PERIODONTAL REGENERATION OF 1-AND 2-WALL INTRABONY DEFECTS USING BIOXCLUDE WITH FREEZE-DRIED BONE ALLOGRAFT

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CERTIFICATE OF APPROVAL

MASTER'S THESIS

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ABSTRACT

PERIODONTAL REGENERATION OF 1-AND 2-WALL INTRABONY DEFECTS USING BIOXCLUDE WITH FREEZE-DRIED BONE ALLOGRAFT

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Introduction: When oral hygiene and non-surgical therapies cannot control periodontitis, surgical therapy is advised to clean deep pockets and try to create periodontal architecture for efficient plaque control to minimize recurrence of disease. In order to restore health to the periodontium, regenerative therapy, aimed at re-growing lost periodontal structures, is often employed to improve post-surgical architecture and improve probing depths (PD) and clinical attachment levels (CAL).

Purpose: The objective of this case series was to determine if BioXclude™, an amnion chorion allograft membrane shown to contain over 55 growth factors, provided predictable periodontal regeneration (formation of new bone, cementum, and connective tissue around teeth) when used in conjunction with freeze-dried bone allograft (FDBA) for the treatment of 1- and 2-wall intrabony defects.

Methods: Subjects undergoing treatment of 1- and 2- wall intrabony defects were assigned to receive guided tissue regeneration with bone graft utilizing FDBA and BioXclude™ membrane. Customized plastic stents were used by blinded investigators to obtain standardized measurements reflecting changes in PD, CAL and recession (REC). Bleeding on probing and plaque were classified as present or absent. PD, CAL and REC were assessed at the deepest defect sites at baseline (time of surgical treatment) and 6-months after surgical treatment. During surgery, the depth, mesiodistal width and buccolingual dimension of the intrabony defect were measured after the defect was debrided and the defect was classified as either 1-, 2-wall or a combination defect. The postoperative healing of each patient was assessed at weeks 1, 2, 4, 6, 8 weeks and again at 3 and 6 months. Data analysis compared changes in PD, CAL and REC. The

improvement in each of these dependent measures was assessed using the paired Wilcoxon signed-rank test.

Results: Seven subjects with eight 1-, 2-wall or combination intrabony defects were evaluated. From baseline to 6 months, notable differences were detected for PD, CAL and REC. The postoperative healing of each patient assessed at weeks 1, 2, 4, 6, 8 weeks and 3 and 6 months was uneventful. The average defect depth was 5.5mm with a range of 5.0-16.0mm measured from the buccal aspect and the average depth of the defects measured from the lingual aspect was 7.5mm with a range of 5.0-14.0mm. The average buccolingual width was 7.5mm with a range of 5.0-9.0mm. The median change for buccal PD was an improvement of 3.0mm from baseline to 6-month re-evaluation and the median change for lingual PD was 4.0mm. Buccal and lingual CAL exhibited a median improvement of 1.5mm and 4.0mm, respectively. Lingual recession demonstrated a median improvement of 1.0mm and buccal recession did not exhibit any median change from baseline to follow-up.

Conclusions: In the current study, the combination treatment of FDBA and BioXclude™ in 1-, 2-wall and intrabony defects was successful in improving PD, CAL and REC. The obtained results were comparable to the limited number of previous studies utilizing amnion chorion membranes for GTR.

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LIST OF ABBREVIATIONS

FDBA	Freeze-Dried Bone Allograft
DFDBA	Demineralized Freeze-Dried Bone Allograft
PD	Probing Depth
CAL	Clinical Attachment Level
CEJ	Cemento-Enamel Junction
PDL	Periodontal Ligament
REC	Recession
GTR	Guided Tissue Regeneration
ePTFE	Expanded Polytetrafluoroethylene
dPTFE	Dense Polytetrafluoroethylene
ACM	Amnion- Chorion Membranes
PDGF-α	Platelet Derived Growth Factor-alpha
PDGF-β	Platelet Derived Growth Factor-beta
FGF	Fibroblast Growth Factor
TGF-β	Transforming Growth Factor- beta
PO	By mouth
NSAIDS	Non-Steroidal Anti-Inflammatory Drugs
Q6h	Every 6 hours
Q8h	Every 8 hours
mg	Milligrams
TSP	Teaspoon
OZ	ounce

CHAPTER I: REVIEW OF THE LITERATURE

Introduction

The goal of periodontal therapy is the preservation of the natural dentition by controlling the inflammatory processes that cause bone loss and eventual tooth loss. When oral hygiene and non-surgical therapies cannot control periodontitis, surgical therapy is advised to clean deep pockets and try to create a periodontal architecture for efficient plaque control to minimize recurrence of disease. More than half a century ago, pocket reduction surgery via osseous re-contouring of diseased bone was the primary approach, but often resulted in un-esthetic and uncomfortable root exposure. As wound healing and etiology became better understood the field moved towards regenerative therapy, aimed at re-growing lost periodontal structures to improve post-surgical architecture. Regeneration is often employed during the treatment of intrabony defects (See Figure 1) that present in patients with periodontal disease (See Figure 2).

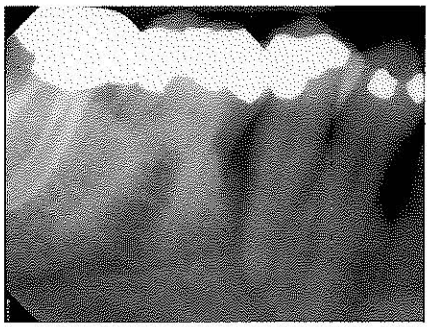


Figure 1: Mandibular right first and second premolar with radiographic evidence of intrabony defects

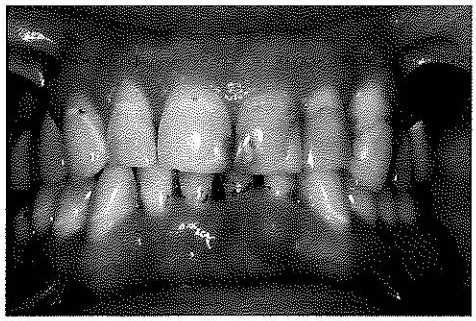


Figure 2: Patient with chronic periodontal disease leading to a periodontal abscess associated with the mandibular anterior teeth

Pathogenesis of Periodontal Disease

Periodontal disease is a pathologic process affecting the periodontium and is observed clinically as a spectrum from gingivitis to periodontitis (**AAP Glossary of Periodontal Terms, 2001**). Prevention of gingivitis is the first step in the prevention of periodontitis (**Burt, 2015**). According to **Löe et al. (1965)**, gingivitis, one of the most common forms of inflammatory disease, is associated with the presence of microbial plaque. However, the pathogenic disease process is multifactorial and as plaque accumulates, inflammation extends to the supporting tissues of the teeth. Progressively destructive changes can lead to loss of bone and the periodontal ligament. The pathogenesis of periodontal disease was extensively evaluated by **Page and Schroeder (1976)** and is described as the stages of disease progression. The process begins with the initial lesion at 2-4 days being characterized by increased migration of leukocytes and increased vasculitis of the vessels underlying the junctional epithelium. Untreated disease then progresses to the early lesion at 7-10 days and is characterized by accumulation of lymphoid cells at sites of acute inflammation, cytopathic alterations in fibroblasts, and continued loss of the collagen network that supports the gingiva (**Page and Schroeder, 1976**). The established lesion develops at 10-21 days and is characterized by early pocket formation due to apical migration and lateral extension of the junctional epithelium, continued loss of collagen, plasma cell infiltrate and presence of extravascular immunoglobulins (**Page and Schroeder, 1976).** The advanced lesion, which does not develop in all individuals, involves formation of periodontal pockets and the presence of irreversible attachment loss and bone loss.

Chronic periodontitis is defined as an infectious disease state that is characterized by progressive attachment and bone loss and is often accompanied by pocket formation

and/or recession of the gingiva (**AAP Glossary of Periodontal Terms, 2001**). **Eke et al. (2012)** determined that approximately 47% of American adults aged 30 years and older have periodontitis, with 38% of the adult population 30 years and older and 64% of adults 65 years and older having either moderate or severe periodontitis. The etiologies for periodontal disease are multifactorial and many patients present with risk factors that increase likelihood of initiation and progression of periodontal disease. However, once periodontal disease occurs, the removal of a risk factor may not result in a cure or elimination of the disease state (**Timmerman, 2006**).

Clinical Indicators of Periodontal Disease

When determining the nature and presentation of periodontal disease, many different clinical parameters can be utilized to evaluate the extent of periodontal destruction. Clinical attachment loss is considered the gold standard and is a measure of how much periodontal support has been destroyed. Clinical attachment loss has also been advocated as the most practical clinical parameter for the assessment of regenerative outcomes (**Kiany and Moloudi, 2015**). The severity of periodontal disease is characterized on the basis of the amount of clinical attachment loss with slight chronic periodontitis measuring 1-2mm of clinical attachment loss, moderate disease measuring 3-4mm of clinical attachment loss and severe disease being ≥5mm of clinical attachment loss (**Armitage, 1999**). Probing depth measurements aid in the diagnosis of the severity of the periodontal disease. According to **Armitage (1999)**, probing depths ≥5 & <7 mm and probing depths ≥7mm with inflammation present indicate moderate or severe periodontal disease, respectively.

Probing depth is assessed by gently inserting a periodontal probe into the gingival sulcus or a periodontal pocket. In health, insertion of the periodontal probe is resisted by the intact connective tissue and probing depths generally will measure 3mm or less. When periodontal disease is present, the periodontal probe inserts more deeply into the pocket because the connective tissue has been destroyed and no longer offers resistance to probe penetration.

Loss of clinical attachment precedes radiographic bone loss by a period of 6-8 months (**Goodson and Haffajee, 1984**). As the disease process continues and more clinical attachment is lost, bone loss may become evident on radiographs once sufficient mineral content in the bone has been destroyed. **Bender (1961)** produced artificial osseous lesions in the mandibles of cadavers, evaluated them radiographically and determined that lesions in cortical bone can only be detected when there is perforation of the cortex and lesions in cancellous bone alone cannot be detected radiographically. However, dental radiographs still provide valuable diagnostic information and aid as a visual tool for detecting and characterizing proximal osseous defects throughout the dental arches (**Rees 1971**).

Treatment Modalities of Periodontitis

Laurell et al. (1998) states that in periodontally diseased patients, periodontal surgery is mainly performed to gain access to diseased surfaces, achieve periodontal pocket reduction or elimination, and to restore the periodontal tissues. In order to achieve these goals, a number of treatment modalities may be employed, including open flap procedures alone or combined with regenerative procedures that utilize bone grafts and/or

exclusion membranes. Regenerative therapy is aimed at reproduction or reconstitution of a lost or injured part. According to the **2005 AAP position paper** on periodontal regeneration, this process is defined histologically as regeneration of the tooth's supporting tissues, including alveolar bone, periodontal ligament, and cementum over a previously diseased root surface (**Wang et al., 2005**).

Given that periodontitis is commonly characterized by the formation of intrabony defects, multiple surgical procedures have been shown to be effective in treating these osseous lesions. (**Kao et al., 2005**). Regeneration may be considered when vertical osseous defects, interproximal and circumferential intrabony defects, and class II and III furcation defects are present. Prior to initiating regenerative therapy, clinicians should consider the prognosis of the tooth or teeth and the anatomy of the osseous defect. Defects that are 4-8mm in depth are generally more amenable to regenerative procedures (**Laurell, 1998**). The number of osseous walls surrounding the defect can also impact clinical outcomes. In a study utilizing Teflon membranes without bone grafts, **Cortellini et al. (1993)** found that 1-wall defects were filled only 39% of their initial depth whereas 3- and 2-wall defects were almost completely filled.

Guided tissue regeneration (GTR) and osseous grafting are two treatment modalities that have been documented to aid in periodontal regenerative therapy through differential tissue responses. Guided tissue regeneration refers to regeneration of the periodontal attachment, which may include use of barrier techniques with the aim of excluding epithelium and gingival corium due to the belief that they will interfere with regeneration (**AAP Glossary of Periodontal Terms, 2001**). Osseous grafting is completed with a demineralized or mineralized bone biomaterial and can be allogenic,

xenogenic, or autogenous in nature. Particulate bone graft that has been processed or milled, to include small particles of either cortical or cancellous or both types of bone, often is used for regenerative procedures. Alloplastic particulate grafting material, such as, bioactive glass, beta tri-calcium phosphate or calcium sulfate, are also available grafting materials, but are not commonly utilized in modern day practice.

Periodontal Regeneration

In **1976, Melcher** hypothesized that cells of the periodontium have the capacity to regenerate the alveolar bone, cementum, and PDL and specifically that regeneration of the cementum in the alveolar part of the periodontium is achieved by cells that appear to take origin from the periodontal ligament. He further discussed that it may not be only PDL cells which are responsible for the regenerative outcomes in guided tissue regeneration, but cells may also come from the alveolar bone and assist in forming a new attachment apparatus (**Melcher, 1987**).

Periodontal regeneration using a barrier membrane was first described by **Nyman (1982)**. A case report utilizing one human subject and a Millipore filter demonstrated that new attachment can become established on a previously diseased root surface; however, no coronal regrowth of alveolar bone occurred. Subsequent studies by **Bowers et al. (1989)** pioneered regeneration using histologic studies that evaluated the formation of new attachment apparatus on pathologically exposed root surfaces. His studies showed that coronal regrowth of bone is possible and that periodontal regeneration of new cellular cementum, periodontal ligament and bone is clinically and histologically achieved. Regeneration was accomplished in submerged and non-submerged defects and

the addition of an allograft bone material was shown to significantly increase the amount of regeneration (**Bowers et al., Part I and Bowers et al., Part II, 1989**). Historically, **Becker (1993)** was one of the first clinicians to demonstrate the long-term follow-up of angular osseous defects treated by GTR and found that after an average of four years, patients had sustained probing depth reductions and clinical attachment level gains.

Extensive evidence-based research on graft materials and regenerative therapy has shown that allografts have high osteogenic potential and are the choice material for periodontal regeneration (**Mellonig, 1981 Part I, Mellonig, 1981 Part II, Sogal and Tofe, 1999**). Research also demonstrates that allograft materials are safe for use in humans. **Mellonig (1995)** completed a comprehensive review of bone graft donor selection, and the testing and inactivation of transmissible diseases in bone allograft material. His study concluded that the chance of obtaining a bone graft from an HIV infective donor is 1 in 1.67 million and the probability of HIV transmission after receiving a bone graft was 1 in 2.8 billion. To date, there are no documented cases of disease transmission from any dental bone graft product.

In comparison to demineralized freeze-dried bone allograft (DFDBA), freeze-dried bone allograft (FDBA) has been shown to be osteoconductive **(Mellonig, 1981).** Osteoconduction is the physical effect in which the matrix of the graft material forms a scaffold allowing cells from the recipient to penetrate the graft and form new bone (**AAP Glossary of Periodontal Terms, 2001**). According to **Schallhorn (1977)**, FDBA has several advantages, such as good induction potential and the mineralized nature of FDBA allows for greater space maintenance properties at graft sites compared to DFDBA. Moreover, FDBA may stimulate earlier, more rapid and substantial new bone formation (**Yukna and Vastardis, 2005**).

In a study by **Mellonig et al., (1976)**, freeze-dried cortical bone allografts of a fine particle size were implanted into wide three-wall, two-wall, one-wall, combination, and furcation defects. It was observed that 23 of the 97 osseous defects treated with grafts obtained complete bone fill, with 39 exhibiting greater than 50% fill, and 23 with less than 50% fill. This study indicated that FDBA resulted in 50% or better bone fill in 64% of the defects and stimulated a partial fill in another 24% of the cases providing strong evidence that FDBA has definite potential as grafting material in periodontal osseous defects (**Mellonig et al., 1976**).

A study by **Rummelhart et al., (1989)**, compared DFDBA to FDBA in promoting bone repair in human periodontal osseous defects and determined that one-walled defects treated with DFDBA and FDBA, when evaluated six months postoperatively, did not display statistically significant differences from each other and resulted in a mean PD reduction with overall gains in CAL and comparable degrees of fill of periodontal defects.

Barrier Membranes

Guided tissue regeneration to achieve periodontal regeneration involves the placement of a barrier membrane around the tooth and under the replaced tissue flap. A membrane is a thin sheet-like usually non-autologous material used in periodontal regenerative procedures (**AAP Glossary of Periodontal Terms, 2001**). A membrane serves four purposes in periodontal regeneration: 1) space maintenance, 2) blood clot stabilization, 3) wound stabilization, and 4) epithelial exclusion. Epithelial exclusion is an important factor in the type of attachment that forms after flap curettage and root

planing procedures and that with regard to regeneration, if the epithelium is excluded, new cementum and new connective tissue can form (**Bowers et al., 1989**). Additionally, the use of barrier membranes allows for selective repopulation of the periodontal defect with mesenchymal cells from the periodontal ligament (**Trejo, 2000**).

Barrier membranes utilized during GTR procedures can be either resorbable or non-resorbable. Non-resorbable membranes used for GTR include expanded polytetrafluoroethylene (ePTFE), and nonporous or dense polytetrafluoroethylene (dPTFE). Non-resorbable membranes can be titanium reinforced to provide superior space maintenance; however, they do require a second surgical intervention for membrane removal after several weeks of healing (**Rakhmatia et al., 2013**). Non-resorbable membranes have also been shown to have higher rates of post-surgical membrane exposure and complications (**Murphy 1995**). Studies have shown no difference in bone fill outcomes between resorbable and non-resorbable membranes (**Garrett, 1997, Cortellini, 1996**). Thus, resorbable membranes are generally preferred for periodontal regenerative procedures.

Resorbable membranes used for guided tissue regeneration include polyglactin, polylactic acid, and non-crosslinked and crosslinked collagen. Recently, human placental-based membranes have been introduced as a promising bioresorbable membrane that can be utilized for guided tissue regeneration in addition to many different types of periodontal surgical treatments such as socket preservation, ridge augmentation and repair of sinus perforations (**Holtzclaw, 2013, Holtzclaw, 2014, Holtzclaw, 2014, Holtzclaw, 2015**). Placental barriers such as amnion- chorion membranes (ACM) demonstrate many unique properties such as incorporation of proteins including collagen

types I, III, IV, V, and VI, laminin-5, platelet derived growth factors alpha (PDGF-α) and beta (PDGF-β), fibroblast growth factor (FGF), and transforming growth factor- β (TGF-β) (**Hodde, 2002, Holtzclaw, 2012**). These proteins have been implicated in providing a bio-active matrix to facilitate wound healing, specifically laminin-5, a known glycoprotein that has significant involvement in epithelial cell motility and junctional epithelium attachment (**Holtzclaw, 2012**). Benefits of using amniotic membrane not only aids in maintaining the structural and anatomical configuration of regenerated tissues, but also contributes to the enhancement of healing through reduction of postsurgical scar formation and provides a rich source of growth factors (**Koob, 2013**).

Amnion-Chorion Membranes

BioXclude™ is a second-generation dental placental allograft and is composed of dehydrated amnion-chorion laminate. (See Figure 3) BioXclude™ is known to contain significant amounts of laminin-5, which is a glycoprotein involved in epithelial cell motility and junctional epithelium attachment (**Kinumatsu, 2009**). Studies have also indicated that the unique properties of ACM also have the ability to positively affect cell proliferation, inflammation, metalloproteinase activity and recruitment of stem cells, all of which are paramount biological processes in regenerative wound healing and soft tissue repair (**Koob, 2013**).

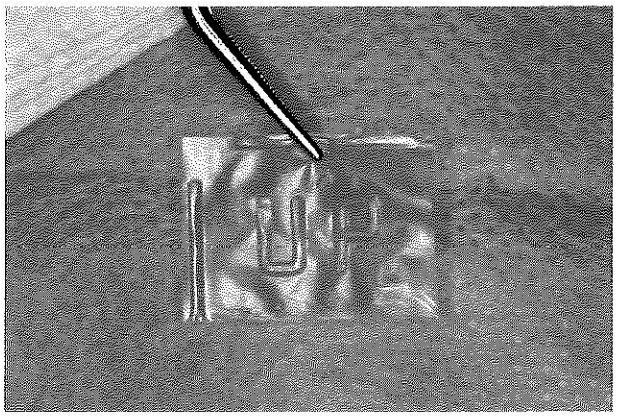


Figure 3: Clinical photo of BioXclude™

Positive benefits have been observed when the ACM, BioXclude™, was utilized for the treatment of intrabony defects in patients with localized moderate to severe periodontitis. **Holtzclaw (2013)** reported statistically significant clinical outcomes were achieved in GTR treatment of intrabony periodontal defects utilizing bone allograft and BioXclude™. At 12-months postoperatively an average probing depth reduction of 5.06 ± 1.37mm and a clinical attachment level improvement of 4.61 ± 1.29mm was observed. BioXclude™ has been shown to provide similar regenerative results in GTR procedures compared to commonly used collagen membranes **(Holtzclaw et. al, 2012 and Kiany and Moloudi, 2015). Kiany and Moloudi (2015)** completed the only known study comparing the efficacy of amnion membrane and a resorbable collagen membrane (Bio-Gide™) for the treatment of intrabony defects. In a split mouth design, chronic periodontitis patients with bilateral intrabony defects ≥4mm were treated with either amnion membrane and Bio-Oss™ or Bio-Gide™ membrane and Bio-Oss™. Results indicated that after six months, both amnion membrane and Bio-Gide™ demonstrated significant improvements in probing depth and clinical attachment, but there were no significant differences between the two treatment groups **(Kiany and Moloudi 2015).**

While recent studies with BioXclude™ appear to demonstrate promising results when utilized for GTR, additional studies are warranted to further evaluate the effectiveness and superiority of ACM for the treatment of intrabony defects. Thus, the objective of the present study is to assess whether BioXclude™ will provide predictable periodontal regeneration when used in conjunction with FDBA in challenging 1- and 2-wall intrabony periodontal defects.

CHAPTER II: MATERIALS & METHODS

This was a case series designed to track the progression of periodontal regeneration in patients treated using BioXclude™ and FDBA. Seven of the planned 54 subjects were enrolled and completed GTR with bone graft utilizing FDBA and BioXclude™. Each subject completed six months of post-surgical follow-up. The study was open to all eligible beneficiaries. The inclusion and exclusion criteria are listed below:

Inclusion Criteria:

a. Patient aged ≥18 years old
b. Patient remaining in the Capital region for at least 6 months following the surgical procedure for follow up appointments
c. Diagnosis of generalized or localized moderate to severe periodontitis
d. Radiographic evidence of a 1- to 2- wall intrabony defect at one or more sites with a probing depth ≥ 5mm
    1. If the patient presents with more than one defect site meeting inclusion criteria, the site with the deepest probing depth was used in the study

Exclusion Criteria:

a. Patient under the age of 18
b. Patient moving from the Capital region area prior to 6 months following the surgical treatment
c. Patients with restorations extending beyond the cementoenamel junction (CEJ) at the intrabony defect site
d. Patients with an indiscernible CEJ either clinically or radiographically

e. Patients with periapical pathology, unrestored caries, defective restorations, root resorption, or vertical root fracture on study teeth.

f. Patients requiring restorative dental care in the proposed surgical area (fillings and crown and bridge work) that cannot be completed prior to fabrication of the customized stent

g. Female patients who were pregnant or nursing

h. Patients who were smoke tobacco or tobacco product users. Former smokers were excluded if they quit smoking < 6 months prior to selection in the study.

i. Patients with clinically significant systemic diseases that could impair healing (e.g. uncontrolled diabetes).

j. Patients with poor oral hygiene unsuitable for periodontal surgery

k. Patients who could not or will not sign the informed consent form

l. Patients receiving immunosuppressive therapy such as chemotherapy and systemic corticosteroids not to include inhaled or topical steroids

m. Patients with severe endocrine-induced bone diseases (e.g. hyper-thyroidism, altered parathyroid function)

n. Patients with bleeding complications (e.g. hemophilia)

o. Patients on warfarin therapy

p. Patient with a history of osteoporosis or taking bisphosphonate medications

q. Patients with a history of radiation therapy in the head and neck area

r. Patients whose teeth with intrabony defect have mobility classified as Miller class 2 or greater

The evaluation and screening process was as follows. The subject was referred for a periodontal evaluation and the findings of their comprehensive periodontal evaluation such as PD, CAL, and REC were recorded on the Navy Periodontal Chart Form - NAVMED 6660/2 (See Appendix B). Dental radiographs were taken and evaluated for evidence of intrabony osseous bone loss and defects. The patient was subsequently treatment planned for GTR with bone graft. The subject was given a half page study brief on the research project. If the subject was interested, the principal investigator discussed the study, and consent forms were signed by the subject. The subject was assigned a study identification number, and the date of enrollment was recorded, as well as age, gender, and tooth number and tooth surfaces with the deepest probing depth. If the subject was not interested in or was unwilling to consent to the study, the treatment plan was continued as planned (See Appendix A).

Following consent, an alginate impression was made of the study arch and was poured up to make a stone model, which was used to fabricate a customized acrylic stent. (See Appendix D). The acrylic stent was used to measure PD, CAL and REC.

Subjects were all assigned to the same surgical protocol utilizing FDBA and BioXclude™. Clinical parameters were measured using the customized plastic stent and a UNC-15 periodontal probe and the PD, CAL, and REC were recorded. Plaque scores and bleeding scores were recorded as positive or negative. Once the intrabony defect was accessed and fully debrided, intra-operative measurements of the depth of the defect from the CEJ, dimensions of the defect mesio-distally and bucco-lingually and the number of walls were recorded. Any osteoplasty that was indicated to improve periodontal architecture and

remove osseous ledging and/or spicules that may prevent flap closure, were removed. Intramarrow penetration of the intrabony defect was completed and the FDBA was hydrated and placed into the intrabony defect and the BioXclude membrane was placed over the bone graft. The flap was replaced and sutured using a non-resorbable suture over the membrane.

Post-operatively, the subject was prescribed appropriate analgesics and a salt water rinse (See Appendix C). The subject was recalled at 1, 2, 4, 6, 8 weeks and 3 and 6 months for plaque removal and to assess healing. Periodontal parameters were assessed at 6 months and compared to the baseline measurements to determine change in PD, CAL and REC. Presence or absence of bleeding on probing and plaque were also assessed at 6 months post-operatively.

CHAPTER III: RESULTS

Seven subjects with eight 1-, 2-wall or combination intrabony defects were enrolled and evaluated. From baseline to 6 months, notable differences were detected for PD, CAL and REC. The postoperative healing of each patient assessed at weeks 1, 2, 4, 6, 8 weeks and 3 and 6 months was uneventful for all enrolled subjects.

Table 1 depicts patient demographics and defects dimensions. Values noted in Table 1 were reported as median changes. Four male and three females, with a median of age 50 years old and ranging from 40 to 55 years of age, were included in this study.

Subjects	*n=7*
Age (y)	50 [40 - 66]
Gender	4M, 3F
Defects	*n=8*
CEJ Depth (mm)	
Buccal	5.5 [5.0 – 16.0]
Lingual	7.5 [5.0 – 14.0]
Size (mm)	
M/D Buccal	3.0 [2.0 – 6.0]
M/D Lingual	3.0 [2.0 – 5.0]
Buccal-Lingual	7.5 [5.0 – 9.0]
Trapezoidal Area	18.75 [14.0 - 49.5]

Table 1: Patient demographics and intrabony defect dimensions

As viewed in Table 1, the average defect depth was 5.5mm with a range of 5.0-16.0mm measured from the buccal aspect and the average depth of the defects measured

from the lingual aspect was 7.5mm with a range of 5.0-14.0mm. The average buccolingual width was 7.5mm with a range of 5.0-9.0mm.

The data for this study was depicted utilizing line and bar graphs, as a small set of observations were completed and it was therefore possible to report all of the data and the changes from baseline to 6-month re-evaluation for each treated site. The x-axis depicted time, while the y-axis denoted changes in treatment variables, such as PD, CAL or REC. Gray bars showed the median changes for all of the defects from baseline to follow-up. Each line was an individual site that was treated. Green lines depicted positive treatment outcomes, while red lines indicated a negative treatment outcome. The improvement in each of these dependent measures was assessed using the paired Wilcoxon signed-rank test.

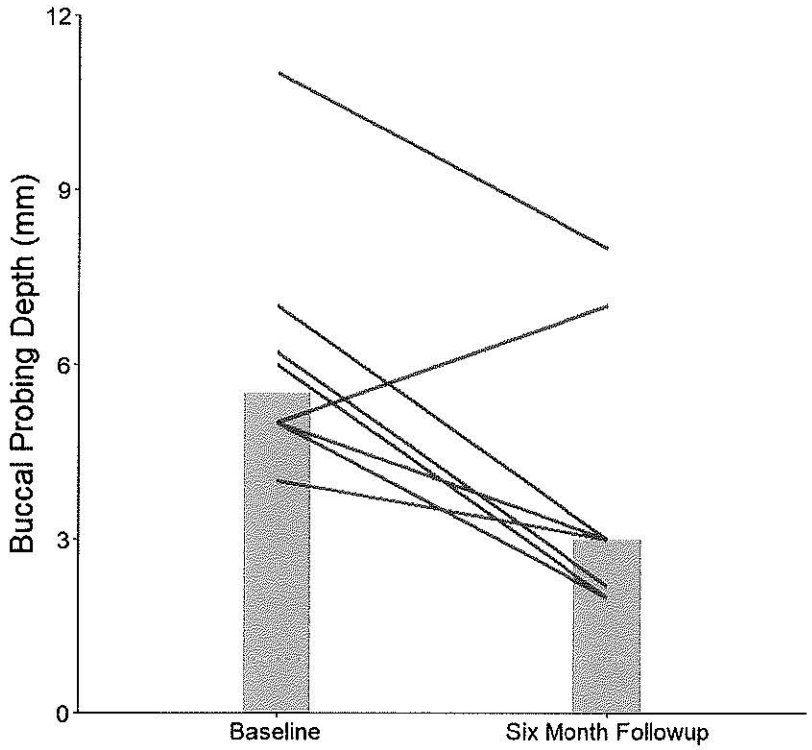


Figure 4: Buccal Probing Depth

Figure 4 shows changes in buccal PD from baseline to 6-month re-evaluation. Seven out of eight defects showed predictable positive trends for improvement in buccal PD. One defect demonstrated increased PD measurements from baseline to 6-months. Statistically significant median change for buccal PD was calculated as an improvement of 3.0mm. The p-value for buccal PD was 0.03.

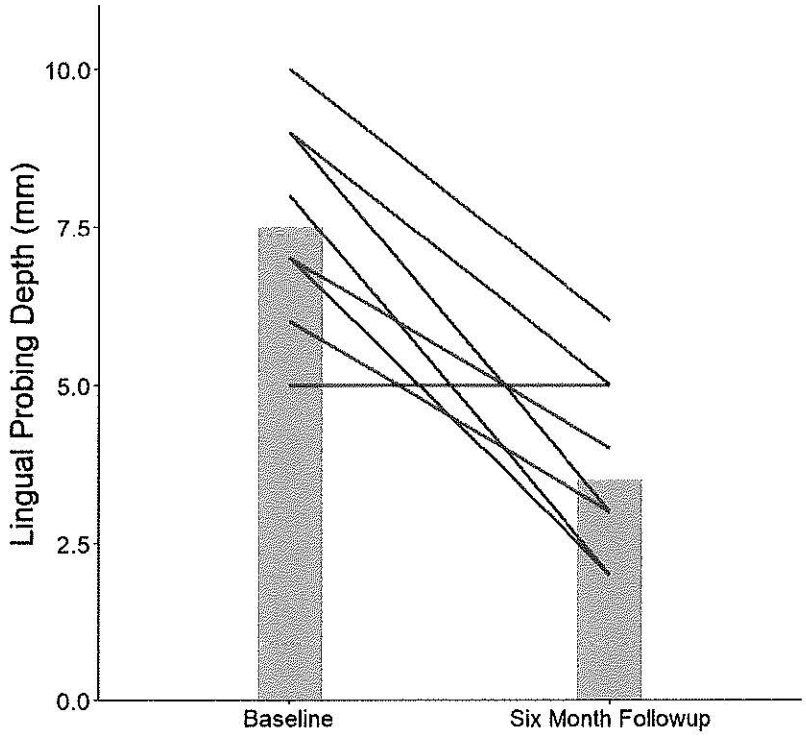


Figure 5: Lingual Probing Depth

Figure 5 shows changes in lingual probing depths for each treated site. All but one of the treated intrabony defects exhibited improvement at 6-month re-evaluation. The site that did not improve is the same defect, noted in Figure 4 that exhibited worsening of buccal PD at 6-months. The median change for lingual PD was 4.0mm and the p-value was 0.02. Therefore, statistically significant improvements were observed.

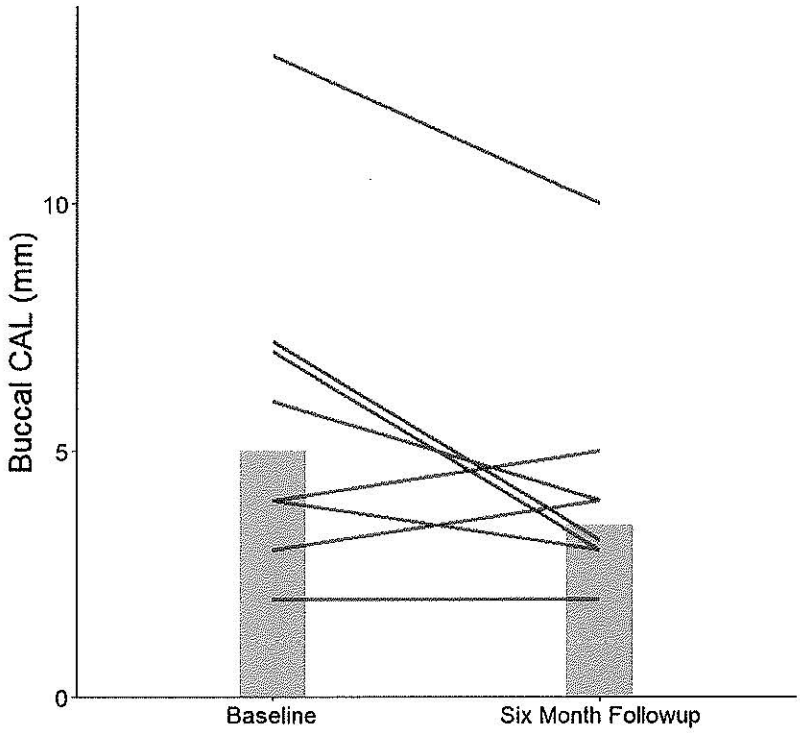


Figure 6: Buccal Clinical Attachment Level

Changes in buccal CAL are depicted in Figure 6. Buccal CAL demonstrated a median improvement of 1.5mm. Five out of eight defects improved, one defect did not change, and two sites worsened. The calculated p-value was 0.14.

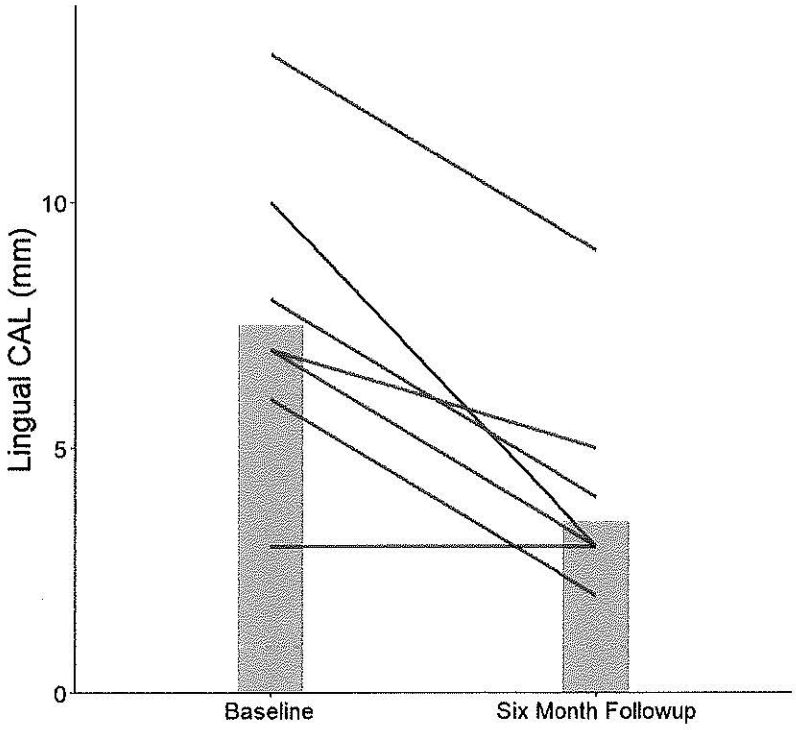


Figure 7: Lingual Clinical Attachment Level

In comparison to buccal CAL, lingual CAL exhibited notable positive trends of improvement from baseline to 6-months. Seven of eight defects improved and one site did not exhibit any change, but remained stable at 6-months. The median change was 4.0mm and the calculated p-value was 0.02, demonstrating statistical significance.

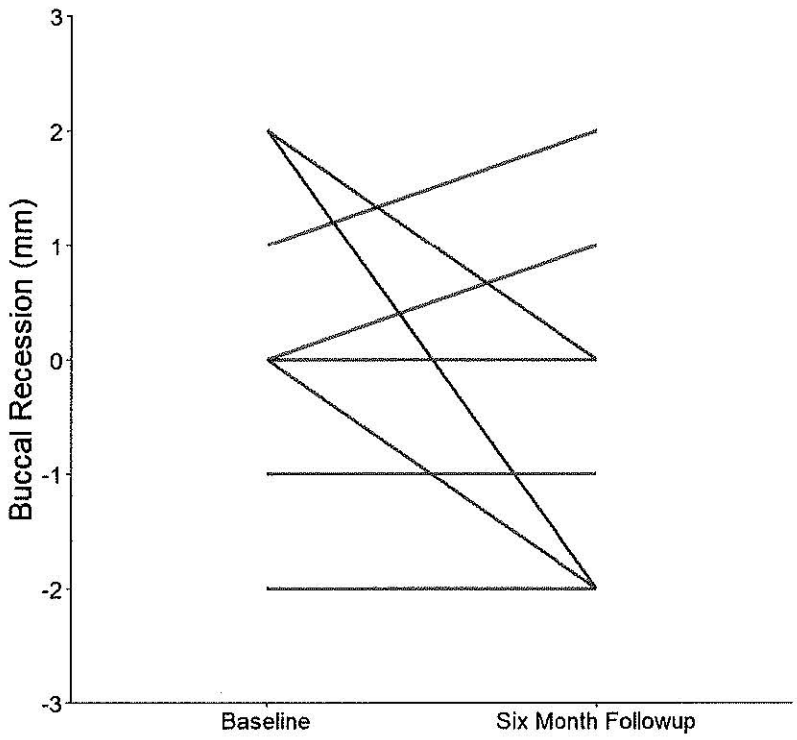


Figure 8: Buccal Recession

Figure 8 shows changes in buccal REC. Two out of eight defects were observed to have buccal recession at 6-months. Three sites did not exhibit improvement and three sites exhibited statistically significant improvements from baseline. The median change was zero and the p-value was 0.44.

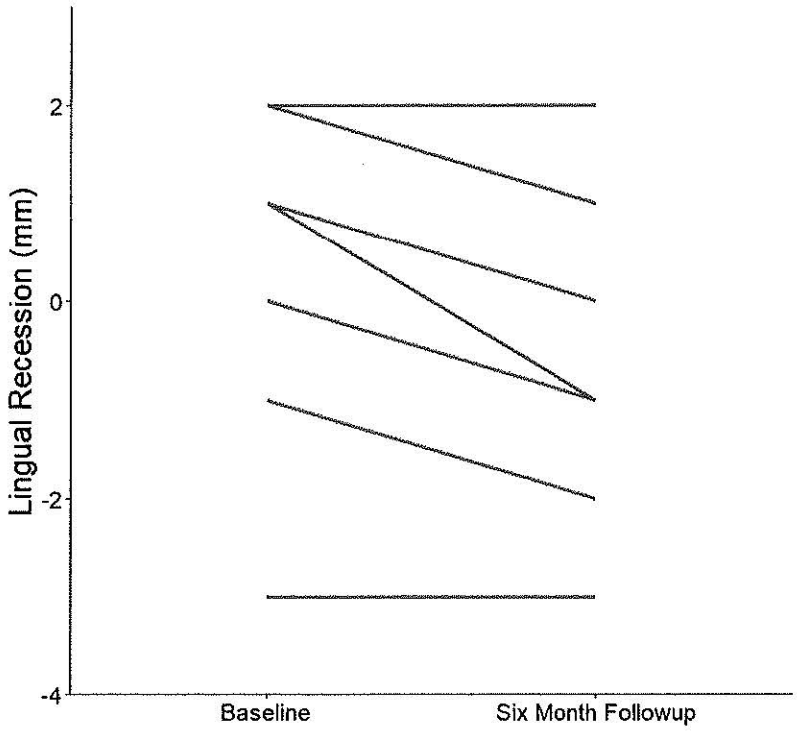


Figure 9: Lingual Recession

The data for lingual REC reflected positive trends as there was an overall median improvement of 1.0mm and no lingual sites exhibited REC at 6 months. The statistically significant improvements were assigned a p-value of 0.03.

CHAPTER IV: DISCUSSION

In the current study, the changes in PD, CAL and REC of teeth presenting with 1- and 2-wall intrabony defects after treatment with FDBA and BioXclude™ were evaluated. According to **Tal (1983)**, in a study examining dry human mandibles, approximately 50% defects were 2-wall, 36% were classified as 1-wall and 15% were 3-wall defects. **Cortellini (1993)** conducted a re-entry study evaluating 1-, 2-, and 3-wall intrabony defects after GTR with a non-resorbable membrane. At one-year surgical re-entry, **Cortellini (1993)** found that relative to the original defect depth, 3-wall defects were filled 95%, 2-wall were associated with 82% fill and 1-wall defects were filled only 39% from baseline. His results demonstrated that as the number of intrabony walls decreases, the ability to fully regenerate a defect significantly decreases.

In a series of studies, **Bowers et al., (Part I, Part II, and Part III, 1989)**, histologically established that regeneration of intrabony defects resulted in new attachment, bone and cementum without evidence of ankylosis, root resorption and pulpal death. This landmark study demonstrated that regeneration with DFDBA supports the formation of a new attachment apparatus on previously diseased root surfaces. However, successful long-term treatment of 1- and 2-wall defects is still often challenging.

In the current study, the combination of FDBA and BioXclude™ was utilized in order to facilitate improved space maintenance associated with 1- and 2-wall defects and employ the unique biologic properties of BioXclude™, which includes over 55 biologic factors implicated in improving healing time, supporting improved angiogenesis, suppression of inflammation, and cellular exclusion. This combination of materials was

hypothesized to be advantageous in improving the PD, CAL and REC of 1- and 2-wall defects.

The clinical significance of the current study was that seven out of eight defects exhibited significant improvement in probing depth and clinical attachment level and only one defect did not improve. This finding was attributed to unfavorable defect morphology. In comparison to the other treated defects, this outlier defect was shallow and wide, and may not have been as amenable to grafting and containment of the particulate bone graft was likely more challenging. Additionally, it was reported that the patient did not adhere to recommended post-operative hygiene instructions. Therefore, the patient's compliance level may have led to deleterious clinical outcomes.
It should also be noted that more postsurgical recession was observed buccally than lingually. This finding may be possibly due buccal sites presenting with initially thinner gingival tissue which may have been more prone to postsurgical recession.

The results of the current study are similar to the outcomes of a 6-month randomized, controlled, blinded, clinical trial by **Kiany and Moloudi (2015)**, which compared bovine bone mineral utilized in conjunction with amnion membrane or collagen membrane, for the treatment of intrabony periodontal defects. The reported values for changes in PD were similar between studies. For the current study, the changes in buccal and lingual PD were 3.0mm and 4.0mm, respectively, and the PD changes reported by **Kiany and Moloudi (2015)** were 3.3mm. Comparison of outcomes for changes in CAL were also comparable between studies, as the current study reported a median improvement of 1.5mm buccally and 4.0mm lingually. **Kiany and Moloudi (2015)** reported an overall positive change of 3.0mm. In comparison to **Kiany and**

Moloudi (2015), the current study exhibited slightly more positive trends with regard to REC from baseline to 6-months. **Kiany and Moloudi (2015)** reported an overall loss of 0.3mm of REC, whereas the current study observed a 1mm improvement on the lingual aspect of treated sites.

While there were similarities in general outcomes of clinical data between these two studies, significant differences in study design and the methodology must be noted. For example, the current study utilized allograft particulate bone whereas the previous study utilized bovine material. In the current investigation, clinical parameters were reported for both the buccal and lingual surfaces, as these are three-dimensional defects, whereas the **Kiany and Moloudi (2015)** study only reported one data point per defect. The present study only included 1- and 2- wall defects while the previous investigation also evaluated healing of 3-wall defects, which have been reported in the literature to be more amenable to treatment **(Cortellini, 1993).**

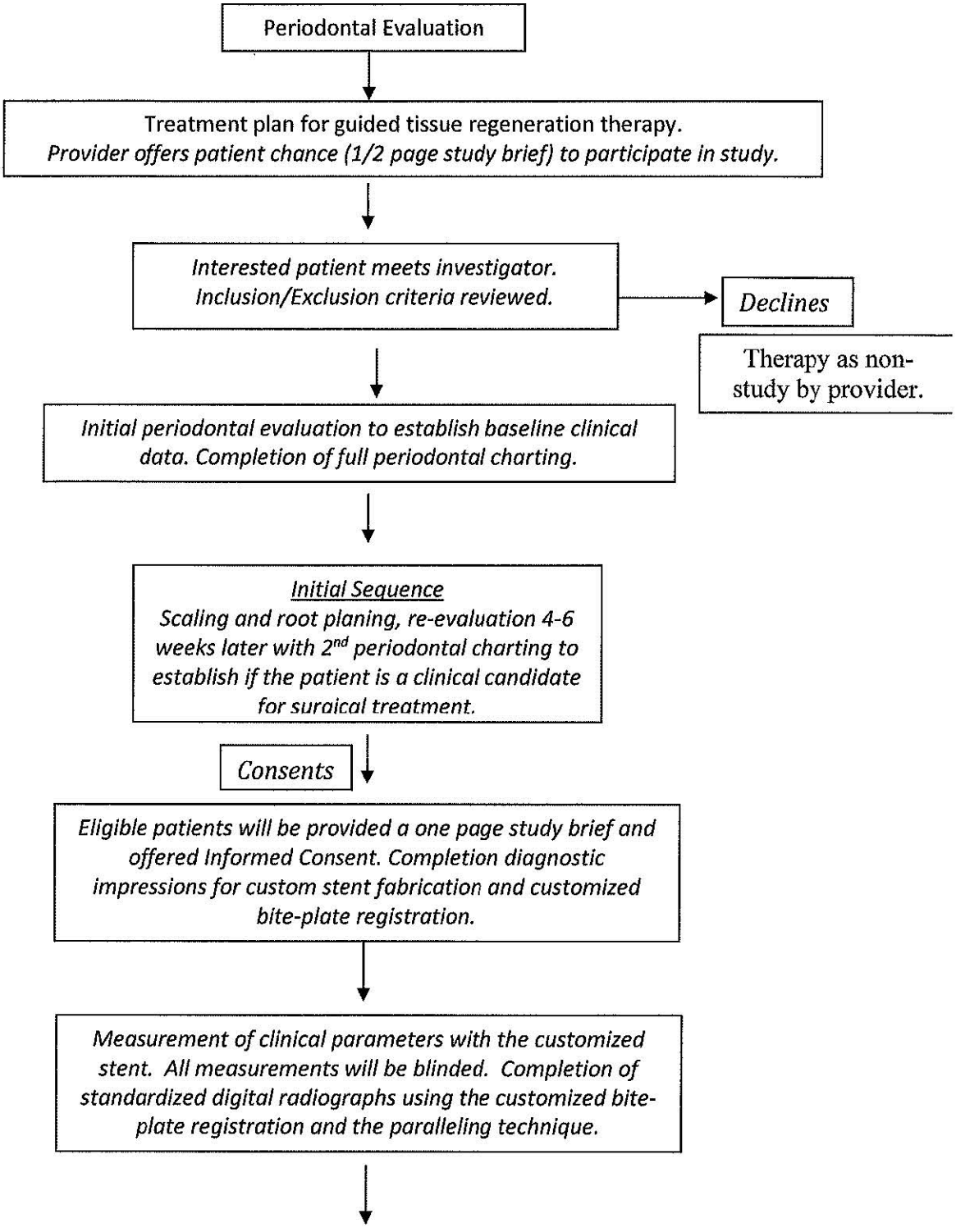
Although the reported results were similar to previous reports **(Holtzclaw et. al, 2012 and Kiany and Moloudi, 2015)**, the current investigation was only a case series and included a limited number of subjects. To the author's knowledge, there are no other studies which utilize FDBA with BioXclude™ for GTR, therefore, it was reasonable to establish treatment efficacy prior to conducting a more robust clinical trial.

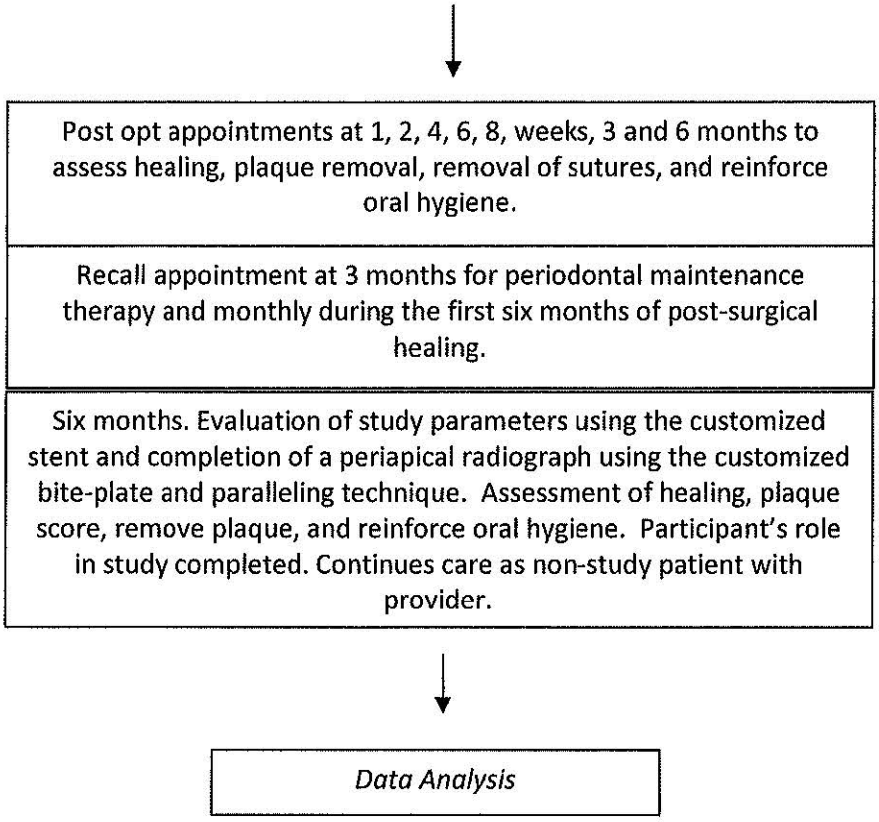
CHAPTER V: CONCLUSION

In conclusion, this case series demonstrated that guided tissue regeneration of 1- and 2-wall intrabony defects, utilizing FDBA and BioXclude™, exhibited notable improvements in PD, CAL and REC six months after treatment. Additional studies with larger subject enrollment and longer-term follow-up are strongly recommended as future research endeavors.

Appendix A: FLOW DIAGRAM OF STUDY DESIGN

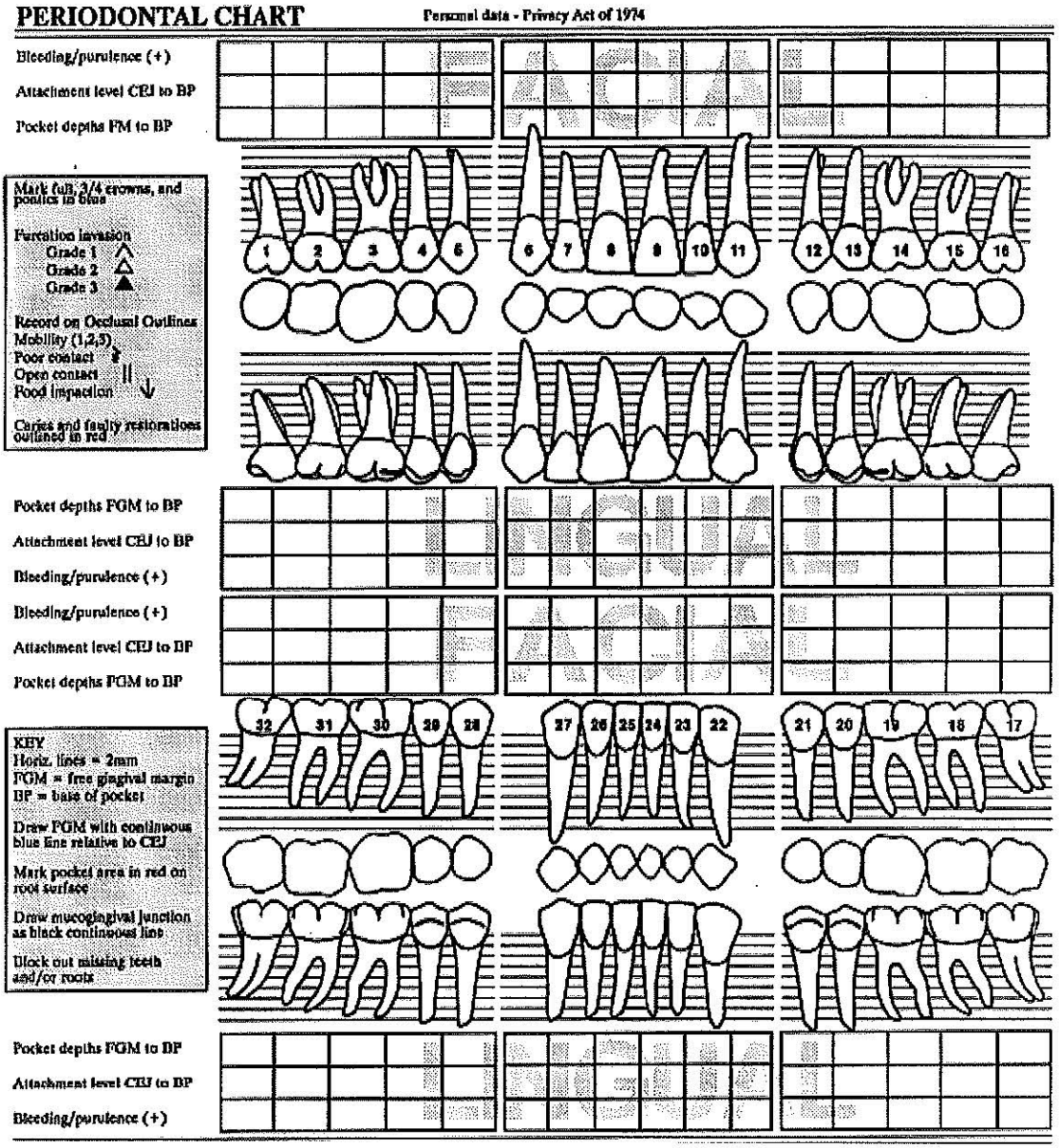
(standard clinical processes in normal font, *research in italics*)




Post opt appointments at 1, 2, 4, 6, 8, weeks, 3 and 6 months to assess healing, plaque removal, removal of sutures, and reinforce oral hygiene.
Recall appointment at 3 months for periodontal maintenance therapy and monthly during the first six months of post-surgical healing.
Six months. Evaluation of study parameters using the customized stent and completion of a periapical radiograph using the customized bite-plate and paralleling technique. Assessment of healing, plaque score, remove plaque, and reinforce oral hygiene. Participant's role in study completed. Continues care as non-study patient with provider.
Data Analysis

Appendix B2: COMPREHENSIVE PERIODONTAL CHARTING FORM

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



APPENDIX C: POST-OPERATIVE INSTRUCTIONS

POST-OP INSTRUCTIONS FOR FDBA/BIO-XCLUDE PROTOCOL
PERIODONTICS DEPARTMENT
NAVAL POSTGRADUATE DENTAL SCHOOL
Bethesda, Maryland

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

PAIN MEDICATIONS:

_____ Motrin 800 mg 1 tablet every 6-8 hours. Do not double up on dosage

_____ Vicodin 5/325 mg or _____ Percocet 5/325 mg 1-2 tablets every 6 hours for pain control. It can be taken in addition to ibuprofen. These narcotic medicines 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 1 tablet three times a day for 7 days

_____ Clindamycin 300 mg 1 tablet three times a day for 7 days

RINSES:

_____ Salt Water Rinse: 1 tsp in 8 oz. water, rinse 30 sec, twice a day 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 side opposite the surgery. 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.

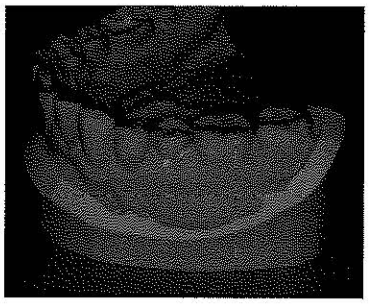
If you have any problems or questions, please do not hesitate to call me at 301-295-0077. If there is an emergency, you may page your doctor through the automated system. Instructions will be given after dialing 1-800-759-8888.

Your follow-up appointment is scheduled for: _____

APPENDIX D: STENT FABRICATION

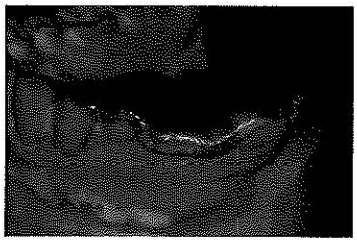
Before surgery a dental impression is made of arch where extraction will occur

- Stone model made from impression
- A customized acrylic stent will be fabricated on model
- Stent will allow 6 standardized measurements of bone level at the surgical site
    - *Before graft placed*
    - *At 6 months, post-surgically*

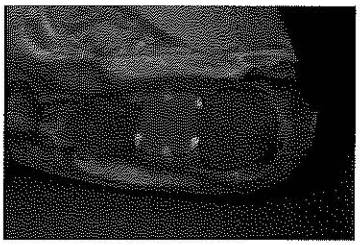


Stent Fabrication Process

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

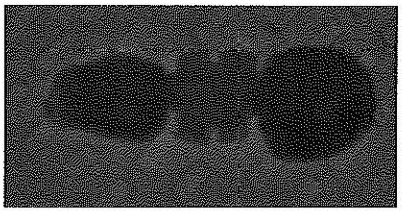


- Acrylic trimmed to just above the height of contour of tooth to be treated
- A 169 fissure bur cuts grooved slots in the stent
- Slots accommodate periodontal probe for 6 measurements of bone levels at same location and angulation



After the surgical exposure before graft placement

- Stent placed on adjacent teeth to measure mesial-distal and buccal-lingual defect dimensions and all study parameters.
- Stent stored in disinfectant container marked by subject's study number
- At 6 months, stent placed on adjacent teeth for measurement of study parameters.



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