COMPARISON OF DENSE POLYTETRAFLUOROETHYLENE (PTFE) AND AMNIOTIC TISSUE MEMBRANES WITH MODERN RIDGE PRESERVATION TECHNIQUES ON HUMAN ALVEOLAR RIDGE PRESERVATION

by

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ABSTRACT

Comparison of dense polytetrafluoroethylene (PTFE) and amniotic tissue membranes with modern ridge preservation techniques on human ridge alveolar preservation

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This single-blind, randomized clinical trial compared two different, commercially available membrane materials to assess their efficacies in socket preservation procedures in 24 patients. This study sought to compare the materials as part of routine, clinical/periodontal therapy in order to determine superiority. Patients were seen at baseline, then 1, 2, 4 weeks, and 4 months post-treatment. Patients were distributed equally into the following groups: Cytoplast (CO), Cytoplast with additional buccal augmentation graft, (CBA), BioXclude (Bx), and BioXclude with additional buccal augmentation graft. (BxBA). Cone beam computed tomography (CBCT) scans were compared between a pre-operative baseline time point and a scan taken four months after healing. The percent of alveolar horizontal and vertical ridge change was measured utilizing subtraction radiology and Dolphin™ 3D software. Secondary outcomes included ease of use, perceptions of pain, complications, cost-effectiveness, and changes in keratinized tissue. Due to the relatively small sample size attained at an early point of

this long-term study, no conclusions with statistical support could be drawn regarding a direct comparison between materials. However, both materials showed loss at all measurement points. There seemed to be no difference in pain scores between the two materials. The continuation of this study may offer some statistical and clinical significance once the population needed is met.

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INTRODUCTION

STATEMENT OF THE PROBLEM

Despite dental advances, tooth loss remains an issue in the modern world, with an estimated 20 million tooth extractions annually in the United States. [1] New techniques are providing more options for tooth replacement; typically these techniques require adequate bone at the treatment site. However, it is common for bone loss from the alveolar process to occur following extraction. This loss has been the subject of extensive clinical study and new techniques to slow or arrest the loss are now at the forefront of research, yet the biologic process after a tooth has been extracted is still not understood fully. It has been shown that a majority of the bone loss will occur within the first six months after extraction. [2] [3] [4] This change is important as the restoration of this edentulous area relies on adequate bone and soft tissue support.

The end goal of any surgery is to provide a predictable outcome with optimal results. Preventing the loss of a tooth is the primary goal of treatment but once the decision has been made to extract the tooth, preventing undue effects of the extraction that can affect the final restorative treatment plan becomes the primary goal. Preventive measures taken at the time of the extraction can provide additional options moving forward with treatment.

Dentists and their patients both have similar desires for the results. Both would like to see a surgery that results in minimal pain and a surgery that uses products that provide superior and predictable results. Newer materials and techniques have increased the probability of such outcomes. Dental material companies rarely provide claims on the superiority of their new products versus existing current products, but rather equivalence

or non-superiority backed with research studies that are mostly funded by the parent company. This funded research then leads to advertised features or advantages of a given product over an alternative.

Newer materials on the market for ridge preservation include high-density polytetrafluoroethylene (PTFE) as well as amniotic tissue-derived membrane materials. Previously, primary closure was strongly encouraged for success with available resorbable materials used in ridge preservation, but based on the techniques provided by the manufacturer, the benefit that these two newer products share over previous material is that primary closure is no longer needed. Since secondary intention healing is seen in tooth extraction, not having to adapt the wound edges allows for maintenance of the periosteum.

Membrane usage in ridge preservation maintains the hard tissue interface, allowing maturation of newly forming bone. Many studies show the ability of PTFE to maintain the healing area for bone by blocking epithelial growth, whereas most of the studies for amniotic-derived membrane explain that the growth factors in the membrane promote the closure of the epithelium, thereby preventing down-growth. [5]

This study aimed to compare commercially-available and similar products and techniques, in order to determine not only equivalence, but any superiority. Since tooth loss remains an important issue in dental services, the results would help determine which membrane provides better results in socket preservation after extraction of teeth.

SIGNIFICANCE

Maintaining alveolar width is a significant concern for dental treatment plans, and utilizing the best products to achieve the best results is paramount. There are many products in the dental field with a wide variety of claims. Most products show research that has been completed or funded by the parent company. By providing an independent direct product comparison, a better understanding of product claims can be ascertained. Reproducibility allows for a standard of care to be set, which in the Army, with multiple clinics around the world, can offer the benefit of continuity of care as soldiers relocate. Also by testing products that may make claims of superiority and have a substantial cost, a clinical trial can prove or disprove the necessity for such expense. As the budget for the military continues to evolve, a basis for spending will be necessary. Determining a predictable outcome to a common surgery such as tooth extraction will decrease the need for additional appointments, thereby decreasing the soldiers' need for additional future dental appointments.

Review of the Literature

INTRODUCTION

Tooth loss continues to affect many people, with an estimated 20 million teeth extracted in the US every year (Marcus). It is estimated that over 40% of people over 60 years old in the US are edentulous. Restorative treatment plans are driven by how the extraction area heals, and improved healing leads to more treatment options available to the patient and better predictability of surgical outcomes.

CONSEQUENCES OF BONE LOSS

Bone loss after an extraction is greatest in the buccal aspect and most (but not all) loss is seen in the first six months. Tan et. al showed that the loss can range from 29 - 63% while overall vertical loss can range from 11-22%. [2] Schropp noted that most of the vertical loss is seen in the first 3 months, and the average horizontal reduction is 5-7 mm over 12 months following the extraction. [3] In a dog study, it was noted that both the buccal and lingual walls resorb after extraction, but the loss in height is more pronounced on the buccal wall. The resorption is seen in two phases; first a replacement of the cortical bone by woven bone, and then the outer resorption of both buccal and lingual walls. [6] A systematic review from 2009 searched 1244 MEDLINE-PubMed and 106 Cochrane papers, finding 12 that met the eligibility criteria. This review showed that the clinical loss in width to be greater than the loss in height, which were assessed both clinically and radiographically. [7] This loss can limit the options for restorative treatment or require additional augmentation to reclaim some of the loss.

STRATEGIES TO PREVENT BONE LOSS

The overall goal for grafting is to retain and preserve the original ridge form. Many different materials and techniques have been suggested and used to slow or arrest the inevitable bone loss following extraction. A 2014 systematic review suggests that most of the histologic studies show remaining unresorbed graft material and this may account for the reported difference between test and control groups. [8] Lekovic showed a significant difference in alveolar ridge dimensional change between the test and control groups; overall 1 mm less vertical resorption and 2 mm more bone fill in the test group that utilized ridge preservation techniques. The average residual ridge width was 6 mm in the test group versus 3 mm in the control sites. [9]

Most techniques try to achieve the result of maintaining isolation by filling the socket and then keeping it isolated while it heals. The materials have varied over the years and with recent technologic changes, materials that assist the healing process have been utilized. While there are many materials available, such as autogenous bone, allografts, xenografts and alloplasts, the allografts such as freeze-dried bone allograft (FDBA) and demineralized freeze-dried bone allograft (DFBDA), are among the most commonly used. A review of techniques showed the material that should be chosen to graft the extraction socket should possess the following properties: 1) the material should maintain space for bone to repopulate the graft and thus recreate the bone volume close to original; 2) the bone formed should have a density to allow for stable placement of the implant; thus, the material placed should have excellent osteoconductive features to enhance bone formation; 3) the material should be relatively inexpensive and readily available, without communicating pathologic conditions. [10]

The benefit to DFDBA is that it has been shown to have osteoinductive potential based on the work completed by Urist in the 1960s. FDBA is osteoconductive and differs only in that the body still must break down the mineral content, but this allows it to help provide initial space maintenance. Autogenous bone offers the best biocompatibility but also may bring the co-morbidity of an additional harvest site and the rapid turnover may provide less space maintenance. Xenografts offer ideal scaffold for new bone formation, but have been shown to resorb very slowly leaving less newly formed vital bone upon reentry for implant placement. Both allografts and xenografts may be refused due to religious beliefs or possible fear of disease (although it has been shown that there is only a 1:2.8 billion chance of contracting HIV *via* a bone graft and no reported cases exist). [11] Wood and Mealey showed no significant difference in dimensional stability between DFDBA and FDBA when used in ridge preservation, but histologically did note more vital bone and less residual graft material with the DFDBA test group. [12]

MEMBRANES USED FOR RIDGE PRESERVATION

Many types of barrier membranes have been used, but none found to be ideal for every clinical situation. Macroporous membranes, such as expanded polytetrafluoroethylene (ePTFE), require primary closure due to a high porosity and a second surgical procedure for their removal. Their macroporosity seems to enhance regeneration by improving wound stability. However, exposure allows for bacterial contamination, soft tissue in-growth, membrane degradation and possible graft exposure. [13] Bartee et. al showed case reports using macroporous membranes that incorporated bacteria when exposed, which would require removal of the membrane. Nowzari noted that presence of pathogens on the tooth-facing surface of the membrane was the critical

determinant for the success or failure of GTR. [14] Resorbable collagen membranes require primary closure of the augmentation site and exhibit variable patterns of resorption of the underlying bone. The e-PTFE membrane and resorbable membrane classically require soft tissue coverage or primary closure to prevent soft tissue ingrowth, bacterial contamination, infection, membrane migration, early membrane degradation, and graft exposure. [15]

D-PTFE pore size is substantially smaller than that of an e-PTFE membrane, which is typically 5-30 micrometers. The d-PTFE membrane does not require primary closure due to its submicron pore size of 0.2 micrometers, which means surgical areas can be large defects, while still preserving the interdental papilla, and preserving the full width of keratinized mucosa without the concerns of bacterial contamination. The small pore size, which is smaller than many bacteria, ensures the underlying graft is not affected by not allowing bacterial cells to penetrate through the membrane. This is a significant advantage over e-PTFE and resorbable membranes. The density of the d-PTFE membrane precludes colonization by the host flora and prevents the biomaterial-centered infections associated with exposed e-PTFE membranes (Barber). Krauser examined d-PTFE membranes that had been exposed for 3 weeks and noted occasional fibroblast-like cells and no bacterial colonization on the inferior surface, with colonies of bacteria on the superior surface. [13]

BioXclude

Fetal tissues have been used in medical procedures for well over 50 years. Amniotic-derived tissue, known as BioXclude, was recently introduced as a new barrier for site preservation. It is relatively thin (300μm) with self-adhering properties once it

becomes moist, eliminating the need for suturing of the membrane. Unlike cadaveric allograft, xenograft and alloplast barrier membranes, placental allografts are composed of immunoprivileged tissue, possess anti-bacterial and anti-microbial properties, reduce inflammation at the wound site, and provide a protein-enriched matrix to facilitate cell migration. [5]

Since neuropeptides and peptide hormones act as cytokines and/or growth factors, placenta and intrauterine tissue offer a tool for investigating multiple functions of peptides. [16] Growth factors such as FGF, PDGF, VEGF, and TGFβ in dehydrated human amnion/chorion allografts (dHACM) grafts provide: 1) angiogenic growth factors retaining biological activity; 2) promote amplification of angiogenic cues by inducing endothelial cell proliferation and migration and by upregulating production of endogenous angiogenic growth factors by endothelial cells; and 3) support the formation of blood vessels *in vivo*. [17] Koob also investigated grafts as a promising wound care therapy with the potential to promote revascularization and tissue healing within poorly vascularized, non-healing wounds.

BioXclude does not have to be secured into place with sutures. Primary closure should be attempted, but is not required. It contains high concentrations of laminin specifically laminin-5 throughout the membrane, potentially providing a bioactive matrix for cellular migration, in addition to growth factors known to facilitate wound healing, such as platelet derived growth factors alpha and beta (PDGFa, b) and transforming growth factor beta (TGFβ). The presence of these proteins is likely to be one of the reasons why the use of BioXclude allows for rapid sealing of the underlying graft material used for site preservation. [5] A difficulty with the interpretation of this

information is that most of these articles were either funded by the parent company or the authors report a direct financial relationship or conflict of interest due to their affiliation with the company.

Cytoplast

Prior forms of PTFE material required that the primary closure of a wound was needed over the graft material during the healing phase, and that if it was exposed it needed to be removed because of increased bacterial colonization. Hoffman showed that dense PTFE allows for secondary intention healing to take place with an exposed matrix. In the Hoffman study, a d-PTFE membrane was placed over an extraction site with no attempt at primary closure and histologic samples were taken from 10 cases during implant placement 12 months later. [18] Bone fill was observed in extraction sockets when Cytoplast was used following tooth removal and subsequent healing. In addition, Bartee showed in a case report the use of d-PTFE and the ability for bone formation to take place in an extraction socket. [19] A literature review spanning 1980 to 2012 found 24 articles discussing the use and properties of d-PTFE, a combination of *in vitro*, clinical studies and experimental studies. The review concluded that there is limited histological and clinical evidence regarding the use of dense membranes, with some limited indications for GTR, GBR, implants, and fresh extraction sockets. [13]

PAIN AND PERCEPTION

A further consideration in the choice of materials for use in treatment is the effect on pain (although this consideration is often overlooked). Pain is defined as the body's response to damaging or potentially damaging contact with the outside world. Nociceptors, the body's tissue receptors for pain, can determine the stimulus, such as

crush, temperature, and chemical stimulation, which can threaten or actually cause damage to tissue. Describing pain can be very subjective and vary greatly between individuals. Pain is considered a unique and individual experience. A person's perception can change with age, overall health, and previous experiences. Stress can increase an individual's perception of pain. The limbic system is the portion of the brain that registers emotion, but the cortex, which handles complex thinking, can exert an undue effect on a person's perception of pain. As an indication of the general population's perspective on dental procedures, studies to determine the total amount of pain experienced in an operation have used a comparison to other "bad experiences, such as 'an average trip to the dentist'". [20] Presurgical anxiety can increase a patient's post-operative pain perception, and this is seen more in implant surgery than periodontal surgery. Generally people experience less post-operative pain with periodontal procedures than implant placement. [21]

Laminins are major non-collagenous glycoproteins that are a part of the basement membrane. They have been implicated in a wide variety of biological processes including cell adhesion, differentiation, migration, signaling, neurite outgrowth and metastasis. Bioxclude is said to contain laminin 5 which is hypothesized to produce faster healing which was shown by reduced patient pain perception. [5] However, the histochemistry to back this claim was referenced from a poster presentation which has yet to have the information published in a peer-reviewed journal.

Materials and techniques chosen should be determined using evidence-based research. Factors such as surgical time, patient comfort, and cost can play a role in technique and material choice. The ultimate goal is to use a material that provides

predictable results for the surgeon, decreases pain for the patient and maintains a low cost. Based on the research available, these desired results are often claimed with available techniques and materials but have yet to be compared directly, and the evidence behind use of the material is often limited. The effect size of a given claim is of paramount importance. A growth factor or protein may reduce pain, but to what degree? Whether the reduction is of any clinical relevance remains to be seen in many situations.

Manufacturers are under no obligation to disclose or quantify a theoretical benefit of a product. The clinician falls into the unfortunate situation of making clinical decisions to optimize patient care, but with imperfect information upon which to base the decision. A systematic review showed that industry investment has increased in biomedical research over the last 20 years while government investment has decreased. [22] Although industry research tends to be of high quality, its primary focus remains on types of research such as randomization and blinding versus using appropriate control therapies, pitting test groups against a placebo and overall dosage. This review also showed that industry sponsored research tends to find pro-industry results. Academic institutions and the federal government are alternative sources for research, but unfortunately take longer to publish data due to funding. For-profit contractors produce 60% of the research that is funded by industry in a quicker and cheaper fashion than academic institutions. Since it is funded by industry, what happens if the results return unwanted results? This can mean publishing delays, data withholding or even destroyed findings. [22]

LITERATURE SEARCH

Little information exists on the metrics of the dental literature; and establishing the relative proportion of studies comparing multiple products cannot be determined readily. As a precursor to this protocol, the investigating resident conducted a manual search of three well-known dental journals (JP, JCP, and JADA), reviewing titles and abstracts for all articles over a two year period (July 2013-July 2015). The search reviewed over 1200 journal articles. Key words and focus for article selection searched for head-to-head comparison of commercially available products or techniques. Nineteen articles were identified that met the search criteria. Of these 19, only 4 completed a direct comparison of two commercially available products that performed similar functions (See Table 1). This means that in three of the main journals used for evidence-based decisions, only 1.5% of the articles actually help to differentiate between products on the market. Most studies comparing or evaluating a product tended to compare the product (or technique) to a placebo or a historical technique (e.g. connective tissue graft). The question must be raised, why is the literature so limited?

A recently published article (2015), discussed that most randomized trials that perform a head-to-head comparison are industry-sponsored trials. These trials typically use non-inferiority/equivalence design and tend to produce "favorable" results. Because these trials typically utilize a larger study population and better methodological quality than non-industry-funded trials, they tend to be more frequently cited with their "favorable" results. This article, however, showed that rarely is the head-to-head comparison completed for superiority and frequently the comparison is to placebo, no treatment, or standard of care, which may not capture the true evidence of head-to-head comparisons and the benefits and harms of alternative interventions. [23]

In an editor's note in JAMA 2015, it was noted that a medical device currently being used in cardiology (IABP) had been invented in the 1960s, prior to the passage of the 1976 Medical Device Amendments that gives the FDA the authority to require evidence for effectiveness and safety. It has since been shown that this device offers no benefit in both randomized clinical trials and observational studies. A newer high risk device (PVAD) that received market clearance did so utilizing the FDA's 510(k) pathway, which does not require clinical evidence of safety or effectiveness, but rather that the device is substantially equivalent to another device on the market. The device on the market for this newer device was most likely the device that had never received approval; both of these devices are high risk machines used on critically ill patients. They are expensive and invasive, and despite evidence are used frequently in clinical practice. [24] While the FDA pushes to get treatment modalities to patients in an expedited process, many high risk devices get approved through the FDA Premarket Approval (PMA) program, which requires premarket clinical evidence providing reasonable assurance of safety and effectiveness and permits post-approval changes. Concern has been shown regarding the studies that support the approval of high risk devices. The FDA can require additional information and studies through its post-approval system (PAS), but between 2005-2011, only a quarter of these studies were completed. Most premarket studies were also limited. The majority of devices were cleared on the basis of 2 studies; 1 non-pivotal and 1 pivotal. The pivotal studies typically enroll less than 300 patients, and are designed without blinding, comparators, or primary endpoint follow-up exceeding 1 year. [25]

In a world where clinicians are encouraged to utilize evidenced-based practices, it is concerning to see how devices and procedures are approved and what true bias may be behind the published articles. It is only recently that the dental community has decided to further review the relationship between published results and industry funding. In 2013, Brignardello et. al reviewed the association between conflicts of interest and positive results, and found in a search of the ten dental journals with the highest impact factor, the odds ratio for positive results varied between 2.40 and 9.19. [26]

Purpose:

The purpose of this project was to test two recently introduced membranes which do not require primary closure in conjunction with ridge preservation in a blinded study. Ridge width measurements were monitored throughout the healing phase and surgical complications were noted. Post-operative pain level was assessed with patient questionnaires and a visual analog scale.

Hypotheses

HYPOTHESIS #1

No difference will be seen in bone loss between dense PTFE and amniotic tissue membrane when used for ridge augmentation.

HYPOTHESIS #2

Post-operative surgical complications, including pain, will be significantly less with amniotic tissue membrane than dense PTFE.

Specific Aims

AIM #1

Determine the quantitative change in alveolar ridge width and height after extraction and ridge preservation using allograft bone material along with either BioXclude or Cytoplast membrane. Determine which product preserved underlying ridge dimensions better, regardless of bone grafting technique utilized. This was accomplished by measuring cone beam computer tomography (CBCT) bone dimensions before extraction and approximately 4 months post extraction.

AIM #2

Determine if post-operative complications, including pain perception, were reduced by using the amniotic tissue membranes compared to dPTFE membranes. General complications were observed and reported using the Post-Operative Assessment form. Pain medication prescriptions and dosing was standardized for all participants. Patient reported pain measurements were reported at 1 week post-operation using a 1-10 scale.

Materials and Methods

Patient Selection and Assessment

This study was a prospective, single-blinded, randomized, human clinical study. Patients with hopeless teeth requiring extraction and potential dental implant placement were considered for inclusion in this study. At the time the initial assessment was confirmed by the periodontic staff and a tooth was deemed to be non-salvageable, the patient was asked if they would consider volunteering to participate in the study. Any dentist from any specialty might designate a tooth as hopeless; however, the periodontics department staff was consulted routinely to assist in the final decision to extract the tooth. All patients who met criteria during the study period were asked to participate. Patients willing to participate signed consent forms after an IRB-approved consent process, and were given a random subject ID number for blinding purposes. Patients had a research note placed inside the dental record to indicate active involvement in the study. Randomization for treatment groups occurred *via* a random number table and stratification occurred across treating practitioners. The PI (a periodontics staff member) maintained all randomization information. The treating resident was given a sealed, opaque envelope the morning of the appointment. At this point, the treating resident was no longer blinded. The AIs (associate investigators) responsible for data collection remained blinded.

This study included all current periodontics residents in order to increase the generalizability of the results to the greater periodontics community. Board-certified staff oversaw the research as a component of the residency. This study strove to

standardize as many aspects of the procedure as could reasonably be achieved, while still allowing enough flexibility for clinicians to make decisions on behalf of the individual patient's best interest. All residents were initially calibrated and trained on nuances of the protocol, and all residents were required to have completed at least two ridge preservation procedures (of any type) within the last 12 months in order to participate in the study. Staff ensured adherence to all standards.

BASELINE DATA

Patients required a small-volume CBCT at baseline. At least one scan is routinely performed for any patient prior to dental implant therapy. The advantage of a CBCT over a medical head and neck CT scan is a much lower radiation dose, while still giving enough resolution to measure the alveolar structures accurately. Dosage for each small volume scan with the 3D Accuitomo 170 CBCT machine is approximately 30 µSv [27], roughly the dose of 1.5 digital panoramic radiographs. By comparison, a flight from London to Los Angeles is 80 µSv [28]. Data was recorded and turned in to the PI (See Figure 1). Diagnostic casts were used for CBCT radiographic guide fabrication to standardize ridge measurements pre- and post-operatively in cases where it would otherwise be difficult to determine references from fixed anatomical landmarks. The operating resident filled out a pre-operative clinical assessment (See Figure 2) and submitted to the PI for data storage (discussed below). Intra-oral clinical photos of the surgical site (no facial features) were gathered at baseline and were compared to future images.

SURGICAL PROCEDURE

The following procedure was entirely consistent with routine practice for socket preservation procedures performed in the residency program. If sedation was used, the technique was documented and was at the discretion of the treating surgeon and patient (sedation is considered a standard of care for this treatment). After local anesthesia application, teeth were extracted in accordance with principles of minimal trauma. All multi-rooted teeth were sectioned before extraction. When appropriate, alveolar bone was removed with fine surgical diamond burs. All sockets were debrided and full thickness mucoperiosteal envelope flaps were reflected to allow access approximately 10 mm apical on the buccal ridge and approximately 5 mm on the lingual ridge for membrane positioning. When needed, any vertical releasing incision was at least one tooth away from the extraction site. Freeze-dried bone allograft (FDBA) (~250-1000 microns; OraGRAFT, LifeNet Health, Virginia Beach, VA)(#MIN-CORT-.5) was placed in the socket. Patients receiving buccal overlay/augmentation received an additional 1-2 mm thick layer on the buccal surface to a depth of 5-7 mm. One of the membranes (according to the randomization schedule) was placed over the bone graft material and adapted to extend 10 mm apically on the buccal surface and approximately 5 mm on the lingual. When using dPTFE (Cytoplast, TXT1224, 12x24; Osteogenics Biomedical, Lubbock, TX),membranes were trimmed to cover the socket but did not encroach within 1 mm of the adjacent tooth. Amniotic tissue membrane (BioXclude, GB-1125, 1.5 x 2; Snoasis Medical, Denver, CO) was adapted and folded as needed for site coverage, again maintaining 1mm of distance from the adjacent tooth. Membranes were not fixed with any kind of tack or screw system. A continuous, running Gore-Tex 5.0 suture was placed for wound stability with two interrupted sutures at mesial and distal papillae. No attempt

was made to achieve primary closure, though the tissue was advanced if applicable as a function of good suturing technique. Clinicians deviated from the protocol if anatomical limitations were warranted (i.e. aberrant nerves). Modifications allowed for surgical details, but did not include altering selected materials or randomization. If modifications were minor (as determined by the PI and other staff) the patient was retained in the study. In brief, the patients were assigned a random identifier at the time of enrollment. The treating clinician submitted the data collection forms to the PI, who then de-identified the document by removing the PII and adding the random number (according to the master key file). The top portion was shredded. The de-identified documents were scanned and uploaded by the AIs for future data analysis.

POST-OPERATIVE MANAGEMENT

Post-operative medication was generally standardized, with caveats. All patients, barring allergy or intolerance, were given amoxicillin or azithromycin, hydrocodone 5mg, non-steroidal anti-inflammatory medications, and a chlorhexidine rinse. Residents could modify the prescriptions as needed to meet the needs of the patient (i.e. no patient was asked to endure unnecessary pain or take a drug he/she was not comfortable taking). Patients were recalled at 1, 2, and 4 weeks and a post-operative assessment form was completed by the treating clinician (See Figure 3). At the one week appointment, pain perception was recorded. At the two week appointment, sutures were removed and at 4 weeks, the dPTFE membrane was taken out; removal of amniotic tissue membrane (BioXclude) was not required. dPTFE (Cytoplast) removal was a relatively painless process, and did not require anesthesia. At approximately four months, an assessment was completed including a second small volume CBCT image that evaluated the implant

site. The implant placement was a second surgery but the research was completed at time of placement. The primary dependent variables were (1) percent of baseline alveolar ridge changes (both horizontal and vertical) and (2) whether or not the anticipated dental implant could be placed appropriately. Secondary outcomes included ease of use, perceptions of pain, complications, cost-effectiveness, and changes in keratinized tissue. A power analysis was calculated based on two different statistical estimates, in conjunction with relevant articles. The sample size for the study was estimated using G*Power with a fixed effects, special, main effects and interactions ANOVA statistical test for a power of 80% with p set to 0.05. A four-group design with the numerator degree of freedom of one (main effect or interaction) was used, with an effect size f of 0.25, considered to be a medium effect size for F-tests. This requires a total sample size of 128 subjects (32 per group). In terms of a clinical effect, this would provide a sensitive test with approximately 6% of the variance being due to the effect. A second sample size calculation was also conducted in the event that a MANOVA test would be utilized for the data. Using G*power, a MANOVA: repeated measures, between factors, effect size .15, power .80, 4 groups and 4 iterations, also gave a sample size of 128.

Interim results from the study were analyzed for the present document, using data collected from 32 subjects (eight per group). This sample size would have a 78% power to detect a main effect size f=0.5 (p=0.05). (If one treatment proved significantly better than the other three, the study would be suspended.)

Estimate Required Sample Size	**128**
Estimate Participant Drop Out / Withdrawal	**22** *(15-20%)*
Total Enrollment Requirement	**150**

DATA MANAGEMENT AND ANALYSIS

Once the final CBCT has been taken, DICOM (Digital Imaging and Communications in Medicine) files were taken from the Accuitomo machine and uploaded into 3D Imaging Software (Dolphin Imaging and Management Solutions, Chatsworth, CA) for analysis. Utilizing this program, the initial pre-extraction and four month healing CBCT images were able to be uploaded and super-imposed on one another. The super-imposition was accomplished utilizing similar anatomic reference points so the images were compared directly (See Figures 5 & 6). Subtraction radiography was completed with the super-imposed images and measurements were taken at the level of the alveolar crest, 2 and 4 mm apical from the crest. A vertical measurement was taken as well to determine if there was any vertical bone loss. The images were measured and analyzed independently by both associate investigators and a correlation coefficient was calculated. For any disputed measurements beyond 0.5, the cases were reviewed and discussed and an agreed conclusion determined.

The primary dependent variables were (1) percent of baseline alveolar ridge changes (both horizontal and vertical) and (2) whether or not the anticipated dental implant could be placed appropriately. Secondary outcomes included ease of use, perceptions of pain, complications, cost-effectiveness, and changes in keratinized tissue. Much of the additional data collected on the study forms will be analyzed at a later date as a secondary outcome of the study once more patients have bene enrolled.

STATISTICAL ANALYSIS

SAS 9.4 was used for statistical analyses. An alpha level of 0.05 was used to assess statistical significance. A preliminary analysis was done to examine the distributions and characteristics of the data. A Wilcoxon Signed Rank test was used to examine whether changes from baseline were significantly different from 0, for each measurement. A Wilcoxon Rank-Sum test was used to examine differences (in changes from baseline) between products and techniques. A Kruskal-Wallis test was used to examine differences between the 4 groups by product and technique. Pain assessment was analyzed using Mood's median test.

Results

All materials and methods were followed according to approved IRB protocol and the study was carried out successfully, with some minor deviations in patient treatment times, which were properly annotated. The clinicians were provided all data sheets along with a surgical checklist to ensure standardization of surgery. All data sheets were returned to the principal investigator after the clinician had seen the patient. The PI then removed all patient information. Workflow was monitored utilizing data spreadsheets in Excel to ensure tracking of patients information as well as dates of surgery. The information from the surgical and CBCT worksheets were logged by one of the two AIs (AL & KH). The information was then confirmed by the other AI to ensure accuracy. The information gathered was taken directly from the surgical assessment sheets (See Figure 1-4).

The initial measurement plan for bone loss was to use a fixed surgical stent to measure affect. It was decided that Dolphin 3D software could complete with high accuracy the superimposition of the two images and subtraction radiography could be completed. The baseline and post extraction (4 mos) CBCT files (DICOM) were downloaded into Dolphin 3D for analysis of bone volume change. The post extraction image was superimposed over the initial scan using 3 similar anatomic points (cusp tips, foramen, CEJ, and restorations) as well as the software image overlay function. This function utilized the surrounding anatomy of the two images in processing the overlay. The accuracy of this superimposition was due to the high resolution of the scans allowing for easy point identification. The investigator was then able to toggle between the two

images and see the differences at the extraction site. The initial scans were measured in horizontal planes and then toggled to the final scans for similar measurements as well as a vertical change. The alternate investigator would take an independent set of measurements. Both were blinded to which technique and material was used.

The CBCT information measurement was then turned over to the principal investigator to be de-identified so that analysis could be completed. After being de-identified, the associate investigators entered all data into a worksheet and averaged the measurements taken at crest, 2 mm and 4 mm. In total 28 patients were measured but 4 patients dropped out of the study leaving 24 patients used for measurement purposes. All groups showed loss in one or both directions. For statistical purposes, the median loss was used because the data was not normally distributed. In the CO group, the crestal loss was 6.03 mm, at 2 mm the loss was 4.45 mm, and at 4 mm, the loss was 2.00 mm. The CBA group showed losses of 7.20 mm, 2.53 mm, and 0.85 mm, respectively. In the BioXclude groups, the Bx showed loss at the crest of 9.63 mm, at 2 mm the loss was 5.80 mm, and at 4 mm the loss was 1.00 mm. In the BxBA, the losses were 8.98 mm, 2.45 mm, and 0.90 mm, respectively.

In direct comparison of the products, regardless of technique, the only statistically significant finding was at the crest where the median loss for Cytoplast was 7.05 mm and BioXclude was 9.05 mm. While found to be statistically significant, it should be viewed with caution due to low sample size. All other findings showed significant reduction from baseline, but the median decreases were not seen as significant. The vertical changes were 0.50 mm for Cytoplast and 0.40 mm for BioXclude, but these were noted to be not significantly different from zero or from each other.

Since the data deviated from normality and other assumptions needed for parametric testing were not met, non-parametric tests were used to assess whether the differences were significant. The statistics were completed not assuming normality or normal distribution. This was most likely due to the small sample group. In the first 27 patients to been treated, no adverse events were noted and three dropouts occurred. One patient moved unexpectedly, one retired from the military, and one was removed from the study due to non-compliance with appointment dates. Overall, patient scheduling and compliance were sometimes an issue regarding follow up care, suture removal and membrane removal, but usually only varied by a few days from the required date. Not all d-PTFE membranes were removed at 4 weeks, some were early and some late. A few cases had sites that were larger than the membranes selected for the study requiring modification or addition of a second membrane. Care had to be taken when enrolling anterior teeth, as reflection of the tissue or overlay grafting may have caused undesired esthetic results.

The pain scale for the two groups was divided into most significant pain for the first week and overall pain average for the week. The Cytoplast group showed an average of most significant pain of 32.9 $\pm$ 22.1 and an average week pain of 16.6 $\pm$ 14.0. The BioXclude group showed an average of most significant pain of 45.4 $\pm$ 25.1 and an average week pain of 20.6 $\pm$ 17.2. The median of the most significant pain and average pain for Cytoplast was 35.5 and 15.0, respectively. The median of the most significant pain and average pain for BioXclude was 36.0 and 15.0, respectively. (See Table 2 & 3)

The Mood's median test for the pain scores showed no significant difference between treatment groups. The p value for most pain was ~1.0 and the average pain p

value was ~0.48. The groups need to be larger to determine if there is any true clinical or statistical difference.

Discussion

For all the progress that has been made in the dental field and advances in our understanding of the biological processes, much still remains unclear regarding how to prevent the bone loss after extractions. High esthetic demand and cultural norms have patients requesting replacements that mirror their current dentition. While clinicians have been able to attempt to slow the body's natural resorption of the alveolar socket after extraction, there are still many unknowns. A lot of those unknowns start with the materials and techniques that are available. While most clinicians utilize evidence-based decisions to drive their practice, the published material they reference may be tainted or incomplete. Many dental materials tout high claims of effectiveness, which may be interpreted as superiority when in reality, the only testing of the material simply demonstrated non-inferiority or similarity. This leads to the clinician discerning how the evidence was gathered. Was it appropriately tested, and are there any conflicts of interest or high levels of bias to the information published?

This exploratory study showed most importantly that materials used in dental practices today are almost never tested against each other. There is no financial gain for a parent company to show superiority for risk that the study may not give statistically or clinically significant results. The manual search of major dental journals returned less than 2% of published articles over a three year period that showed a direct comparison of materials. This means when a clinician is trying to review a new material, they most likely are reading material that was published or funded by the parent company, and may not receive truly unbiased information.

Some limitations with this pilot portion of the study were observed. While randomization ensured a proper distribution of those patients needing a tooth extracted and desiring an implant into the test groups, it also had its short comings. Anterior teeth were particularly rare in this study, and these teeth are often in need of additional bone in order to ensure proper implant placement. On the contrary, posterior teeth can often show ample amounts of buccal bone and would not require augmentation. This was encountered in some cases, where clinically the patient was randomized into an overlay group but was already exhibiting over 2 mm of buccal bone. All teeth were randomized in one of four groups regardless of position in the mouth.

The parent company that makes the amnion chorion membrane suggests that clinicians be familiar with the product before making final judgments regarding its use. Most clinicians in the study had completed 2-5 cases prior to involving study patients. The material is very flexible and will passively lay over the socket and is easy to maneuver, provided it is placed where it is needed. One issue encountered while working with this material was attempting to complete overlays; trying to place a membrane that rapidly adheres to a wet surface while trying to maintain minimal flap reflection and place bone material for augmentation. Another touted benefit of the amnion chorion membrane is reduced pain during the healing period. Since the power of the study has not been achieved, no definitive conclusions can be drawn, but at this time, there does not seem to be a difference in pain scores. So while Cytoplast is not showing less post-operative pain, it is also not showing more, which with a higher n-value may prove that the claim of less post-operative pain may have no value.

The study size, at this current time, cannot provide significant findings consistent with the initial power analysis showing that 128 patients would be required to provide significance. At this point any conclusions drawn based on the results so far should be interpreted with caution. The non-parametric tests should be noted that they are less powerful than the parametric testing, but were needed based on the deviation from normal. Any non-significant results at this point are tied to the small sample size and do not necessarily imply that a difference does not exist; rather that there is not enough evidence in the data to conclude that a difference exists. The continuation of this study will be relevant to not only statistically significant results but also provide blinded, unbiased information regarding the direct comparison of two products touting similar clinical usage. The goal of the continued study would be to achieve the necessary number of patients in order to prove superiority of one product over the other with significant clinical implications.

TABLES

Table 1. Journal Search for Direct Comparisons

3 Year Journal Review	Total Articles Reviewed	Articles that met search criteria	Percentage of Comparison
Journal of the American Dental Association	515	0	0%
Journal of Clinical Periodontology	279	10	3.5%
Journal of Periodontology	445	9	2.0%

Table 2. Most Significant Pain Scores for each material

MATERIAL	MEAN	MEDIAN	STD DEVIATION
CYTOPLAST	32.9	34.5	22.1
BIOXCLUDE	45.4	36	25.1

Table 3. Average Pain Scores for each material

MATERIAL	MEAN	MEDIAN	STD DEVIATION
CYTOPLAST	16.6	15	14.0
BIOXCLUDE	20.6	15	17.2

Table 4. Horizontal Change at Crest by Product

Product	Variable	Mean	SD	Min	Max	Median	p-value*
Cytoplast	Baseline	9.11	1.72	6.35	12.05	8.65	
	Final	3.02	4.10	0	11.15	0.00	
	Change from baseline	**-6.09**	**3.21**	**-11.50**	**-0.90**	**-7.05**	**0.0005**
BioXclude	Baseline	10.20	1.68	7.95	14.3	9.93	
	Final	1.73	3.72	0	12.2	0.00	
	Change from baseline	**-8.47**	**2.62**	**-11.10**	**-2.10**	**-9.05**	**0.0005**

*Wilcoxon Signed Rank test

Median horizontal width at crest changed (decreased) significantly from baseline for both products. Also, the median decrease for Cytoplast was significantly less than the median decrease for BioXclude (Wilcoxon Rank Sum test, p = 0.0284).

Table 5. Horizontal Change at 2 mm by Product

Product	Variable	Mean	SD	Min	Max	Median	p-value*
Cytoplast	Baseline	11.07	2.79	7.1	16.6	10.70	
	Final	7.60	3.54	3.5	13.8	7.53	
	Change from baseline	**-3.47**	**2.53**	**-10.05**	**-0.75**	**-4.45**	**0.0005**
BioXclude	Baseline	11.89	1.88	9.35	15	11.38	
	Final	7.43	4.38	0	13.4	8.00	
	Change from baseline	**-4.46**	**3.99**	**-11.65**	**-1.1**	**-2.53**	**0.0313**

*Wilcoxon Signed Rank test

Median horizontal width at 2mm changed (decreased) significantly from baseline for both products. Although the median decrease for BioXclude was less than the median decrease for Cytoplast, the decreases for the two products were not significantly different from each other (Wilcoxon Rank Sum test, p = 0.9317).

Table 6. Horizontal Change at 4 mm by Product

Product	Variable	Mean	SD	Min	Max	Median	p-value*
Cytoplast	Baseline	11.73	2.66	8	16	11.58	
	Final	9.78	3.30	5.15	15.3	9.63	
	Change from baseline	**-1.96**	**2.31**	**-7.65**	**-0.2**	**-1.05**	**0.0005**
BioXclude	Baseline	12.88	2.37	9.6	17.4	13.05	
	Final	11.65	2.60	8.25	15.8	10.68	
	Change from baseline	**-1.23**	**1.19**	**-4.3**	**0.4**	**-0.90**	**0.0015**

*Wilcoxon Signed Rank test

Median horizontal width at 4mm changed (decreased) significantly from baseline for both products. Although the median decrease for Bioxclude was less than the median decrease for Cytoplast, the decreases for the two products were not significantly different from each other (Wilcoxon Rank Sum test, p = 0.8864).

Table 7. Vertical Change by Product

Product	Variable	Mean	SD	Min	Max	Median	p-value*
Cytoplast	**Change from baseline**	**0.16**	**1.19**	**-1.3**	**2.35**	**-0.50**	**0.7334**
BioXclude	**Change from baseline**	**-0.26**	**1.47**	**-2.1**	**2.95**	**-0.40**	**0.5195**

*Wilcoxon Signed Rank test

Although the median vertical height for both products indicates a slight decrease from baseline, the decreases were not statistically significant. Also, the median decreases for the two products were not significantly different from each other (Wilcoxon Rank Sum test, p = 0.3952).

FIGURES

Figure 1. CBCT Assessment Form

CBCT Assessment Form

Tooth number: _____

Date of Scan: _____

Is this scan pre-op or post-op? _____

If post-op, how long from time of surgery? _____

Was a reference marker used? _____

B-L ridge width as measured at mid-extraction socket (or using reference point):

At crest: _____

2mm below crest: _____

4mm below crest: _____

Cortical bone thickness:

At crest: _____

2mm below crest: _____

4mm below crest: _____

Figure 2. Baseline & Surgical Assessment Form

Baseline & Surgical Assessment Form

Tooth Number: _____

Keratinized Gingiva: _____ mm

Biotype: _____ (thick / thin)

Reason for extraction: _____ (be brief)

Presence or Absence of active local infection? Yes or No

Anticipated Elian classification: _____ (I II or III)

Difficulty of extraction: _____ (routine or complex)

Would GBR be required regardless of socket preservation? _____

Residual buccal plate thickness at crest: _____ mm (estimate with probe @ mid-ext site)

Residual ridge width at crest: _____ mm (estimate with probe @ mid-ext site)

Dehiscence or fenestrations? _____ (yes or no, if yes then how severe (% of buccal plate)?)

De facto Elian classification at closure: _____ (I II or III)

Ease of use of product: (very easy) 1 2 3 4 5 (very hard)

Figure 3. Post-Operative Assessment Form

Post-Operative Assessment Form

Time since surgery: _____ (days or weeks)

(1 week POT only) Please have patient place a mark along the scale indicating pain level:

Patient's report of most significant pain:

No pain _____ Extreme Pain

Patient's report of average pain experience:

No pain _____ Extreme Pain

Complications: _____ (substantial pain, graft failure, infection, etc)

Membrane removal: _____ yes or no *(this appointment only)*

This form is required at the one week post-op appointment AND for any complication or membrane removal appointment

Figure 4. Implant Surgery Assessment Form

Implant Surgery Assessment Form

Tooth Number: _____

Keratinized Gingiva: _____ mm

Residual ridge width at crest: _____ mm (estimate with probe @ mid-ext site)

If you were not able to place the implant today, please state why:

_____

If you were able to place the implant:

Implant platform placed: _____

Primary stability: _____

Was the implant size the intended implant or did you have to use a smaller implant?

_____

Were any additional augmentation procedures performed?

_____

If so, were these entirely necessary to place the implant IAW with prosth guidelines and have 2mm of buccal bone on the implant? Or was augmentation performed for MRB/CYA?

_____

If so, was GBR going to be required from the beginning?

_____

Figure 5. A pre-operative CBCT with measurements for illustration purposes

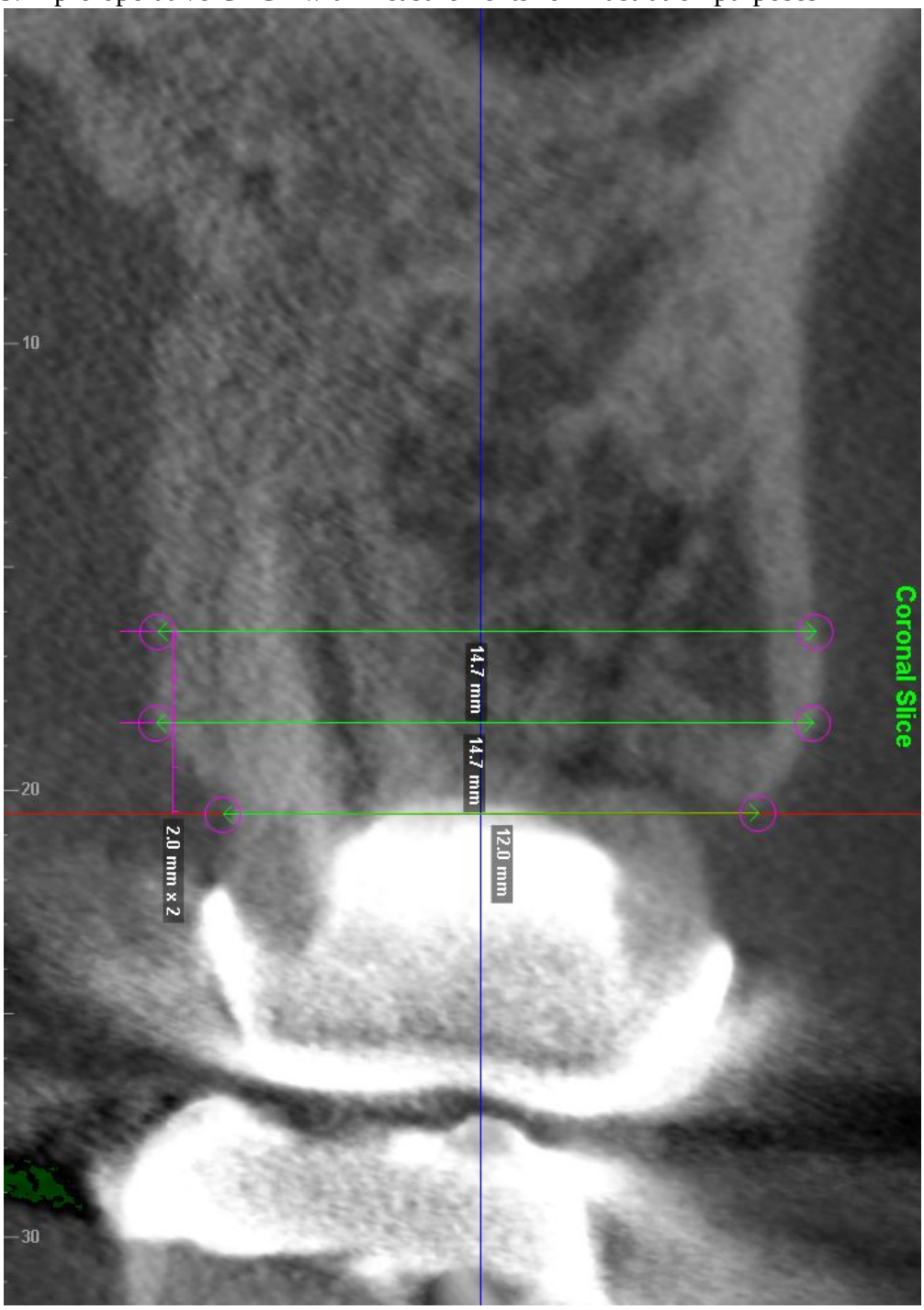


Figure 6. A super-imposed post-operative CBCT for illustration purposes

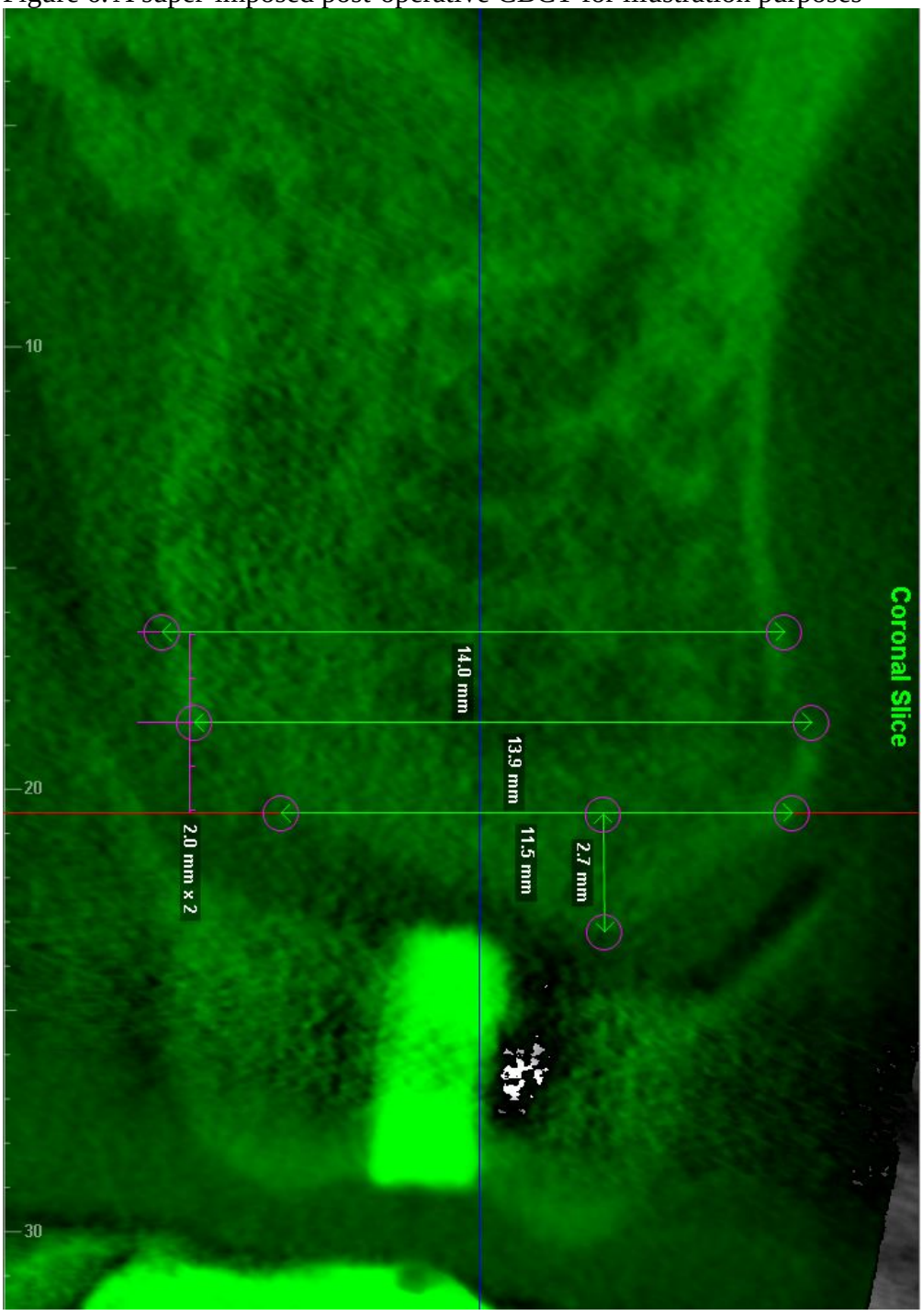
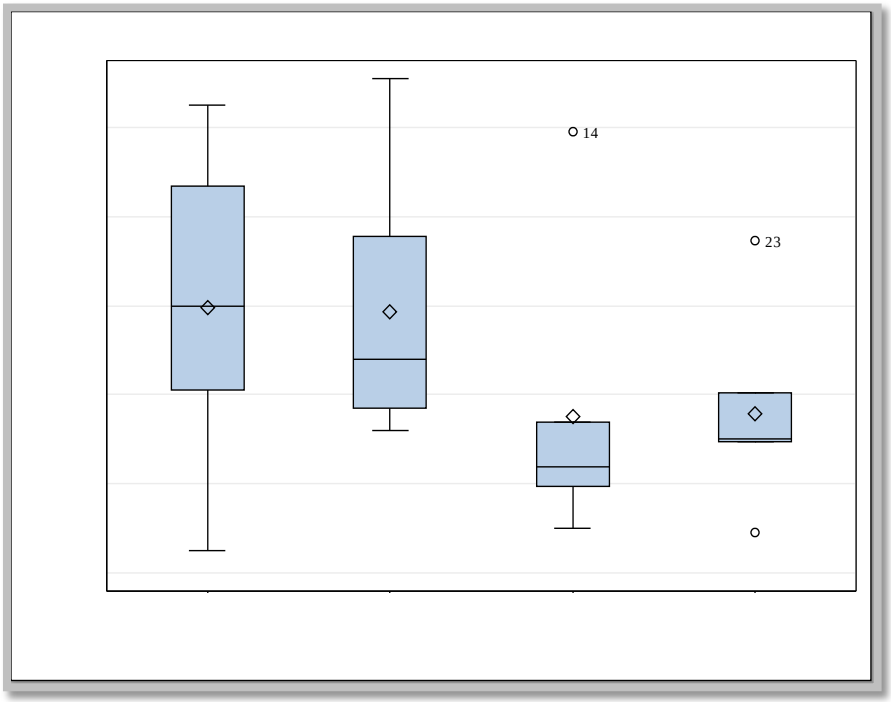


Figure 7. Comparison of 4 (Product/Technique) Treatment Groups

Horizontal Width at Crest (Change From Baseline) by (Product/Technique) Treatment Group.

Although horizontal width at crest had a median decrease for all treatment groups, there was no significant difference in change (decrease) from baseline between treatment groups (Kruskal-Wallis test: p = 0.1278).

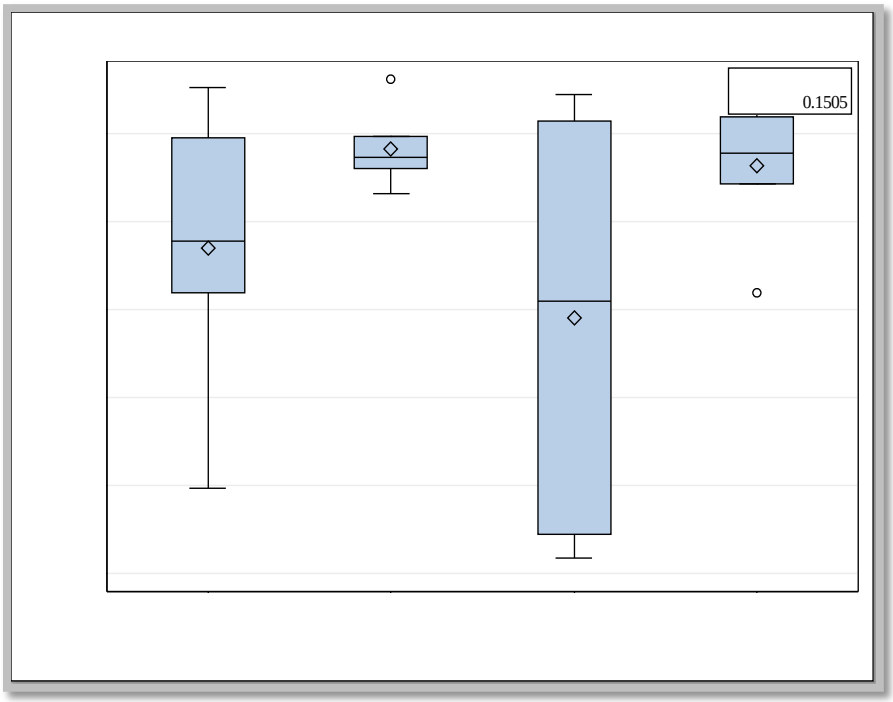


Treatment Group	N	Median*	Mean*	SD
1 Cytoplast	6	-6.03	-6.04	3.67
2 Cytoplast, Overlay	6	-7.20	-6.14	3.02
3 BioXclude	6	-9.63	-8.50	3.24
4 BioXclude, Overlay	6	-8.98	-8.43	2.16

. *change from baseline

Horizontal Width at 2mm (Change From Baseline) by (Product/Technique) Treatment Group.

Although horizontal width at 2mm had a median decrease for all treatment groups, there was no significant difference in change (decrease) from baseline between treatment groups (Kruskal-Wallis test: p = 0.6013).

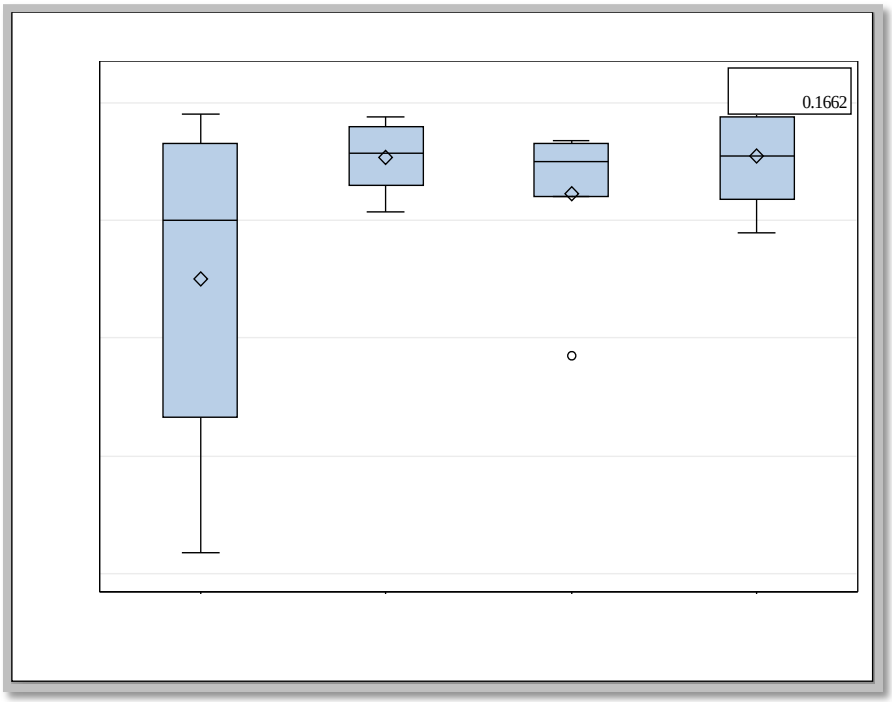


Treatment Group	N	Median*	Mean*	SD
1 Cytoplast	6	-4.45	-4.60	3.19
2 Cytoplast, Overlay	6	-2.53	-2.33	0.90
3 BioXclude	6	-5.80	-6.19	5.02
4 BioXclude, Overlay	6	-2.45	-2.73	1.59

*change from baseline

Horizontal Width at 4mm (Change From Baseline) by (Product/Technique) Treatment Group.

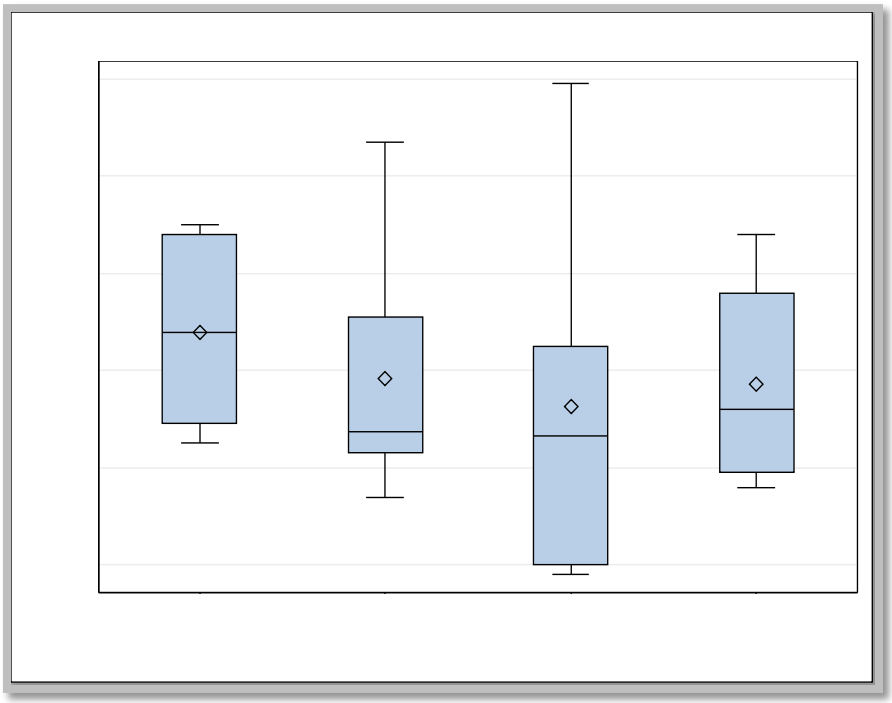
Although horizontal width at 4mm had a median decrease for all treatment groups, there was no significant difference in change (decrease) from baseline between treatment groups (Kruskal-Wallis test: p = 0.6333).



Treatment Group	N	Median*	Mean*	SD
1 Cytoplast	6	-2.00	-2.98	2.97
2 Cytoplast, Overlay	6	-0.85	-0.93	0.62
3 BioXclude	6	-1.00	-1.54	1.41
4 BioXclude, Overlay	6	-0.90	-0.92	0.94

*change from baseline

Vertical Change From Baseline by (Product/Technique) Treatment Group.
There was no significant difference in mean vertical height change from baseline between treatment groups (Kruskal-Wallis test: p = 0.5920).



Treatment Group	N	Median*	Mean*	SD
1 Cytoplast	6	0.40	0.40	1.08
2 Cytoplast, Overlay	6	-0.63	-0.08	1.34
3 BioXclude	6	-0.68	-0.38	1.91
4 BioXclude, Overlay	6	-0.40	-0.14	1.05

*change from baseline

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