment maxillary mesial-distal distance \((r=0.797; \ p=0.01)\) meaning that if the initial palatal bone thickness was preserved during extraction then it helped to preserve mesial-distal space. Otherwise, approximately \(1.2\pm0.7\) mm narrowing in maxillary M-D distance was observed.

In mandible, baseline CAL at mid-buccal site of extracted tooth was negatively correlated with post-treatment ridge height at the site \((r=-0.562, \ p=0.046)\) meaning that if the initial CAL was worst, the final bone height loss measured at this specific mandibular site was less. Similar correlation was not present for initial CAL detected at mandibular interproximal surfaces. Thus, the statistically significant effect at mid-buccal site may be due direct effect of periodontal CAL on post-extraction defect morphology creating a defect with more pronounced intrabony component instead of an initial horizontal defect.

Also, initial mandibular buccal and lingual bone thicknesses had statistically significant effect on post-treatment mesial-distal distance \((r=0.771; \ p=0.002, \ r=0.811; \ p=0.001)\) meaning that if the initial lingual and buccal bone thicknesses were larger in size this might help to preserve mesial-distal space. In general, a widening of \(1.15\pm0.45\) mm in M-D distance was observed in mandible. Similarly, root length of the extracted tooth had statistically significant negative effect on post-treatment mesial-distal distance \(r=0.816; \ p=0.001, \) respectively) meaning that if the mandibular root length was long, post-treatment M-D distance became narrower.

Study by Garn et al, showed a positive correlation between root length and mesiodistal diameters in the mandible, though to a low order of magnitude. Overall, the root-length mesiodistal diameter correlations approximate 0.12. Moreover, mesiodistal crown-size measurements apparently show higher correlations with the root lengths \((r = 0.13)\) than do the analogous buccolingual values \((r = 0.11)\) in this pilot study. Our study differs from these findings since a negative correlation was found between the root length and the mesio distal dimension at re entry in mandibular sites.
The traditional histological/histometric analysis generally requires fixation, decalcification and sectioning of the bone tissue. This, in turn, is critical when using small specimens since it causes to loose significant details. Micro-CT analysis was included into this study to analyze the original bone cores without any processing. This technique allowed to analyze the morphological characteristics of the original bone core and to determine mineralization, the amount of bone and bone graft material. Micro CT is recently introduced into the field of dental research. Micro CT results revealed no significant difference in trabeculation between maxilla and mandible (p>0.466). However, mineralization per unit volume was higher in newly forming bone than in residual bone graft in mandible (p<0.03), while this difference was not significant within maxilla (p=0.375).

Histometric analysis revealed similar distribution of residual biomaterial, new cellular bone and nonmatured tissue in maxilla and in mandible (P>0.05). There was no statistically significant difference between maxillary and mandibular bone cores for the four parameters included into this study.

Within the limits of this study, it appears that in different anatomical locations different factors may determine the clinical treatment outcome following ARP. Histometric and micro-CT treatment outcome evaluations results mostly reveal no statistically significant differences in regenerated/regenerating bone at the grafted site. The data generated in this study may help clinicians to make evidence-based decisions for ARP indications and to reach more predictable treatment outcome. Further studies are needed to better understand wound healing at the cellular level at grafted alveolar sockets and to determine clinical limitations of ARP.


