EXTRACTION WITH SITE PRESERVATION USING FREEZE-DRIED BONE ALLOGRAFT WITH A HUMAN AMNION-CHORION MEMBRANE VS FREEZE-DRIED BONE ALLOGRAFT WITH A BI-LAYER PORCINE DERIVED COLLAGEN MEMBRANE: A RANDOMIZED CLINICAL TRIAL

by

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NAVAL POSTGRADUATE DENTAL SCHOOL
ELIZABETH A. POLAK

2019

ABSTRACT

EXTRACTION WITH SITE PRESERVATION USING FREEZE-DRIED BONE ALLOGRAFT WITH A HUMAN AMNION-CHORION MEMBRANE VS FREEZE-DRIED BONE ALLOGRAFT WITH A BI-LAYER PORCINE DERIVED COLLAGEN MEMBRANE: A RANDOMIZED CLINICAL TRIAL

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Introduction: Alveolar ridge preservation, also known as site preservation, using various techniques and materials, has been shown to decrease three-dimensional bone loss following tooth extraction. This randomized, single blinded study compared clinical outcomes of extraction with site preservation using freeze-dried bone allograft (FDBA) with either a porcine collagen (Bio-Gide ®) or human amnion-chorion (BioXclude®) resorbable membrane.

Methods: Patients treatment planned for tooth extraction and subsequent implant with intact adjacent teeth were enrolled. At time of extraction the site was preserved with FDBA and randomly allocated to receive either a Bio-Gide® or BioXclude® membrane. Probing depths and clinical attachment level were assessed on teeth adjacent to the extraction site at the time of extraction and at 18-20 weeks post-extraction. Vertical alveolar ridge change was measured using a customized stent at baseline and 18-20 weeks post-extraction. Horizontal alveolar ridge change was measured using stone models from alginate impressions that were taken at baseline, 12 weeks, and 18-20 weeks

post-extraction. The rate of soft tissue closure post-extraction was recorded using intra-oral measurements at baseline and at 1, 2, 4 and 6 weeks (or until closed).

Results: Thirty-six patients and 40 teeth completed the study. The Bio-Gide® group consisted of 19 teeth and the BioXclude® group consisted of 21 teeth. From baseline to 18-20 weeks no significant differences were detected between the groups for any of the measured parameters. Both membranes had horizontal ridge width loss of 2.0mm and both membranes had an average vertical height loss of <1.0mm**.** For adjacent teeth, probing depth reduction was just under 1.0mm for each group and clinical attachment level loss was <1.0mm for both groups, with no significant difference between groups. Soft tissue closure was completed on average at 4 weeks for both groups.

Discussion: The outcomes of this study are consistent with reports in the literature for decreasing horizontal and vertical ridge resorption.

Conclusions: Extraction and site preservation using FDBA with either Bio-Gide® or BioXclude® membrane was successful in limiting the amount of alveolar ridge resorption in both a horizontal and vertical dimension. Neither technique was detrimental to the clinical parameters of adjacent teeth. Soft tissue closure was also similar between membranes and occurred on average at approximately 4 weeks.

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LIST OF ABBREVIATIONS

ASA	American Society of Anesthesiologists
CAL	Clinical Attachment Level
CEJ	Cemento-Enamel Junction
FDBA	Freeze-Dried Bone Allograft
mg	Milligrams
mm	Millimeters
PD	Probing Depth
PO	Post-Operative
Q6h	Every 6 hours
Q8h	Every 8 hours

CHAPTER I

REVIEW OF THE LITERATURE

Healing of extraction sockets

Teeth are extracted for a multitude of reasons: caries, fracture, periodontal disease, orthodontics, or trauma. If a tooth requires extraction, dental implants are often the treatment of choice for replacement. Dental implants allow edentulous sites to be functionally and esthetically restored; however, to surgically and prosthetically place implants, sufficient volume of alveolar bone and ideal ridge architecture is required.

Naturally, teeth in the maxilla and mandible are surrounded by native bone. This bone consists of basal bone that develops with the overall skeleton and an alveolar process that develops following tooth eruption and contains the tooth socket. Bundle bone lines the alveolar socket and extends coronally forming the crest of the buccal bone.[1]

Many changes occur during the healing process of an extraction site. Shortly after a tooth extraction, a blood clot forms and fills the socket creating a mixture of proteins and damaged cells within the first 24 hours.[2] This newly formed clot not only acts as a barrier, but initiates a cascade of inflammatory reactions to occur.[3] Neutrophils and macrophages enter into the extraction socket and phagocytize bacteria and tissue debris. A variety of cytokines and growth factors induce migration and synthesis of mesenchymal cells.[2] Granulation tissue is first seen peripherally around 2-3 days post extraction and has completely infiltrated and replaced all of the centrally positioned blood clot by 7 days. Young immature connective tissue first appears on day 4 and rapidly increases and fully replaces granulation tissue by about day 20.[4] The first evidence of bone is seen on days 7-8 post extraction in the marrow vascular spaces adjacent to and

along the entire length of the lamina dura.[5] By day 10, new bone is seen in the extraction socket all along the lateral aspects. By day 18, the fundus is around two-thirds filled with bone. Approximately at day 105, bone in the extraction site is similar to that of the surrounding bone.[6]

Although bone is reformed after tooth extraction, the alveolar process is a tooth-dependent tissue and concurrently as bone growth happens, so does resorption of the alveolar ridge. Resorption occurs in 2 phases. In the first phase, bundle bone is rapidly resorbed and replaced with woven bone leading to a reduction in bone height, especially in the buccal aspect. In the second phase, the outer surface of the alveolar bone is remodeled causing horizontal and vertical tissue contraction.[1] The reason for remodeling and resorption is not well understood in the literature. It is commonly thought that disuse atrophy, decreased blood supply, and localized inflammation may play a role.[1]

Following tooth extraction, bone loss has been documented in both a vertical and horizontal dimension. As an extraction site heals, Schropp et al. reported 50% of crestal width was lost in a 12 month period which corresponded to a reduction ranging from 5-7mm. Their study estimated two-thirds of the hard and soft tissue changes occurred in the first 3 months of healing, and also reported that the bone levels never regenerated to the level of bone attached to tooth surfaces mesial and distal to the extraction site.[7]

Van der Weijden examined vertical and horizontal changes post tooth extraction in a systematic review. Their systematic review found, in the individual selected studies, a mean clinical bone loss of 3.87mm horizontally and 1.67mm vertically.[8] Similar results were reported in a systematic review by Morjaria, with a range of horizontal width loss

measuring between 2.46-4.56mm and vertical height loss measuring between 0.90-3.6mm.[9]

In another systematic review, a horizontal reduction of 3.79mm and a vertical reduction of 1.24mm was noted by 6 months post extraction. Similar to the findings by Schropp, the mean horizontal reduction was 32% at 3 months.[10]

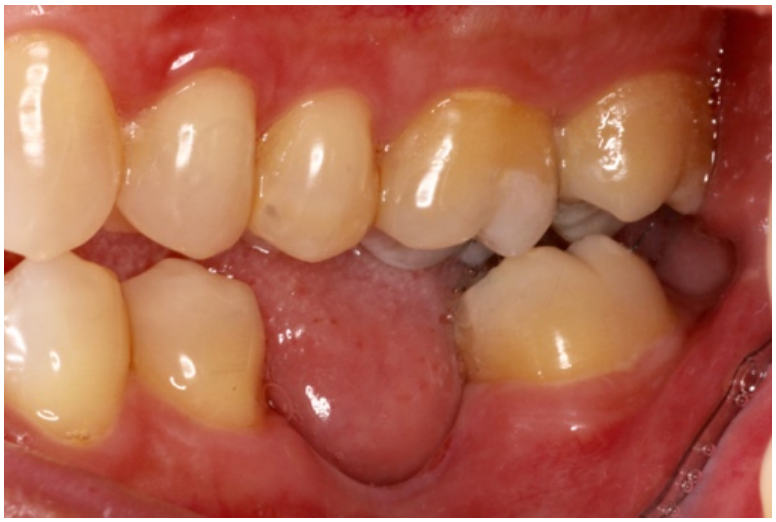


Figure 1: Healing post-extraction without site preservation, demonstrating vertical ridge resorption

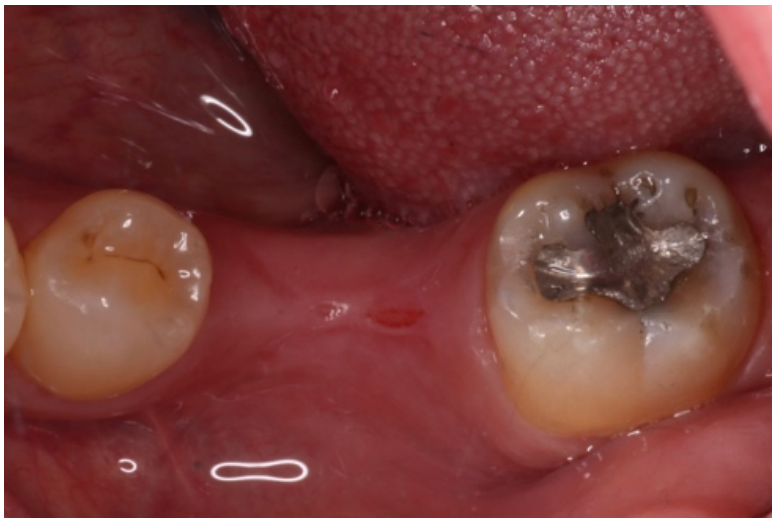


Figure 2: Healing post-extraction without site preservation, demonstrating horizontal ridge resorption

Site preservation

The principles of guided bone regeneration and guided tissue regeneration have been successful in reducing bone resorption to allow for ideal placement of a dental implant. These two methods advocate regeneration through the application of bone graft and the use of occlusive membranes.

Different bone graft materials have been used in site preservation to foster selective cell repopulation of the extraction site. Bone graft materials may be classified as osteoconductive, osteoinductive or osteogenic. According to the American Academy of Periodontology glossary of terms, an osteoconductive graft material functions to serve as a scaffold for deposition of osteoid. Osteoinductive refers to the quality of a biologic adjunct, growth factor, or graft material which leads to differentiation of osteoprogenitor cells into osteoblasts; this potential is often achieved via release of bone-inductive proteins from the material. Osteogenic is the quality of an autogenous graft which enables it to lead to bone formation via the transplant of viable osteoblasts within the graft.[11] Occlusive membranes can be used to exclude non-osteogenic cell populations from the surrounding soft tissues, thereby allowing osteogenic cell populations to occupy the extraction site. These membranes are placed over the graft material and under the epithelium to provide epithelial exclusion, space maintenance, clot stabilization, and graft containment.

In a study by Iasella, the dimensional changes of hard and soft tissue were investigated for extraction alone versus extraction with site preservation using freeze-dried bone allograft and a collagen membrane. Site preservation reduced horizontal loss by 1.6mm and vertical loss by 2.2mm. When both groups were examined histologically, the site preservation group revealed significantly more bone than in the extraction site

alone group. When looking at the soft tissue thickness it was found that the site preservation group had a decrease of 0.1mm in thickness compared to the extraction site alone which gained 0.5mm of soft tissue thickness. This loss of thickness may have been due to the decreased vascularity to the flap by the membrane. They concluded that site preservation improved the ridge width and height dimensions compared to extraction alone, which allows for more suitable implant placement in areas where loss of ridge volume would compromise the esthetic result. [12]

In a systematic review by Weng and Schliephake, it was found that the mean loss in horizontal ridge dimension was 0.87mm in the extraction with site preservation group and 2.12mm in the extraction alone group. In the vertical direction, the site preservation group gained 0.14mm and the extraction alone lost 1.48mm. In the site preservation group, this represents a reduction of horizontal ridge loss of 59% and reduction of vertical ridge loss by 109%. This study concluded that ridge resorption after extraction was less if site preservation was performed.[13]

In another systematic review by Avila-Ortiz examining horizontal and vertical ridge dimension changes, site preservation was found effective in limiting ridge reduction. The horizontal ridge loss was 1.89mm and in a vertical direction was a reduction of 2.07mm on the buccal and 1.18mm on the lingual. When performing subgroup analysis, it was found that flap elevation with graft material and membranes had superior results compared to extraction alone, especially in the vertical height at the mid-buccal and mid-lingual.[14]

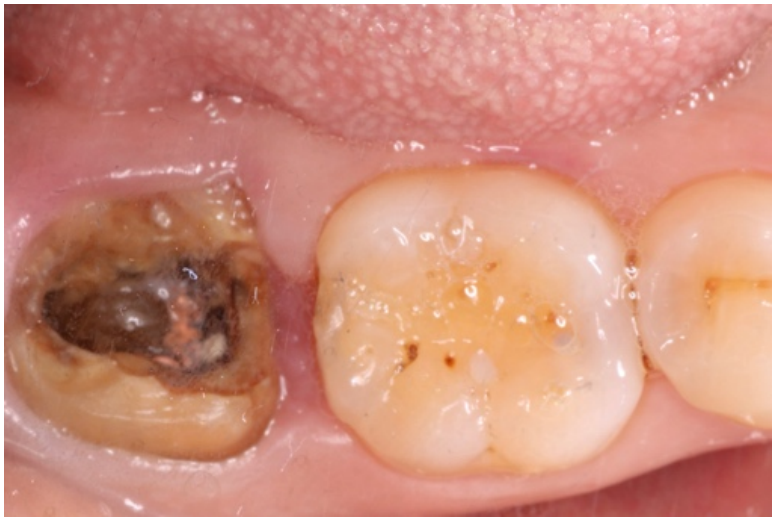


Figure 3: Non-restorable tooth requiring extraction

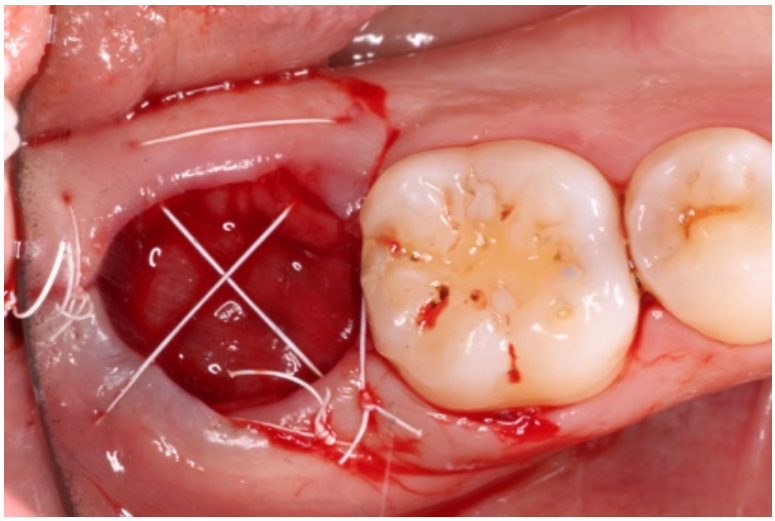


Figure 4: Post extraction and site preservation

Allografts

As noted previously, osseous grafts are often used in performing extraction with site preservation. There are multiple types of grafts that can be used, which include, autografts (same donor), allografts (same species), xenografts (different species) and alloplasts (synthetic).

Of the osseous grafts, allografts have become a popular choice when preserving the existing dimensions of the extraction site. Allograft material can be either mineralized

(FDBA) or demineralized (DFDBA) and can come in block or particulate form. Allografts are either harvested from cadavers or from living donors undergoing procedures such as hip replacement surgery. FDBA is commonly used in extraction site preservation and is classified as an osteoconductive material. The mineralized nature of the graft allows it to be an excellent scaffold and provides optimal space maintenance to allow formation of new bone. Moreover, freeze-dried bone allograft may stimulate earlier, more rapid and substantial new bone formation.[15]

The safety of allografts was investigated and established in a study by Mellonig. Their review showed that allograft materials are safe and concluded that the calculated risk of HIV transmission from a bone graft was 1 in 2.8 billion.[16] At the time of Mellonig's 1995 article, FDBA had been used for over 25 years and there had been no documented cases of disease transmission. This finding of FDBA safety was re-enforced by October 2013 posting by the Centers for Disease Control and Prevention.[17] Mellognig's article also reviewed the process of bone graft donor selection, graft testing, and treatment of the graft. They also recommend only using allografts from a company that is accredited by the American Association of Tissue Banks.

In a monkey study by Yukna and Vastardis, they compared nylon mesh cylinders containing either DFDBA or FDBA implanted into surgically created vertical grooves on the facial aspects of the posterior maxilla and mandible. They found that FDBA chambers contained more new bone and total bone than either the DFDBA or the empty chambers. It was concluded that FDBA may stimulate more rapid and substantial bone formation that DFDBA.[15]

In a comparison of FDBA and DFDBA in site preservation in humans, Wood and Mealy found that there were no significant differences in alveolar ridge dimensional changes between the two groups. The average loss of ridge height was less than 1mm and the average loss of ridge width was approximately 2mm. When they compared the amount of residual graft histologically, they found a lower mean percentage of residual graft particles at 8.88% for DFDBA versus 25.42% for FDBA. This study concluded that FDBA is successful in reducing the amount of resorption in molar extraction sites.[18]

Collagen membranes

Many studies have utilized a collagen membrane when performing extraction with site preservation. The ideal properties of a membrane are biocompatibility, cell-occlusiveness, permeability for nutrients and ease of use. Collagen is a major protein found in the connective tissue of all mammals. It is composed of three polypeptide chains with each containing nearly one thousand amino acids. Collagen molecules are synthesized by endothelial cells, smooth muscle cells and predominantly by fibroblasts.[19] They are important proteins involved in numerous biological activities including extracellular matrix and blood vessel formation, cell adhesion and migration, as well as tissue morphogenesis and repair.[19] Type I collagen is the most prevalent member of the 28 different collagen types. Collagen has many additional advantages such as hemostasis and chemotaxis for periodontal ligament fibroblasts and gingival fibroblasts.[20] Different collagen membranes of animal origin have been engineered and are extensively used in dentistry.

Collagen membranes utilized in dentistry are often derived from tendon, dermis, or the pericardium and are commonly from porcine or bovine origin. Collagen membranes demonstrate varying absorption rates depending on the source and if they are

cross-linked or not. Cross-linking is done by various physical, chemical and enzymatic processes that cross-links the existing collagen fibers.[21] Cross-linked membranes are more resistant to tissue degradation and are often associated with higher incidence of post-operative complications.[21] Cross-linked collagen membranes can last up to 24 weeks while non-cross-linked membranes can last 8-12 weeks.[22] It was reported by Tal that this degradation can start as early as 4-28 days post-placement for collagen membranes and that by 28 days over 80% resorption has occurred for non-cross-linked membranes compared to only slight resorption at 24 weeks for cross-linked membranes.[20]

Resorption rates also vary if the membrane is exposed to the oral cavity or completely covered by an overlying soft tissue flap. When a collagen membrane is exposed, it is susceptible to the oral environment which allows bacteria to adhere to the membrane surface.[23] These bacteria produce collagenase that have the ability to degrade collagen membranes, leading to premature loss of the membrane and exposure of underlying tissue.[24] In dental procedures such as guided bone regeneration, this early exposure can lead to loss of graft material and epithelial down growth.

Bio-Gide® is a non-cross-linked, porcine-derived, type I and type III collagen membrane. The membrane has a bi-layer structure that prevents the ingrowth of soft tissue into the augmented site and optimally degrades to allow the cascade of biologic events leading to regeneration.[25] The overlying portion is cell occlusive and allows for fibroblasts to attach, allowing for favorable soft tissue healing. The underlying area facing the grafted site is fibrous and functions as a guide for angiogenic and osteoblastic cells.[25] These two layers work together to aid in early wound stabilization through chemotactic properties to attract fibroblasts and remain semi-permeable for nutrient

transfer.[26] Bio-Gide® undergoes biodegradation within 2-4 weeks and can last up to 12 weeks.[27] Bio-Gide® is indicated for multiple uses such as extraction sites, periodontal defects, ridge augmentation and sinus floor elevation.

Amnion Chorion Membrane

A new class of membranes, sourced from human amniotic tissue, has recently become available for use in dental surgical procedures. BioXclude® is an example of a human amnion-chorion membrane. This unique material combines the benefits of growth factors and the properties of a barrier membrane. Amnion tissue, the inner layer of the amniotic sac, has collagen types III, IV, and V and the chorion layer, the outer layer of amniotic sac, has collagen types I, III, IV, V, and VI.[28] This membrane has been shown to contain over 250 biological factors including, extracellular matrix proteins, fibronectin, laminins, cytokines, growth factors, interleukins and tissue inhibitors of metalloproteinases which are known to play a role in wound healing.[29] These different factors help aid in rapid wound closure, rapid angiogenesis, inflammation suppression, and recruitment of mesenchymal stem cells.[30] Immunohistochemical analysis has also shown high concentrations of laminin-5 throughout the membrane which has high affinity for cellular adhesion of gingival epithelial cells. BioXclude® also lacks antigenicity and innately contains antibacterial properties.[31]

The allograft tissue used in BioXclude® is procured from mothers who donate their placenta during elective caesarian section surgery. All tissue is obtained in compliance with federal regulations and tissue standards of the American Association of Tissue Banks. BioXclude® is processed using Purion®, a proprietary process that provides a cleansed, dehydrated, and sterilized graft of human placenta amnion and

chorion tissue.[32] Unlike other collagen membranes that are between 700-800 microns thick, BioXclude® is approximately 300 microns thick, allowing it to be quite pliable. This allograft also requires minimal to no trimming and can be folded onto itself.

In a case report series by Holtzclaw, the use of FDBA with BioXclude® was shown to enhance site preservation by demonstrating rapid epithelialization and closure of the surgical site versus FDBA alone. At 48 hours after site preservation of a maxillary right first molar, rapid healing was demonstrated by opacification of the membrane and granulation tissue over the extraction site. At 96 hours complete closure of the extraction site was demonstrated. At day 10 the tissue continued to mature with keratinization of the tissue by day 21.[32]

Comparison of Bio-Gide® and BioXclude® Membranes

To date there are no studies that compare the use of Bio-Gide® with FDBA to BioXclude® with FDBA for extraction with site preservation. The purpose of this study is to compare clinical outcomes of extraction with site preservation using freeze-dried bone allograft (FDBA) with either a porcine collagen membrane (Bio-Gide®) or an amnion chorion membrane (BioXclude®) . Due to its innate biologic properties, we hypothesize that utilizing FDBA with BioXclude® will result in faster soft tissue closure and less alveolar ridge resorption as compared to sites grafted with FDBA and Bio-Gide® .

CHAPTER II

METHODS AND MATERIALS

This is a randomized, prospective, single-blinded study. The study population consisted of male and female military healthcare beneficiaries age 18 years and older who were treatment planned for extraction, ridge preservation treatment and subsequent dental implant placement. The inclusion and exclusion criteria are listed below:

Inclusion Criteria:

1. Patient aged ≥ 18 years old.
2. Patient will remain in the Capital region for at least 18-20 weeks following the extraction with site preservation for follow up appointments and implant placement.
3. Patient requires an extraction of a single tooth that has intact adjacent teeth.
    i. If the patient presents with two teeth in different quadrants requiring extraction, both sites can be included and randomized.
4. Patient treatment planned for a dental extraction with site preservation and subsequent dental implant placement at 18-20 weeks following tooth extraction.

Exclusion Criteria:

1. Patient under the age of 18.
2. Patient will be moving from the Capital region area prior to 18-20 weeks following tooth extraction.
3. Female patients who are pregnant or nursing.
4. Patients with clinically significant systemic diseases, which may affect healing, placing them in an ASA status of 3 or higher (e.g. uncontrolled diabetes).

5. Patients allergic to any medications or materials utilized within the study.
6. Patients with poor oral hygiene unsuitable for periodontal surgery.
7. Patients with an active infection.
8. Patient currently uses tobacco.
9. Patients who cannot or will not sign the informed consent form.

Patients that met the criteria were then referred for a periodontal comprehensive exam and then treatment planned for extraction with site preservation and implant placement 18-20 weeks later. The findings of the exam, such as probing depths (PD), clinical attachment levels (CAL), and recession were recorded on the Navy Periodontal Chart Form-NAVMED 6660/2 (See Appendix A). The subject was given a half page study brief of the project. If the subject was interested, the principal investigator discusses the study, and consents were then signed. The patient was assigned a study identification number. The date of enrollment was recorded, as well as other information including age, gender, and tooth to be extracted. Smoking history and diabetic condition were also recorded (See Appendix B for flow diagram of study design).

Following the consent and evaluation, impressions of the study arch were taken with alginate and poured up in stone to make a study cast. This study cast was used to make a customized acrylic stent and a vacuum formed retainer (See Appendix C). This stone cast also served to measure the ridge width of the extraction site approximately 4mm apical to the adjacent teeth cemento-enamel junctions. The acrylic stent was used to measure the alveolar crest height intra-operatively at the time of extraction and at 18-20 weeks post extraction.

Subjects were stratified into groups by the location of the tooth to be extracted. These groups consisted of maxillary anterior, maxillary posterior, mandibular anterior and mandibular posterior. Subjects were randomized to receive either BioXclude® or Bio-Gide® . The type of membrane was recorded on an index card and placed in a sealed envelope that was opened on the day of tooth extraction. The study investigator was blinded to the type of selected membrane until the envelope is opened.

During the surgical procedure, probing depths and clinical attachment levels of the mesial and distal adjacent teeth were recorded prior to extraction. Recordings were done at four sites per tooth: mid-buccal, mesio-buccal, mesio-lingual, and mid lingual on the distal tooth and mid-buccal, disto-buccal, disto-lingual and mid lingual on the mesial tooth. The cemento-enamel junction or restorative margin was used as the reference point. Following measurements, the tooth was extracted as atraumatically as possible. The acrylic stent was used to measure the vertical alveolar ridge dimension at 6 points around the extraction site with a periodontal probe. The presence or absence of a facial dehiscence was determined and whether it was greater or less than 50%. At this point, the envelope was opened and the membrane type was revealed. The freeze-dried bone allograft was hydrated with sterile saline and placed in the extraction socket. The selected membrane was placed over the bone graft and the flap was sutured using a non-resorbable suture. The soft tissue opening was measured bucco-lingually with a periodontal probe and mesio-distally with caliper at the center of the ridge.

Post-operatively, the subject was prescribed an appropriate analgesic, a salt water rinse, and an antibiotic at the surgeon's discretion. All patients were provided with standard post-operative instructions. Patients were recalled at week 1, 2, 4 and 6 to assess

post-operative healing, remove plaque from adjacent teeth, and measure amount of soft tissue closure. De-plaquing and healing assessment was also done at week 8, 12 and 16. Alginate impressions were taken at week 12 and at week 18-20 for ridge width measurements.

At the time of implant placement, the probing depths and clinical attachment were taken on the adjacent teeth prior to tissue reflection. After flap reflection, the acrylic stent was used to measure alveolar ridge height at the 6 predetermined areas. The implant was then placed and the patient had completed the study.

CHAPTER III

RESULTS

Fifty-four patients were enrolled into the study with 18 patients having to be excluded. The total number of included subjects was affected due to the sample population being primarily military members with permanent change of duty stations and deployments occurring frequently. The remaining 36 patients accounted for 40 teeth to be extracted. Of the extractions, 18 were mandibular posterior, 6 were maxillary anterior, and 16 were maxillary posterior. Of the 40 teeth, 19 were in the Bio-Gide® group and 21 in the BioXclude® group (See Table 1).

Table 1: Number of sites and locations for Bio-Gide® and BioXclude®

	Bio-Gide®	BioXclude®	Total
Sites	19	21	40
Mandibular Anterior	0	0	0
Mandibular Posterior	10	8	18
Maxillary Anterior	3	3	6
Maxillary Posterior	6	10	16

The box plot below (See Figure 5) reflects the change in probing depth (PD) measurements of the mesial and distal adjacent teeth. The x-axis represents the two membrane groups of Bio-Gide® and BioXclude®. The y-axis shows the average change in probing depth from baseline to 18-20 weeks in 1mm increments. The box represents the interquartile range with the bottom of the box being Q1, 25th percentile and the top of

the box is quartile 3, 75th percentile. The error bars represent 95% confidence intervals and the solid black bold line represents the median value for each group.

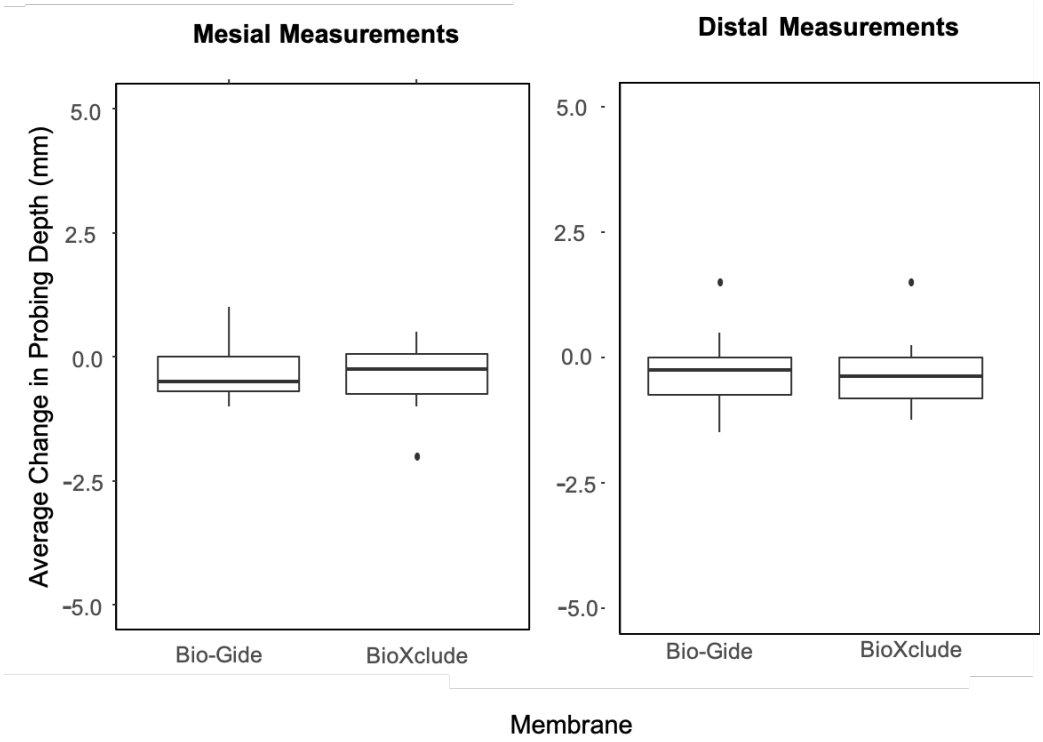


Figure 5: Boxplot graphs of average change in probing depth of mesial and distal teeth adjacent to the extraction site

The mesial and distal measurements show very similar interquartile ranges and median values, all showing no change to slight decrease in PD at 18-20 weeks. At 18-20 weeks Bio-Gide® and BioXclude® had an average reduction of probing depth of 0.29mm. When comparing Bio-Gide® to BioXclude®, there were no statistically significance differences between the two groups.

The box plot below shows the change in clinical attachment levels (CAL) from baseline to 18-20 weeks. The x-axis represents the two membranes and the y-axis the change in CAL in 1mm increments.

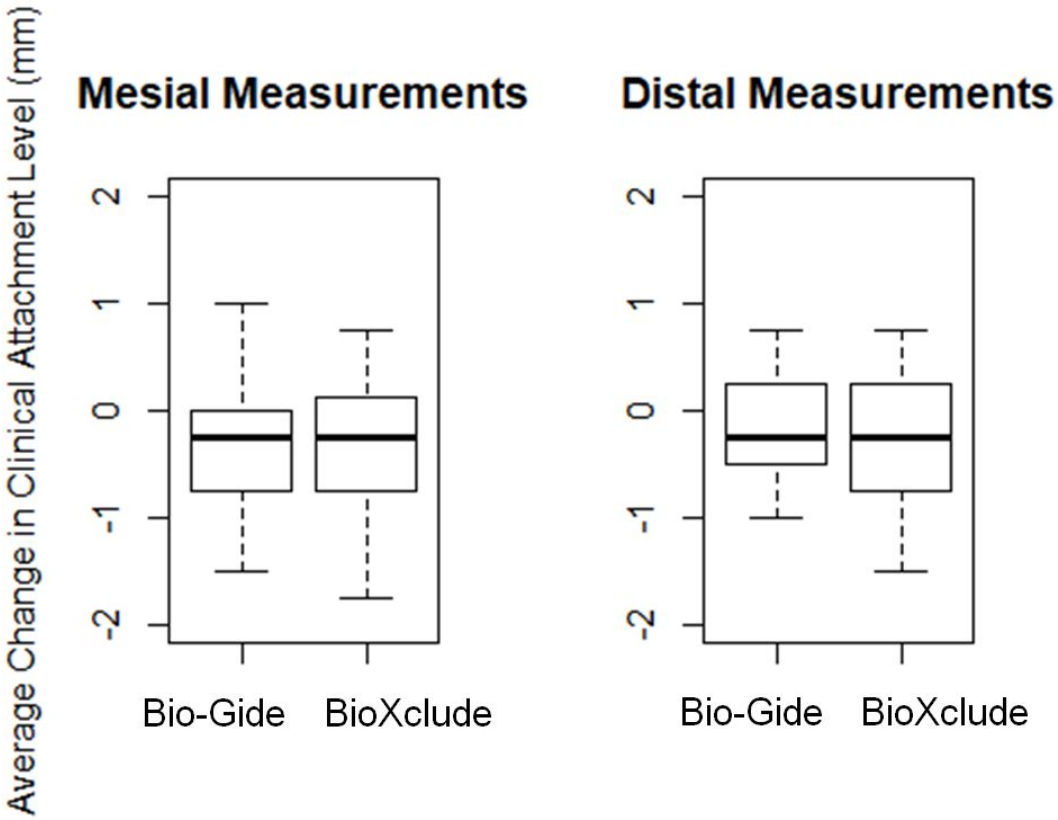


Figure 6: Boxplot graphs of average change in clinical attachment levels of mesial and distal teeth adjacent to extraction site

Once again, the median values and interquartile ranges are very similar between the two. Bio-Gide® had an average CAL loss of 0.31mm and BioXclude® had an average loss of 0.32mm. When comparing the two groups, there were no statistically significant differences between the groups.

The boxplot below represents the vertical ridge change from baseline to 18-20 weeks as measured by the acrylic stent. The x-axis represents the two different membranes and the y-axis shows the change in height in 1mm increments.

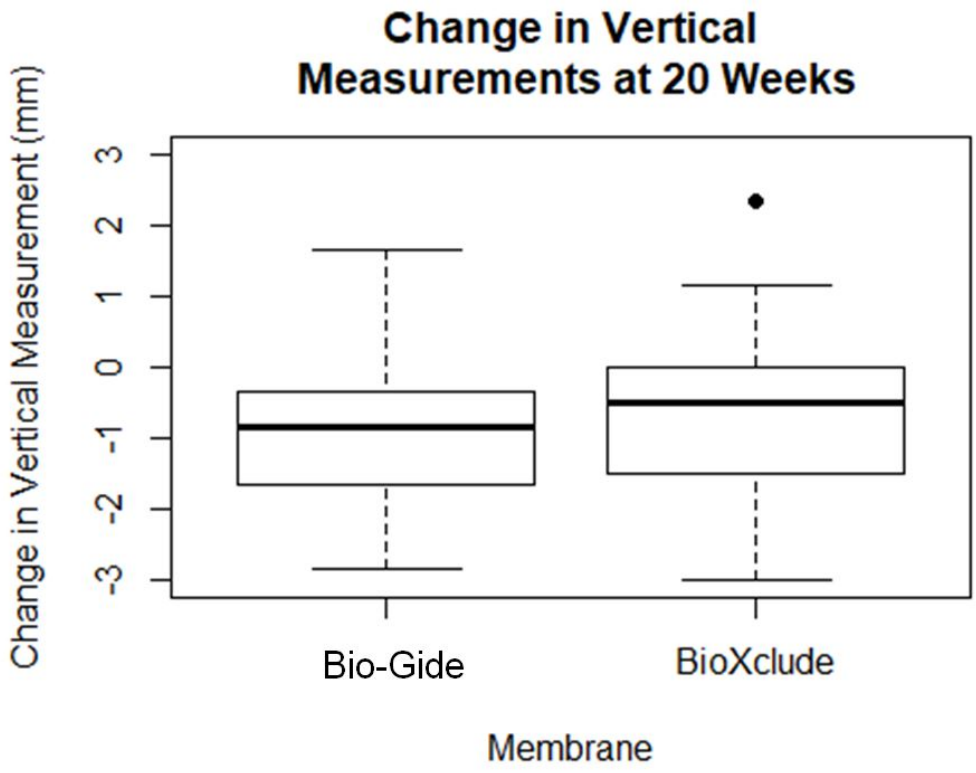


Figure 7: Boxplot graphs of average change of vertical ridge height at 18-20 weeks

Significant differences in vertical ridge dimension were not detected with both groups showing none to less than 1.5mm loss in ridge height.

Figure 8 shows the horizontal ridge change from baseline to 18-20 weeks that were taken on the stone casts. Of the 36 patients, only 21 patients had cast data due to providers not taking casts at the 18-20 week visit. On the x-axis is the membrane type and the y-axis is the change in horizontal ridge dimension in 1mm increments. For the Bio-Gide® group, the interquartile range from Q1(25^{th} percentile) to Q3 (75^{th} percentile) was the same as the median value.

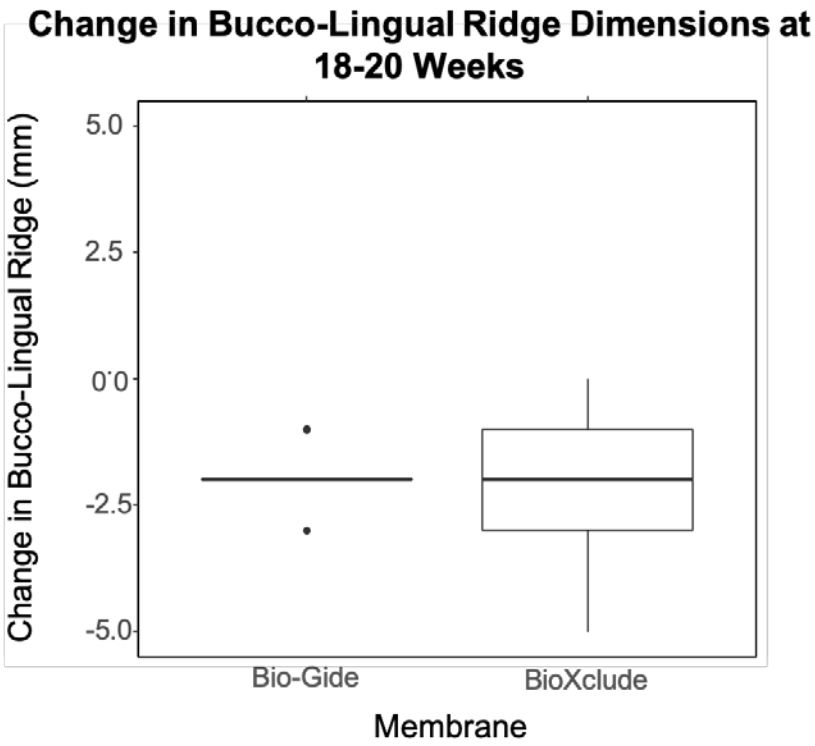


Figure 8: Boxplot graphs of average change of bucco-lingual ridge width dimensions at 18-20 weeks

Significant differences in horizontal ridge width were unable to be detected between the two membrane groups, with both demonstrating approximately 2mm ridge width reduction.

Figure 9 reflects the change in bucco-lingual soft tissue closure over the two membranes. The x-axis represents the post-operative weeks at which the measurement was taken. The y-axis represents the amount of exposed membrane in millimeters.

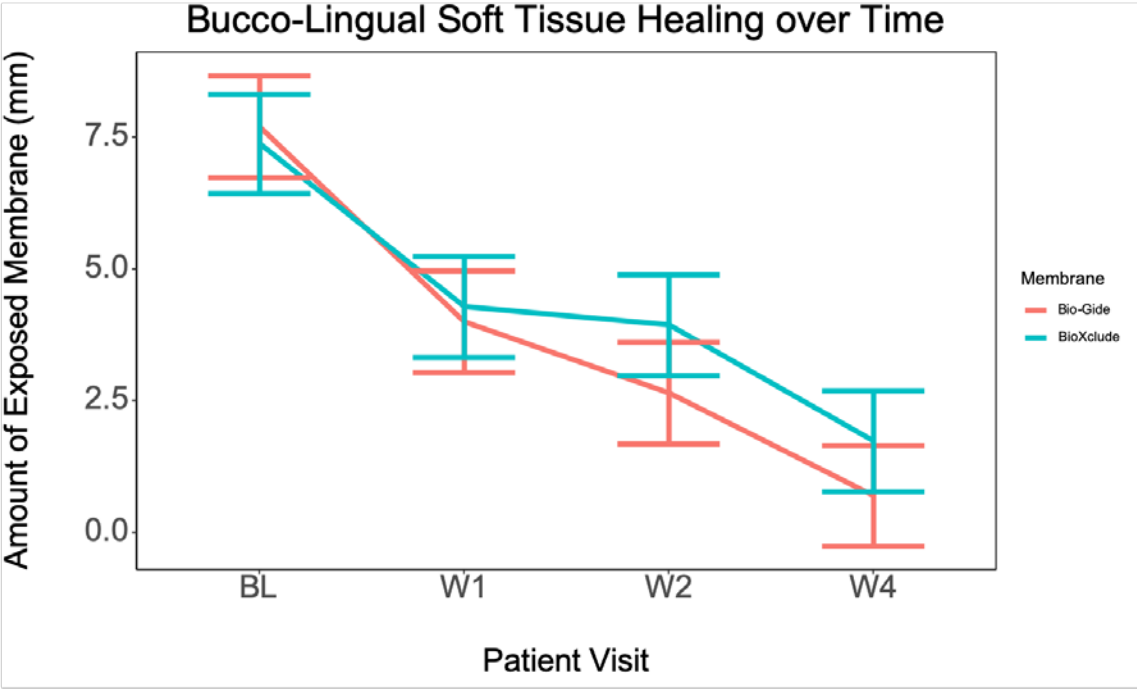


Figure 9: Line graph of rate of bucco-lingual soft tissue closure over the exposed membranes at baseline and weeks, 1, 2, and 4 post-operative visits

Significant differences in bucco-lingual soft tissue closure were unable to be detected between the groups, with both groups being closed by week 6. It could be speculated that Bio-Gide® had a slightly faster closure rate.

Figure 10 reflects the mesio-distal change in soft tissue closure over the two membranes. Similarly, significant differences were not detected between the two membrane groups, with Bio-Gide® also showing slightly faster closure rate.

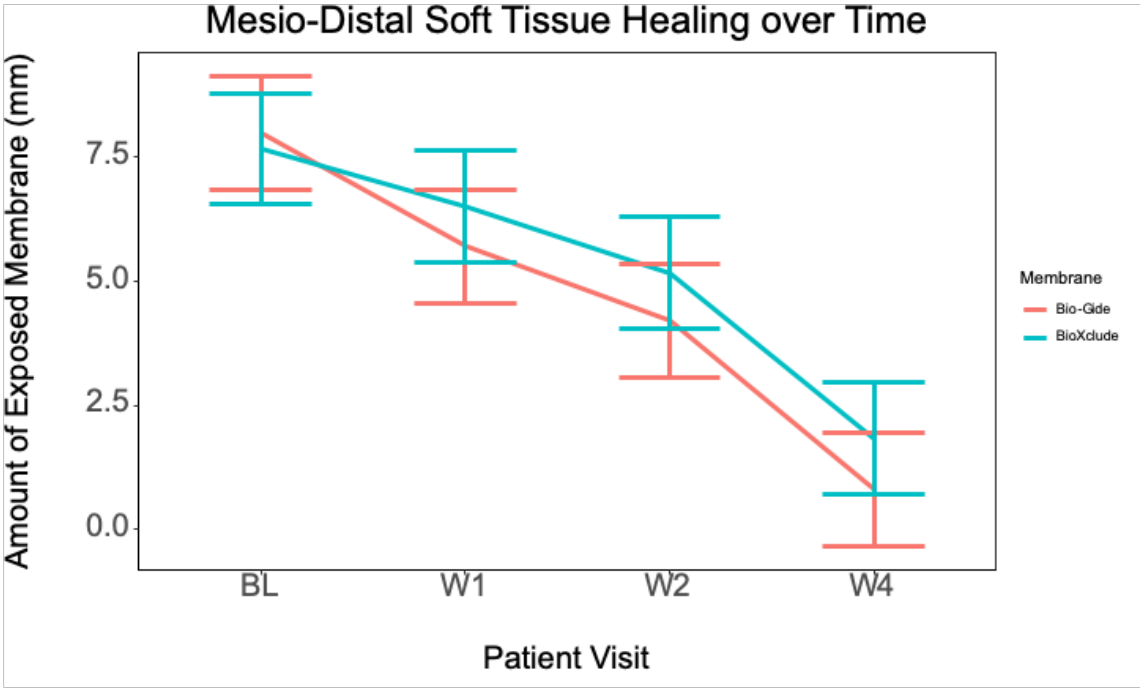


Figure 10: Line graph of rate of mesio-distal soft tissue closure over the exposed membranes at baseline and weeks 1, 2, and 4 post-operative visits

CHAPTER IV

DISCUSSION

The two main goals of ridge preservation are to maintain adequate width and height of the alveolar ridge and to promote vital bone formation. This study demonstrated that ridge preservation with FDBA utilizing either Bio-Gide® or BioXclude® membrane had horizontal ridge loss on average 2.0mm and vertical ridge dimension loss of <1.0mm at 18-20 weeks post extraction. These results correlate well with a previous study by Iasella who examined site preservation with FDBA and BioMend Extend® demonstrating a horizontal ridge reduction of 1.2mm and a mid-buccal vertical ridge height reduction of 1.3mm.[11] The horizontal ridge loss in this study was slightly more than Iasella's; however, we measured horizontal loss on stone models which does not consider soft tissue changes. The present study only obtained casts on 21 of the 40 subjects, so the data must be interpreted with caution. The materials and methods used by Iasella differed from the present study in many ways. Two differences noted were the time at which the measurements were taken and the number of vertical measurements taken at re-entry. The present study measured at 6 sites around the extraction site at baseline and 18-20 weeks post-extraction, whereas Iasella measured 4 points at baseline and 24 weeks post extraction. The studies also differed in membranes used. Iasella used BioMend Extend® versus extraction alone and the present study compared Bio-Gide® versus BioXclude®. Both studies were similar in that they used freeze-dried bone allograft in conjunction with the membranes.

These findings can also be compared to a recent systematic review by Avila-Ortiz. They reported that alveolar ridge preservation, using a variety of types of grafts

with a collagen membrane or sponge, decreased horizontal ridge resorption by a mean of 1.99mm and decreased vertical loss by 1.72mm on the mid buccal and 1.16mm on the mid lingual.[34]

In a study by Cardaropli using Bio-Oss® (xenogenic graft) and Bio-Gide® , they reported a probing depth reduction of 0.49mm and a clinical attachment level loss of 0.19mm.[35] At 18-20 weeks, the present study demonstrated a probing depth reduction of 0.29mm and clinical attachment level loss of 0.31mm for the Bio-Gide® group, and probing depth reduction of 0.29mm and clinical attachment loss of 0.32mm for the BioXclude® group. There were no significant differences found between the two groups for either probing depth reduction or clinical attachment level loss. The two studies differ in bone graft, but show similar results and compare well with the systematic review by Avila-Ortiz.

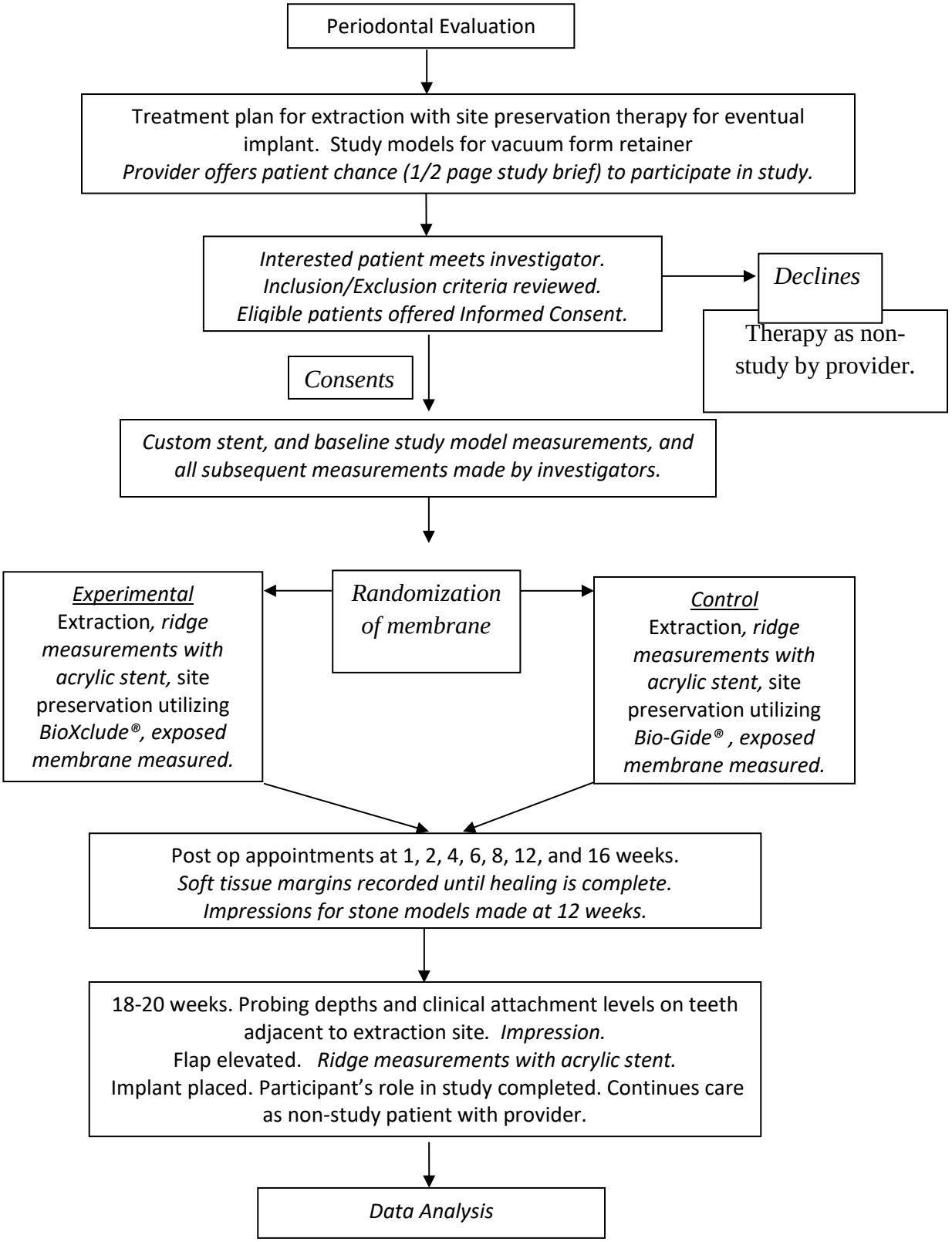
To the best of the author's knowledge, this is the first study to examine the rate of soft tissue closure over the exposed membranes after extraction site preservation. The average rate of soft tissue closure over the Bio-Gide® was 4.67 weeks and for the BioXclude® it was 4.75 weeks. These differences were not statistically significant; the results differ from our hypothesis. The majority of sites had soft tissue closure at 4 weeks with a few outliers taking up to 6 weeks. When comparing soft tissue closure in natural healing, it is seen in a study by Evian that epithelial soft tissue closure happened on average between 24-35 days[36]. The present study concurs with these healing time frames showing that the Bio-Gide® and BioXclude® did not delay or accelerate soft tissue closure.

CHAPTER V

CONCLUSION

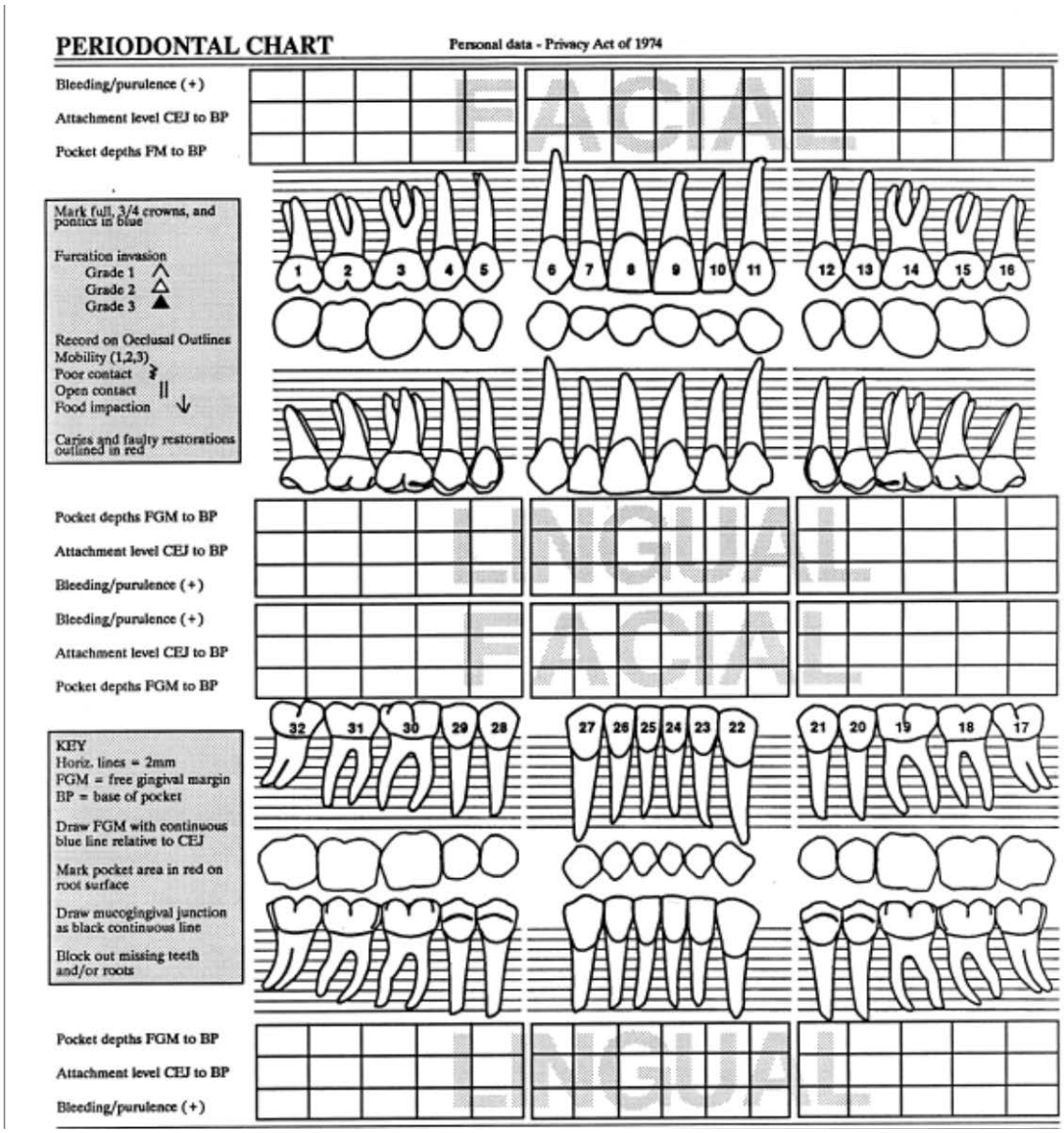
To the author's knowledge, this is the first study to compare alveolar dimensional changes and soft tissue closure between Bio-Gide® and BioXclude® membranes with FDBA in an extraction with site preservation procedure. It was found that both membranes were capable of limiting both horizontal and vertical ridge loss and compared similarly with soft tissue closure rate over the membrane.

APPENDIX A: FLOW DIAGRAM OF STUDY DESIGN
*(*standard clinical processes in normal font, *research in italics)*



APPENDIX B: COMPREHENSIVE PERIDONTAL CHARTING FORM

Subject ID # _____
Date enrolled _____
Gender _____
Age _____
Tooth Site # _____



APPENDIX C: POST-OPERATIVE INSTRUCTIONS

POST-OP INSTRUCTIONS FOR BIO-GIDE/BIOXCLUDE PROTOCOL
PERIODONTICS DEPARTMENT
NAVAL POSTGRADUATE DENTAL SCHOOL
BETHESDA, MARYLAND

For best healing and to minimize complications, please read and follow these instructions carefully. You may have been given one or more of these medications:

PAIN MEDICATIONS: _____ Ibuprofen 800 mg 1 tablet PO Q6-8 h prn moderate pain or _____ Acetaminophen 325 mg 1-2 tablets PO Q6h prn moderate pain.

_____ Hydrocodone 5/325 mg or _____ Oxyocodone/Acetaminophen 5/325 mg 1 tablet PO Q6h for severe/breakthrough pain.
* These can be taken in addition to Ibuprofen or Acetaminophen. These narcotics can make you drowsy. Therefore, do not drive or operative or operate machinery while taking this drug. Additionally, do not take with alcoholic beverages; the alcohol will make you sleepier, but will not decrease your discomfort.

ANTIBIOTICS: _____ Amoxicillin 500 mg or _____ Clindamycin 300 mg 1 tablet PO TID for 7 days

RINSES: _____ Salt Water Rinse, 1 TSP in 8 OZ water, rinse 30 sec, BID for two weeks

The following are post-operative considerations during healing:

BLEEDING: There may be slight bleeding from the surgical site for 1-2 days after surgery. Your saliva may appear slightly reddish. This is common. If you notice an increase in bleeding, please contact us.

SUTURE/STICHES: You may have sutures placed in your mouth. They may have to be removed in the future. Please leave the sutures alone as much as possible. Early removal or the loss of sutures may impair healing.

DIET: It is very important to maintain a soft diet for at least a week. Chew as much as possible on the opposite side of the surgical site. This is not the time to start a diet. Please maintain your caloric and fluid intake as at pre-surgical levels. You will not heal well if you are dehydrated or undernourished. Please do not drink using a straw.

ORAL HYGIENE: It is very important not to brush or floss the surgical site until given

express instructions. Normal brushing and flossing procedures can traumatize the tissue and impair healing. You may brush and floss those areas not affected by the surgery. To keep bacteria under control, salt water rinse is recommended. Later, you may be instructed to use a cotton-tipped applicator, dipped in salt water rinse, to swab along the gum line of the surgical site. Please do not use a Water-Pik or other irrigator unless instructed to do so.

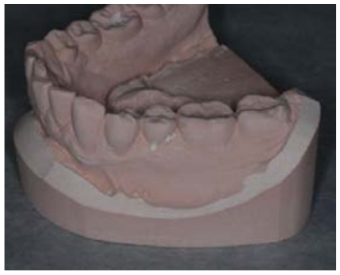
PHYSICAL ACTIVITY: Avoid strenuous physical activity (to include running and heavy lifting) for 72 hours. Additionally, no vigorous spitting, rinsing, or speaking (yelling). Forceful movements at the site of the surgery will negatively affect healing. You may experience some swelling. This is common and usually peaks at 2-3 days after surgery. Thereafter, you should expect to see a return to normal. To decrease swelling you can apply ice to the site for the first 3-4 hours after surgery. Please call if the swelling appears to increase after the third day, or if you are concerned.

SMOKING: Smoking is deleterious to healing. We advise you to stop smoking for as long as possible after surgery.

If you have any problems or questions, please do not hesitate to call our clinic at 301-295-0077. If there is an emergency, you may page your doctor through the automated system. Instructions will be given after dialing 1-800-759-8888.

Your follow-up appointment is scheduled for: _____

APPENDIX D: STENT FABRICATION

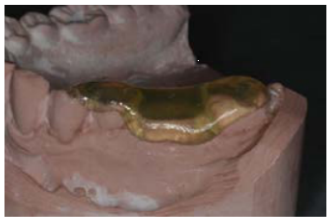


Before surgery a dental impression is made of arch where extraction will occur

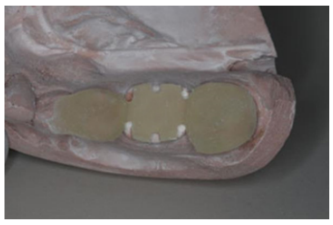
- Stone model made from impression
- A customized acrylic stent will be fabricated on model
- Stent will allow 6 standardized measurements of bone level at extraction site
    - *Before graft placed*
    - *At 18-20 weeks before implant placement*

Stent Fabrication Process

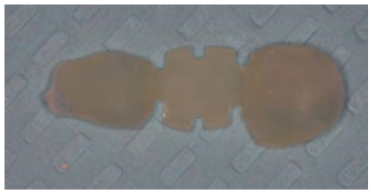
- Tooth to be extracted reduced to the height of contour using a slow speed hand piece.



- Moldable light cured acrylic is adapted to model over the site of tooth to be extracted and the adjacent teeth
    - Acrylic on adjacent teeth orients and stabilizes stent for measurements



- Acrylic trimmed to just above the height of contour of tooth
- to be extracted
- A 169 fissure bur cuts grooved slots in the stent
- Slots accommodate periodontal probe for 6 measurements of bone levels at same location and angulation



After the extraction before graft placement

- Stent placed on adjacent teeth to measure bone levels at 6 sites in extraction site
- Stent stored in disinfectant container marked by subject's study number
- At 18-20 weeks, just before implant placement, gingival flap reflected, stent placed on adjacent teeth and 6 measurements of bone at extraction site (where implant will be placed) are repeated

Note: impressions made pre-surgically and at 12 weeks and 18-20 weeks after extraction

- Stone models made
- Width of each stone model measured with calipers
- Width measurement made at mid-point of the extraction site, 4mm apical to the cemento-enamel junctions of the adjacent teeth

REFERENCES

1. Pagni G, Pellegrini G, Giannobile WV, Rasperini G. Postextraction alveolar ridge preservation: biological basis and treatments. *Int J Dent* 2012:1-13.
2. Tomlin E, Nelson S, Rossmann J. Ridge Preservation for Implant Therapy: A Review of the Literature. *The Open Dent Journal.* 2014;8:66-76.
3. Amler MH. The time sequence of tissue regeneration in human extraction wounds. *Oral Surg* 1969;27:309-318.
4. Jahangiri L, Devlin H, Ting K, Nishimura I. Current perspectives in residual ridge remodeling and its clinical implications: a review. *J Prosthet Dent* 1998; 80(2): 224-37.
5. Amler MH, Johnson PL, and Salman I. Histological and histochemical investigation of human alveolar socket healing in undisturbed extraction wounds. *J Am Dent Assoc* 1960;61:32-44.
6. Boyne P J. Osseous repair of the postextraction alveolus in man. *Oral Surg Oral Med Oral Pathol* 1966: **21**: 805–813.
7. Mangos JF. The healing of extraction wounds. An experimental study based on microscopic and radio- graphic investigations. *New Zealand Dental Journal* 1941: **37**: 4–24.
8. Schropp L, Wenzel A, Kostopoulos L, Karring T. Bone healing and soft tissue contour changes following single-tooth extraction: a clinical and radiographic 12-month prospective study. *Int J Perio Restorat Dent* 2003; 23(4): 313-24.
9. Van der Weijden F, Dell'Acqua F, Slot DE. Alveolar bone dimensional changes of post-extraction sockets in humans: A systematic review. *J Clin Periodontol* 2009;36:1048–1058.

10. Morjaria KR, Wilson R, Palmer RM. Bone healing after tooth extraction with or without an intervention: a systematic review of randomized controlled trials. *Clin Implant Dent Relat Res* [Epub] 2012; 1-20.
11. Tan WL, Wong TLT, Wong MCM, Lang NP. A systematic review of post-extractional alveolar hard and soft tissue dimensional changes in humans. *Clin Oral Impl Res* 2012;23(Suppl. 5):1-21.
12. Iasella JM, Greenwell H, Miller RL, Hill M, Drisko C, Bohra AA, Scheetz JP. Ridge preservation with freeze-dried bone allograft and a collagen membrane compared to extraction alone for implant site development: a clinical and histologic study in humans. *J Periodontol* 2003;74:990–999.
13. Weng D, Stock V, Schliephake H. Are socket and ridge preservation techniques at the day of tooth extraction efficient in maintaining the tissues of the alveolar ridge? *Eur J Oral Implantol* 2011;4(suppl):S59-S66.
14. Avila-Ortiz G, Rodriguez JC, Rudek I, Benavides E, Rios H, Wang HL. Effectiveness of three different alveolar ridge preservation techniques: a pilot randomized controlled trial. Int J Periodontics Restorative Dent 2014;34:509–521.
15. Yukna RA, Vastardis S. Comparative evaluation of decalcified and non-decalcified freeze-dried bone allografts in rhesus monkeys. I. Histologic findings. *J Periodontal* 2005;76:57-65.
16. Mellonig JT. Donor selection, testing, and inactivation of the HIV virus in freeze-dried bone allografts. *Practical Periodontics & Aesthetic Dentistry* 1995;7(6):13
17. CDC. Infection Control, frequently asked questions-Bone Allografts. Centers for Disease Control and Prevention. 25 October 2013. Web. 27 May 2019.

18. Wood RA, Mealey BL. Histologic comparison of healing after tooth extraction with ridge preservation using mineralized versus demineralized freeze-dried bone allograft. *J Periodontol* 2012; 83(3): 329-36.
19. Kadler KE, Baldock C, Bella J, Boot-Handford R.P. 2007. Collagens at a glance. J Cell Sci 120:1955-8.
20. Tal H, Kozlovsky A, Artzi Z, Nemcovsky CE, Moses O. Long-term biodegradation of cross-linked and non-cross-linked collagen barriers in human guided bone regeneration. *Clin Oral Impl Res* 2008;19:295-302.
21. Jiménez G, Berghezan S, Caramês JMM, Dard MM, Marques DNS. Effect of cross-linked vs non-cross-linked collagen membranes on bone: A systematic review. *J Periodont Res*. 2017;52:955–964.
22. McAllister BS, Haghighat K. AAP commissioned review. Bone augmentation techniques. *J Periodontol* 2007;78(3):377-396.
23. Sela, M.N.C., Steinberg, D.C., Klinger, A., Krausz, A.A.S. & Kohavi, D.C. Adherence of periodontopathic bacteria to bioabsorbable and non-absorbable barrier membranes in vitro. Clinical Oral Implants Research. 1999. 10: 445-452.
24. Bunyaratavej P, Wang HL. Collagen membranes: A review. *Journal of Periodontology* 2001;72(2): 215-229.
25. Schwarz F, Rothamel D, Herten M, Sager M, Becker J. Angiogenesis pattern of native and cross-linked collagen membranes: an immunohistochemical study in the rat. *Clin Oral Impl Res* 2006;17:403-409.
26. Zitzmann NU, Naef R, Scharer P. Resorbable versus nonresorbable membranes in combination with Bio-Oss for guided bone regeneration. *Int J Oral Maxillofac Implants* 1997;12:844-852.

27. Rothamel D, Schwarz F, Sager M, Herten M, Sculean A, Becker J. Biodegradation of differently cross-linked collagen membranes: an experimental study in the rat. *Clin Oral Impl Res* 2005;16:369-378.
28. Niknejad, H; Peirovi, H; Jorjani, M; Ahmadiani, A; Ghanavi, J; Seifalian, AM; (2008) Properties of the amniotic membrane for potential use in tissue engineering. *Eur Cells Mater,* 15:88-99.
29. Koob T, Rennert R, Zabek N, et al. Biological properties of dehydrated human amnion/chorion composite graft: implications for chronic wound healing. *Int Wound J* 2013: doi: 10.1111/iwj.12140.
30. Koob T, Li W, Gurtner G et al. Angiogenic properties of dehydrated human amnion/chorion allografts: therapeutic potential for soft tissue repair and regeneration. *Vas Cell* 2014; 4(10): doi:10.1186/2045-824X-6-10.
31. Holtzclaw D, Toscano N. Amnion chorion allograft barrier: indications and techniques update. *Journal of Implant and Advanced Clinical Dentistry* 2012;4(2):25-38.
32. Holtzclaw D, Toscano N. BioXclude® placental allograft tissue membrane used in combination with bone allograft for site preservation: a case series. *Journal of Implant and Advanced Clinical Dentistry* 2011;3(3):35-50.
33. Avila-Ortiz G, Chambrone L, Vignoletti F. Effect of alveolar ridge preservation interventions following tooth extraction: A systematic review and meta-analysis. Doi:10.1111/jcpe.13057.
34. Cardaropoli D, Tamagnone L, Roffredo A, Gaveglio L, Cardaropoli G. Socket preservation using bovine bone mineral and collagen membrane: a randomized

controlled clinical trial with histologic analysis. *Int J Periodontics Restorative Dent* 2012;32:421-430.

35. Evian CI, Rosenberg ES, Coslet JG, Corn H. The osteogenic activity of bone removed from healing extraction sockets in humans. *J Periodontol* 1982: **53**: 81–85.