Polylactic acid (PLA) Membrane as a Sole Treatment For Alveolar Ridge Preservation

Thesis

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by

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Abstract

Background:

The combination of membranes and bone grafting materials has been shown to preserve the post-extraction dimensions of the alveolar ridge and constitutes the currently accepted protocol for socket preservation. However, the use of bone grafting materials in extraction sockets has been questioned because of possible interference with bone formation in the wound sites; particles of graft material have been found in alveolar sockets more than 6 months after placement. Histologic examination of extraction sockets filled with allograft bone revealed the presence of graft particles with no evidence of bone formation on the particle surface, suggesting that the allograft bone may delay healing and affect the quality of regenerated bone. The purpose of this preliminary study is to determine whether use of a polylactic acid (PLA) membrane alone (without bone graft) after tooth extraction results in sufficient bone formation for implant placement and to determine the quality of the newly formed bone.

Methods:

Patients with single rooted non-esthetic teeth deemed hopeless for various reasons and in need of extraction, socket preservation and implant placement were recruited at the Graduate Periodontology Clinic, College of Dentistry, The Ohio State University. Extraction sites were randomly assigned to either control group (extraction alone) or test group (extraction + PLA membrane). Clinical

measurements, including relative ridge height and soft tissue level, were recorded using a pre-fabricated plastic stent before and immediately after extraction, and pre-implant placement. Clinical parameter measurements were recorded at six different positions (MB, B, DB, ML, L, and DL) per extraction site. Bone cores (2 x 6 mm) were retrieved with a trephine immediately before implant placement and subjected to micro-computed tomography (micro-CT) and cone beam CT (CBCT) scans followed by histomorphometric analysis.

Results:

A total of 18 subjects fulfilled the inclusion criteria and were recruited for the study. Twelve subjects (5 control and 7 test) successfully completed the bone core harvesting during implant placement. Six subjects (4 experimental and 2 control) were removed from the study due to the need for bone grafting at the time of implant placement. Fifteen subjects (8 control and 7 test) completed the clinical measurements before and immediate after tooth extraction. When comparing the relative ridge heights and soft tissue levels prior to and immediately after tooth extraction, there were no statistically significant differences in either control or test groups ($p \geq 0.05$). When comparing the relative ridge height immediately after extraction and before implant placement (4 months after extraction), there was a statistically significant bone loss at MB (1.43 mm; p=0.037), B (2.06 mm; p=0.031), L (2.0 mm; p=0.001) and DL (1.87 mm; p=0.013) aspects in the control group. However, no statistically significant difference was found regarding

changes in bone height in the test group in all six surfaces ($p>0.05$). After grouping the measurements at the mesial and distal sites of the extraction tooth, again only the control group showed statistically significant difference in loss of bone height at mesial (1.19 mm; $p=0.0373$) and distal (1.38 mm; $p=0.0464$) aspects. Furthermore, when all 6 measurements were averaged into one value, the control group showed a significant amount of 1.5 mm ($p=0.0063$) bone loss, whereas no significant changes in bone level were identified in the test group. Changes in soft tissue levels, after tooth extraction and before implant placement, were not statistically significant in either control or test groups, when either the mesial and distal sites of the socket were examined or all 6 measurements were averaged into one value. CBCT and Micro-CT analyses - Using a paired t-test, the mean value of grey level (equal to degree of bone mineralization (DBM) was compared between control and test groups. There was no statistically significant difference in the CBCT ($p=0.616$) and micro-CT ($p=0.319$) analyses. Histograms of DMB obtained from micro-CT scan of bone core specimen showed similar plots and peak values between the control and test groups. Histomorphometric analysis - Examination of each bone core sample showed similar distribution of viable bone in both groups with abundant lacunae present. A different level of connective tissue versus bone was present.

Conclusions:

Within the limits of this study, the use of PLA membrane alone after tooth extraction may prevent post-extraction vertical bone loss. Nevertheless, bone quality may not improve beyond control by the use of PLA membrane. More studies with a larger sample size are needed to confirm these findings.

Dedication

Dedicated to the Residents at The Ohio State University

Acknowledgements

I would like to take this opportunity to acknowledge everyone who was able to lend me their knowledge and expertise. Dr. Joeng, who was able to assist with the processing of the samples and coordinating with the imaging center at The Ohio State University. Dr. Mallery and her staff for working closely with us in analyzing the histology and providing the equipment for photomicroscopy. Vedat Yildiz for assisting us in the statistical analysis and diagram configurations of the data. Thank you to Munirah Burashed and my fellow residents in the Graduate Periodontal Program for assisting in the recruiting process and then further adding in subsequent steps. I would like to acknowledge the collaborative effects and guidance provided to me by my committee and my advisor especially. This would not have come to fruition without him. Lastly I would like to thank my wife and children, without their love and support over the past 11 years journey, none of this would have been possible

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Chapter 1: Introduction

The natural process of healing after tooth extraction is well documented. Following extraction of a tooth, the alveolus diminishes in both height and width[1]. This phenomenon is due to both internal processes leading to new bone formation and to external processes resulting in bone loss[2]. There is a large variation from patient to patient and it is nearly impossible for a clinician to predict the exact response that a patient will experience after a tooth has been extracted. Studies detailing this also only limit their scope to atraumatic extraction. Despite best efforts even the most experienced clinician may have to extract a tooth surgically. This can lead to an even greater amount of bone loss[3].

Jahangiri and others reported a current perspective of bone remodeling and healing of sockets following extraction[4]. Once the tooth has been extracted there is an immediate inflammatory reaction that occurs. This reaction along with the blood filling the socket leads to a blood clot. This clot contains proteins and cells which continue to produce various growth factors. The blood clot itself consists heavily of platelets and fibrin. Neutrophils and other leukocytes migrate into the wound and begin the process of phagocystosis in an attempt to sterilize the wound. After a few days the clot undergoes fibrinolysis and break down, during which time the socket begins to be infiltrated with granulation tissue. In one week a dense network of anastomosing blood vessels forms. Within 2 weeks a

connective tissue layer is formed. Mesenchymal cells continue to multiply and differentiate, eventually leading to the formation of woven bone. This bone becomes mineralized over the next 4-6 months. The end bone that results from this product is not as much as the initial bone[3, 4].

Loss of bone following an extraction is usually more pronounced on the buccal surface of bone than the lingual[5]. Several studies have reported that a horizontal resorption anywhere from 0.34 mm to 7.7 mm may occur from 6 to 12 months after tooth extraction. The loss of this bone becomes problematic for restoring esthetic and non-esthetic areas alike with implants[6].

Following extraction of teeth in the posterior maxilla, the maxillary sinuses can pneumatize and thus prevent implant placement. Aside from this added complication there is also the always present vertical loss of bone height associated with all extraction sockets. Short implants can be placed and achieve primary stability, however the need for ridge augmentation or sinus augmentation may still be present[7]. It has also been shown that ridge height loss is more extensive in mandibular than in maxillary sites[8].

It has been documented that the use of membranes and bone graft material may aid in the reduction of post-extraction bone loss[8]. Socket preservation is a procedure in which graft material or a scaffold is placed in the socket of an extracted tooth at the time of extraction to preserve the alveolar ridge[9]. Various

materials have been implemented in the process of socket preservation; however all have the same goal of forming mature bone for future implant placement.

The ability of clinicians to determine the quality and density of bone is of importance when considering treatment options for implant therapy. There have been many ways to assess bone quality, however most are not practical to apply routinely[1,7,20]. The simplest form of using this knowledge is assessing risk for possible alveolar fractures during surgical procedures. These surgeries could range from a low-risk extraction to implant placement. With the advances in imaging today it is a great asset to the surgeon to be able to view important anatomical structures prior to surgery. Structures such as the floor of the maxillary sinus, or the position of the mandibular canal can be evaluated and taken into consideration during treatment planning. Being able to assess these risks and plan accordingly is a great advantage to the practitioner as well as the patient.

A system to describe bone quality has been described and is broken into four groups, simply Q1, Q2, Q3 and Q4[20]. The determination of bone quality is based on the amount of cortical bone in relation to cancellous bone quantity. The extremes, Q1 and Q4, are considered to have poor implant success while Q2 and Q3 have been implicated to be more successful. Many methods of assessing the quality of bone have been implemented, including histomorphometry of bone biopsies, densitometry, digital analysis of radiographs, and ultrasound[7]. A new

aim of pre-operative assessment is to find less invasive ways to measure the type of bone in a patient prior to surgery.

Bone density has been determined and evaluated using three-dimensional imaging system. A method was utilized by Trisi and Rao[10] based on bone volume (BV) in percentage to estimate density and place it into one of four groups D1, D2, D3 and D4. They experienced a high degree of variation between the D2 and D3 groups and suggested combining these groups, making into 3 groups as hard, normal and soft. They set parameters that allow for classification of this bone type based on micro-computed tomography (microCT) scans and CT scans. A BV of greater than 76.54 % can be classified as hard, greater than 28.28 and smaller than 76.54 as normal, and smaller than 28.28 as soft[1]. Bone density has not always been considered an essential aspect for implant therapy, however recently there has been an increasing awareness of the importance of bone density when considering implant treatment planning and placement[10].

The Guidor® matrix barrier is a bioresorbable membrane and is made of amorphous polylactic acid blended with a citric acid ester. It was the first synthetic material to appear on the market, and its safety and efficacy have been well documented in clinical studies, including treatment of intrabony defects, furcation defects, root coverage, implant and guided bone regeneration (GBR), and sinus grafting [11-13]. The Guidor® barrier has a unique, double-layered design

in which the external layer of the membrane has rectangular perforations allowing integration of the overlying gingival connective tissues and efficiently preventing epithelial downgrowth. The internal layer of the barrier has minute circular perforations that retard tissue penetration but still allow for nutrition [14]. Complete degradation of the Guidor® barrier has been reported 6-12 months after implantation, with maintenance of function for at least six weeks[15]. The process of degradation is hydrolysis to lactic acid, which is subsequently absorbed by the surrounding tissues. The polymer fragments are then removed by a foreign-body reaction characterized by macrophages and multinuclear cells, as demonstrated by histological animal studies, and are finally metabolized to water and carbon dioxide. It has been reported that the Guidor® matrix barrier could be used for GBR in conjunction with implant installation in rabbit tibia and in humans without the addition of any bone grafting materials.[16] Previous research has shown that if graft material is utilized, residual graft material is present at the time of implant placement.[17] It may be interesting to compare the density of bone obtained from different techniques of socket preservation.

While there are numerous attempts to use a wide variety of materials, to the best of our knowledge there were no studies using a PLA membrane alone with evaluation of the bone density along with the ridge dimension changes occurring to the socket at the same time. Limiting the amount of materials used during alveolar ridge preservation (ARP), it will be possible to benefits the

clinician and the patient. Less products means less cost passed on to the patient and less foreign material exposure to the patient. Also, for various reason, patients may refuse to receive bone graft material whether allograft or xenograft. It also means that the clinician has less overhead cost and less worry about documenting and monitoring materials placed into patient in case of recalls or contamination.

Currently, the potentially beneficial effects of a PLA membrane in the preservation of alveolar ridge after tooth extraction have not been well documented. Thus, the purpose of this present study was to investigate the effects of a uniquely-designed PLA membrane (Guidor® matrix barrier) in the preservation of alveolar ridge after extraction of non-molar teeth. To achieve this purpose, a randomized, controlled clinical trial was conducted. This study pursued two specific aims.

Specific Aims:

1. To determine whether ridge preservation using a uniquely-designed PLA membrane can prevent the ridge resorption compared to extraction alone.

2. To determine whether the use of a uniquely-designed PLA membrane can improve the quantitative and qualitative aspects of new bone formation compared to extraction alone.

Significance:

The combination of membranes and bone grafting materials has been shown to preserve the post-extraction dimensions of the alveolar ridge. This combination approach constitutes the currently accepted protocol for socket preservation. However, the use of bone grafting materials in extraction sockets has been questioned because of possible interference with bone formation in the wound sites; particles of graft material have been found in alveolar sockets more than 6 months after implantation. Graft materials that need a longer time period for complete resorption decrease the total amount of newly formed bone due to their continuous existence. Furthermore, some patients object to receiving cadaveric or animal-derived bone graft materials, whether for religious or other reasons. In this study, the effects of a uniquely-designed PLA membrane in the preservation of alveolar ridge after extraction of non-molar teeth were investigated by using clinical and radiographic outcomes, as well as histologic, CBCT and micro-CT analyses, to determine the quantitative and qualitative aspects of new bone formation. The information obtained from this study has the potential to drastically change the current paradigm for socket preservation and to allow practitioners to use a sole product for this common procedure. Successful implementation of the PLA membrane as the sole means to preserve the post-extraction socket will decrease costs and increase acceptability of the procedure.

Hypothesis:

The null hypothesis is that there is no difference between the control (extraction alone) and test (PLA membrane alone) groups.

Chapter 2: Materials and Methods

Study Design

The study was a prospective randomized clinical trial to evaluate the effects of PLA membrane in the preservation of alveolar ridge after extraction of non-molar teeth. A priori power analysis was conducted to determine the reasonable sample size required for this study. A total of 28 patients (14 in each group) are required for power of 90% to detect a difference in change of bone height. Patients treatment planned for single non-molar tooth extraction (excluding maxillary incisors and canines) and implant restoration in The Ohio State University College of Dentistry (OSUCoD) Graduate Periodontology Clinic were invited to participate in this study. The dental implant was placed 12-16 weeks after extraction with or without ridge preservation, Relative ridge height was examined and recorded prior to extraction, immediate after extraction before membrane placement, and prior to implant placement, using a prefabricated plastic stent (made from 0.06" thermoforming material) to standardize the measurements. A Cohen's kappa analysis was completed to determine intraexaminer aggrement. Measurements were carried out on 5 subjects at two different time points.

Bone core samples were taken from the patients during the implant placement appointment (12-16 weeks after tooth extraction) and used to assess

bone quality by micro-CT, CBCT and histomorphometric analyses. A random number generation website (www.randomizer.org) was used to randomly assign participants to either control (extraction alone) or test (extraction and membrane) groups. Table 1 shows the timeline of the study, which includes a total of 6 clinical visits related to research study per patient. The study was approved by The Institutional Review Board of The Ohio State University (IRB Protocol Number: 2012H0228).

Study Population

Patient recruitment was limited to those patients attending The Ohio State University College of Dentistry and currently seeking implant therapy. A questionnaire (Table 2) was used to screen for qualifying participants. Criteria for eligibility were: single rooted tooth in non-esthetic zone that had been treatment planned for extraction and implant placement (pre-molars with 2 roots needed to have ≥6mm of root trunk length). Inclusion criteria were: >17 years of age , physically and mentally healthy, American Society of Anesthesiologists class 1 or 2, periodontally healthy, non-smoker; and able and willing to provide informed consent and adhere to study procedures and visits. The exclusion criteria included uncontrolled diabetes, drug or alcohol abuse, pregnant or lactating women, history of bisphosphonate therapy, radiotherapy and/or chemotherapy in the head and neck region for malignancies within 6 months, and other systemic conditions or

medications affecting surgical procedure and healing process. Participants were exited from the study immediately upon change of mind regarding participation, development of acute dental/oral conditions requiring treatment, development of conditions requiring further surgical manipulation of the site beyond the study protocol (e.g. need for GBR and delayed placement), and failure to comply with study instructions/requirements. Written informed consent was obtained for all subjects participating in the study. Alginate impressions and study casts were fabricated. Plastic stents were made, trimmed, and six sites were selected and holes prepared to guide the clinical measurements. The same stent was retained for use throughout the study.

Surgical Protocol

A. First Surgery (atraumatic extraction): Atraumatic extraction was carried out by residents of the Advanced Education Program in Periodontics at OSUCoD under the supervision of periodontal faculty. After adequate anesthesia was achieved, the selected tooth was removed using periotomes and taking care to preserve the four-walled configuration of the socket. Sockets were thoroughly debrided and irrigated with sterile saline to remove residual tissue; sockets had to have four intact walls otherwise the patient was excluded from further participation in the study. Relative ridge height (stent to alveolar crest) was measured in the apical-coronal (vertical) dimension through 6 holes (3 sites each on the buccal and

lingual/palatal) made in a prefabricated plastic stent using a periodontal probe (UNC-15 probe) (Figure 1-A). The soft tissue thickness was also recorded at each of these sites by removing the stent and bone sounding through the bleeding points already created via the stent. A caliper was utilized at the center of the targets to measure the thickness of the buccal and lingua/palatal wall. All measurements were taken by a trained examiner to eliminate inter-examiner errors. Patients were then randomly assigned to either the test (Guidor® membrane; Sunstar Americas, Inc., Chicago, IL) or control group (extraction alone) using a randomization program on the internet (http://www.randomizer.org). After decortication of the proximal socket walls, the Guidor® membrane was placed over the socket orifice and secured with resorbable sutures in the test group. The decortication procedure was done by drilling the socket wall with high-speed carbide burr. The biologic rationale for decortication of the socket wall is to allow progenitor cells easy access to the extraction socket and to facilitate prompt angiogenesis. A conservative mucoperiosteal flap, not extending past the mucogingival junction, was elevated if the patient was assigned to the test group in order to secure the membrane under the flap. The participants from the control group received extraction and decortication with no membrane; sutures were placed in the both groups as needed to maintain soft tissue position. Membrane left exposed was measured after sutures were in place (Figure 1-B). Patients from both groups received the same post-operative instructions, including 600 mg Ibuprofen (3 times

a day, as needed) and 0.12% chlorhexidine mouthrinse (twice daily for 2 weeks). The test group subjects were also given an antibiotic (Amoxicillin 500mg q.i.d.) for seven days.

B. One- to six-week post-operative check-up: Membrane integrity was examined and recorded at 0- (baseline), 1-, 3-, and 6-week on participants from the test group. The pain assessment was carried out on individuals from both control and test groups, including 0 – 10 numeric rating scale and analgesic usage (Table 3).

C. Second Surgery (implant placement): After adequate healing time (12-16 weeks) had transpired, a full thickness flap was raised for implant placement. Relative ridge height was measured again using the original stent and recorded as previously described. A core biopsy of 6mm in length was harvested (Figure 1-C), by a well-trained periodontist with 20 years of implant surgery experience, from the central portion of the socket using a 2.0/3.0 mm internal/external diameter trephine drill (the outer diameter is 3 mm) (Figure 1-D). Bone cores obtained were placed in sterile Eppendorf tubes and placed on ice. Immediately following surgery, bone cores were stored in a -20^{o}C freezer for future analysis. The osteotomy was completed and the implant seated. The implant diameter used and the possible need for additional grafting at time of implant placement was also recorded.

Bone Core Analysis

To determine new bone volume, bone cores stored in freezer were thawed and arranged in patterns that would allow for micro-CT and CBCT image analysis. The cores were then scanned parallel to the longitudinal axis in 360^{o}. A micro-CT scanner (SkyScan 1172-D, Kontich, Belgium) was used to obtain high resolution 3-D images of the cores with the scanning and reconstruction voxel sizes set at 20 x 20 x 20 μm^3. Scanning conditions (49 kV, 200µA, 0.4^{o} rotation per projection, 8 frames averaged per projection, and 40 ms exposure time) were utilized for all specimens. Total counts and Ct attenuation values were collected. Enveloping and thresholding was completed. Values were converted to histograms using method previously described [17,18,19]. CBCT images were obtained using a CBCT scanner (i-Cat; Imaging Science International, Hatfield, PA) at 200 µm/voxel resolution under standardized conditions (70 kV, 141 µA and 20 min scanning time). Histograms of grey levels were established for each voxel in the process of bone segmentation. This was considered to be equivalent to degree of bone mineralization (DBM). Mean and standard deviation (SD) of DBM histograms were obtained. After the micro-CT and CBCT scans were completed, the most apical extent of the bone cores were marked with India ink and immediately placed in 10% neutral-buffered formalin for histomorphometric analysis. Histomorphometric analysis was performed according to Beck and Mealey[21] for the qualitative study of the microscopic organization and structure of

bone. Demineralization was carried out and then the cores were imbedded in parafilm and sectioned. Light microscopy was carried out and analyzed by an outside expert. Qualitative descriptions were given for each slide.

Statistical Analysis

Clinical Data Analysis

The data were analyzed using Statistical Analysis Software, version 9.3 (SAS Institute Inc., Cary, NC, USA). Prior to analysis, the data were examined for outliers; no extreme values were found. Descriptive statistics are reported as mean±se. Prior to extraction and post extraction data was reported as an average as baseline. Normality testing was performed for all parameters. Logarithmic transformation was applied when it was needed. Within group differences between time points and between group differences at each time points were obtained using paired t-test and unpaired t-test, respectively. A random effect (intercept and slope) regression analysis was conducted to estimate slopes of each outcome over continuous time for test and control groups. Standard orthogonal contrast was performed to test for slope differences between two groups.

Radiographic Analysis

Student t-testing was used to compare the percentage differences of grey level parameters between the control and test groups in the micro-CT and CBCT images of specimens. Significance was set at 0.05 or less.

TABLES and FIGURES

Table 1: Study Time Line

Study Visit	Procedures	Duration
1	• ***Review and collection of signed informed consent, HIPAA forms*** • Dental screening X-ray film • ***Questionnaire**** • Oral hygiene instruction	30 minutes
2	• First surgery: atraumatic extraction of non-molar tooth • ***Socket dimension measurements through a prefabricated stent using a periodontal probe in the apical-coronal (vertical) dimensions.*** • ***Control group: extraction with decortication*** • ***Experimental group: extraction with decortication and Guidor® membrane placement*** • Taking traditional dental X-ray films (periapical radiographs)	1.5 hours
3	• ***One-week post-operative check-up for membrane integrity and pain assessment through questionnaire (including 0 – 10 Numeric Rating Scale and the analgesic usage)***	15 minutes
4	• ***Three-week post-operative check-up for membrane integrity***	10 minutes
5	• ***Six-week post-operative check-up for membrane integrity***	10 minutes
6	• Second surgery: implant placement • ***Ridge height measurements using the original stent*** • ***Bone core biopsy (2.0 x 6 mm) taken during implant osteotomy preparation and immediately stored at -20^{o}C for micro CT, CBCT and histologic analyses*** # • Taking traditional dental X-ray films (periapical radiographs)	1.5 hours

* Note: procedures done only for the study are shown in bold and Italic.

No additional bone was removed during the bone biopsy. The size of the bone being removed is smaller than the diameter of the final drills that was used to prepare the hole for the implant placement.

Table 2: Questionnaire

STUDY SUBJECT # _____

QUESTIONNAIRE (PRE-OPERATIVE)

Date Completed _____

Please answer as best as you can. If you have difficulty answering a question, please leave it blank. If you would like an explanation, please feel free to ask. Your answers are strictly confidential and will remain anonymous.

1. How old are you? _____years _____months
2. _____Male _____Female; If you are female, are you currently pregnant? ____Yes ____No
3. Are you currently receiving dental treatment in addition to your periodontal treatment?
   _____Yes _____No
   If yes, please describe_____
4. Have you been diagnosed with diabetes mellitus?
   _____Yes _____No
   If yes: a) what type of diabetes mellitus? (please circle) Type I Type II
   b) when were you diagnosed? _____
   c) What's your HbA1C or blood sugar level? _____
5. Are you currently taking any medications?
   _____Yes _____No
   If yes, please list the medication, reason for taking it, and dosage (if known)

	Medication	Reason	Dosage
1			
2			
3			
4			
5			

6. Do you have any allergies? _____Yes _____No
   If yes, what to? _____
7. Do you currently smoke cigarettes or use other tobacco products? ____Yes _____No
   If yes: a) what do you use? (circle all that apply) cigarettes pipe cigar chewing tobacco
   b) If you smoke cigarettes, how many do you smoke each day? _____
   c) If you smoke cigarettes, how long have you been smoking? ____years ____months
8. Do you have history of radiotherapy in the head and neck region for malignancies?
   ___Yes _____No; If yes: What is the diagnosis of the tumor? _____
   When did you have the radiotherapy? _____
9. Are you currently under chemotherapy for treatment of malignant tumor?
   ____Yes _____No; If yes: What is the diagnosis of the tumor? _____
   When did you have the chemotherapy? _____
10. Do you have drug addiction or alcohol abuse problem? ____Yes _____No.

Thank you for completing this questionnaire

Table 3: Post-operative Discomfort Statement

Subject ID Number: _____

Post-operative Discomfort Statement

Please answer the Questions below to the best of your ability. If Not applicable, please circle N/A.

1. Please mark on a scale of 0→10 to corresponding degree that best represents the amount of discomfort you experienced after the surgery.

   0------1-------2-------3-------4--------5--------6-------7-------8-------9-------10
   No pain Moderate Pain Worst Pain
2. How long did the discomfort last for?

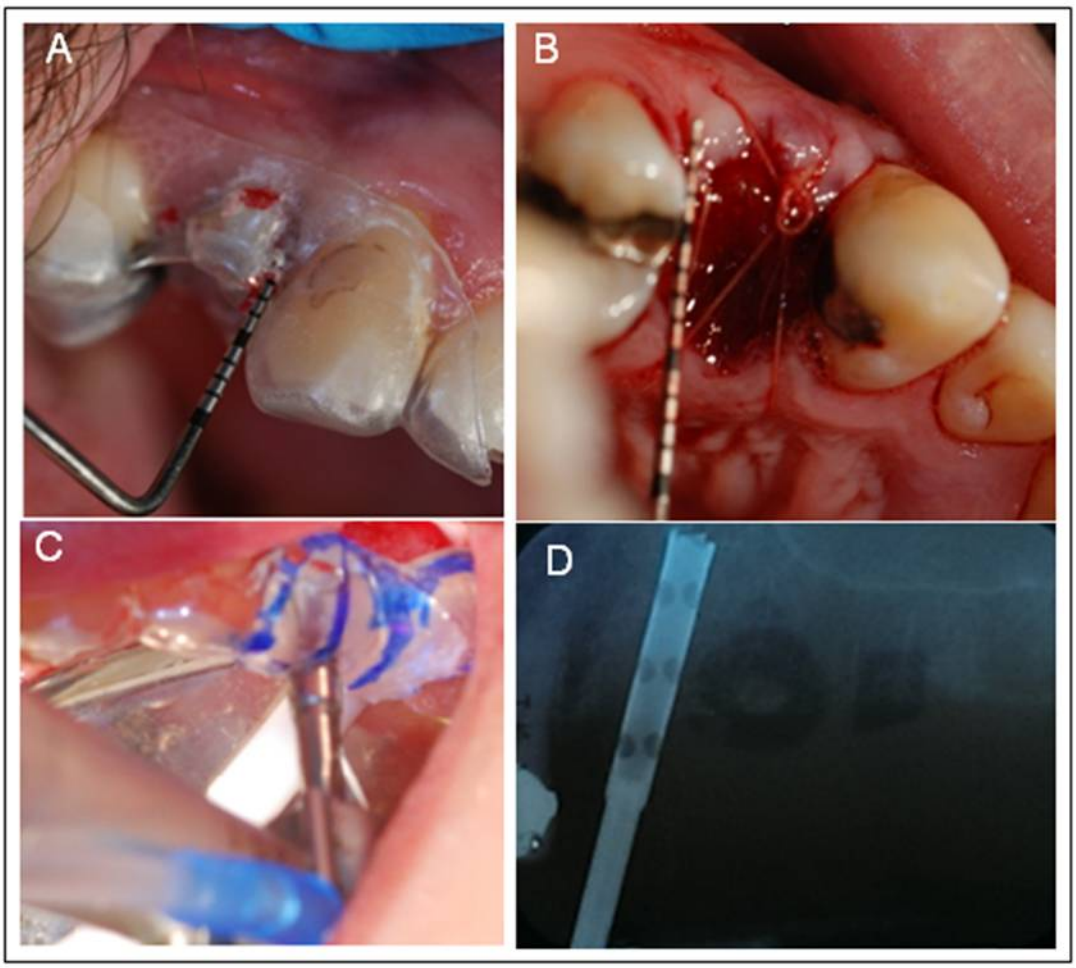
3. What day after surgery did you experience the most discomfort? (e.g. Day 1, or day 3 after the surgery)

4. Which medications did you take for the discomfort? How often?

Drug Name	Dose	Frequency

5. Did you use any non-medicinal approaches for the discomfort? (Ice, heat, herbs, etc.)

Figure 1. Photographs depicting clinical measurements and bone core harvesting. A) Relative ridge height measured through stent. B) Initial measurement of the exposed membrane after suturing. C) Bone core harvest through stent. D) Radiograph of trephine in place to confirm osteotomy angulation and position before bone core was harvested.



Chapter 3: Results

Study Population

Eighteen patients were recruited into the study and 12 were able to complete the study; 6 subjects were excluded from the study due to immediate loss of alveolar dimensions and need for subsequent GBR on the day of implant placement. Of these 6, 3 were determined prior to implant surgery to need subsequent GBR, while the remaining 3 had clinical data measurements taken and then upon flap reflection it was determined that implant placement was not possible due to inadequate bone available and were then exited from the study. Clinical data analysis is based on 15 subjects and bone core analysis on 12 cores. Each patient contributed one site for a total of 12 sites analyzed. Seven sites were from the maxilla while 5 sites were mandibular, and all teeth included were premolars. There were no significant ($p=0.5$) differences between control and test groups for gender, age or location (maxillary versus mandibular)(Table 4). Alveolar bone was well established upon re-entry on the day of implant placement with implant successfully installed in all 12 sites; one site in the mandible was grafted with mineralized freeze dried bone allograft simultaneously with implant placement due to buccal dehiscence defect.

Clinical Data

Relative Ridge Height

The average healing time after tooth extraction was 132 ±11 days. The intra-examiner reliability of clinical data measurement was determined by Cohen's kappa value, which was calculated on five separate subjects at two different time points. A kappa value of 0.765 was obtained indicating a good level of reliability. When comparing the relative ridge heights and soft tissue levels prior to (Time 0) and immediately after tooth extraction (Time 1), there were no significantly differences in either control (Table 5) or test (Table 6) groups ($p \geq 0.05$). Hence, the baseline was defined as the time when tooth was extracted. The average amount of vertical bone height lost in the control group, from the time of tooth extraction (baseline) to implant placement, was 1.43 mm on the mesiobuccal, 2.06 mm on the midbuccal, 1.25 mm on the distobuccal, 0.93 mm on the mesiolingual, 2.0 mm on the midlingual, and 1.87mm on the distolingual aspects (Table 7). The loss of vertical bone height was statistically significant on the mesiobuccal ($p = 0.037$; Figure 2), midbuccal ($p = 0.031$; Figure 3), midlingual ($p = 0.001$; Figure 3), and distolingual ($p = 0.013$, Figure 5) aspects. In the test group, average amount of vertical bone height gained was 0.07 mm on the mesiobuccal, 0.21 mm on the mesiolingual and 1.07 on the distolingual aspects. Meanwhile, average loss of bone height was 1.71 mm on the midbuccal, 0.64 mm on the distobuccal, 0.5 mm on the midlingual aspects (Table 8). There were no

statistically significant differences between the baseline and 16 weeks of tooth extraction in the test group. When comparing the relative ridge height between the control and test groups at baseline, there was no difference statistically ($p > 0.05$) on 5 sites (Table 9). However, one site at the mesiobuccal aspect was significantly different ($p = 0.048$; Figure 2), with control having an average of 5.1 (0.4 S.E.) mm from stent to bone and 6.2 mm (0.4 S.E.) in the test group. At 16 weeks after tooth extraction, there was no significant difference in relative ridge height between control and test groups ($p = 0.05$, Table 10). The means of each time point were then used to create a linear regression and the slopes of each group were compared to see if there was a difference. The slope comparisons showed a significant difference between the two groups on the distolingual aspect with a p-value of 0.02 (Table 11).

However, there was a statistically significant loss of vertical bone height between the baseline and 16 weeks after tooth extraction in the control group when only the mesial and distal sites of the socket were examined (Table 12). There was an average of 1.19 mm ($p = 0.037$) and 1.38 mm ($p= 0.0464$) loss in the mesial and distal aspects, respectively. In contrast, the test group did not have any significant amount of vertical bone loss at either site. Furthermore, when all 6 measurements were averaged into one value, the control group had a significant amount of vertical bone loss, (1.5 mm; $p = 0.0063$) whereas the test group

experienced no significant change of bone height (0.07 mm; $p = 0.9$) between the baseline and 16 weeks after tooth extraction (Table 13).

Soft Tissue Levels

The soft tissue levels were also compared between the baseline and 16 weeks after extraction in both control and test groups. Intragroup comparison of soft tissue levels between baseline and 16 weeks after extraction showed no significant difference ($p > 0.05$) in the control group (Table 7). There was an average of 0.5 mm loss of soft tissue level in mesiobuccal, 0.43 mm in midbuccal, 0.62 mm in distobuccal, 0.43 mm in mesiolingual, and 0.18 mm in distolingual aspects; whereas, an average of 0.12 mm gain in soft tissue level was noted in midlingual aspect. However, intragroup comparison of soft tissue levels between baseline and 16 weeks after extraction showed statistically significant difference on the lingual aspect in the test group (Table 8). There was an average of 0.92 mm loss of soft tissue level in mesiolingual ($p = 0.018$; Figure 6), 1.14 mm in midlingual ($p = 0.025$; Figure 7), 1.64 mm in distolingual ($p = 0.028$; Figure 8) aspects, respectively. Comparison of soft tissue levels at baseline between control and test groups showed significant difference on midlingaul ($p = 0.017$; Figure 7) and distolingaul ($p = 0.044$; Figure 8) aspects (Table 9). The soft tissue levels in the test group was 0.96 mm and 1.18 mm higher than the control group in midlingual ($p = 0.017$) and distolingual ($p = 0.044$) aspects, respectively.

However, there was no statistically significant difference in the soft tissue levels at 16 weeks after tooth extraction between the control and test groups (Table 10). Again, these time points were placed into a linear regression model and the slopes were compared between the control and test groups; no statistically significant differences were identified.

When either the mesial or distal sites of the socket were examined (Table 12) or all 6 measurements were averaged into one value (Table 13), there were no statistically significant differences in changes of soft tissue levels between the baseline and 16 weeks of tooth extraction in either control or test groups.

Buccal and lingual plate thickness was recorded at the time of extraction. Average thickness of the buccal plate was 1.53 mm and 1.98 mm for the lingual plate for all sites. There was no significant difference between control and test sites, nor was there a statistical difference between maxillary and mandibular sites.

Radiographic Analysis

Bone core imaging analysis, including CBCT and micro-CT, were completed and then analyzed. Using a two sample t-Test the bone core grey levels (equal to degree of bone mineralization – DMB) were compared between control and test groups. After enveloping and thresholding was accounted for, there was no significant difference ($p = 0.616$) between control and test groups in

the CBCT imaging analysis (Table 14). The same technique was employed for micro-CT scans (Table 15) and again there was no significant difference ($p = 0.319$) in the grey levels histogram data. Representative histogram of DMB obtained from micro-CT scan of bone core specimen was illustrated in Figure 9, which showed similar plots and peak values between the control and test groups.

Histological Analysis

Histological analysis was carried out manually after H & E staining was completed. Photomicrographs of representative bone core section were shown in Figure 10. Each analysis was similar in that mature bone was noted interspersed with areas of connective tissue. Empty lacunae were noted in each specimen indicating that bone viewed was indeed vital. Using polarizing filters the collagen arrangement for each specimen was noted. There was no difference between groups in collagen arrangement, which indicated both groups had maturely formed bone. Due to the processing to get the micro-CT and CBCT images for radiographic analysis, followed by histological analysis, there was a freeze-thaw effect noted in all specimens in which empty lacunae with no osteocyte was observed. The qualitative analysis was indiscernible between the control and test groups.

Post-op follow-up questionnaire

When statistically comparing the categories that subjects were asked at subsequent follow-up visits, there were no statistical differences found between the groups (Table 16). Notably, the control group had 2 subjects that required narcotics, albeit for 2 days or less, for pain management control. When questioned about which day had the most pain, there was an average recording of 1.29 days for the test group and 1 day for the control. Membrane exposure in the test group averaged 6.86 mm on the day of extraction, and subsequently decreased to 4.86 mm and 2.43 mm at 1-week and 3-weeks after extraction, respectively. All extraction sockets were completely covered by soft tissue at 6 weeks after tooth extraction with no membrane observed.

Tables and Figures

Table 4. Demographics

Characteristic	Control (n=8)	Test (n=7)	P-value
Age, mean (SD), years	56.4 (14.0)	56.4 (14.0)	0.793
Gender N (%)			
Male	3 (38)	4 (57)	0.619
Female	5 (62)	3 (43)	
Location			
Maxillary	5 (63)	5 (71)	0.999
Mandibular	3 (37)	2 (29)	

	VARIABLE	Time 0 Mean(SE)	Time 1 Mean(SE)	Differences	p-value
Control	vmb	5.1(0.52)	5.0 (0.27)	0.125	0.8868
	vb	4.4(0.73)	4.5 (0.53)	-0.125	0.8988
	vdb	5.5(0.60)	6.0 (0.60)	-0.5	0.4973
	vml	5.0(0.50)	5.1 (0.52)	-0.125	0.8482
	vl	1.5(0.07)	1.6 (0.09)	-0.04262	0.6626
	vdl	4.8(0.45)	5.0 (0.46)	-0.25	0.786
	smb	3.0(0.19)	3.3 (0.25)	-0.25	0.7573
	sb	2.8(0.67)	2.9 (0.40)	-0.125	0.8507
	sdb	1.2(0.14)	1.3 (0.15)	-0.06385	0.7166
	sml	1.0(0.08)	1.2 (0.08)	-0.1226	0.3539
	sl	1.0(0.09)	1.0 (0.09)	4.44E-16	1
	sdl	3.4(0.26)	3.3 (0.25)	0.125	0.8808

Table 5. Relative ridge height and soft tissue levels in the control group prior to (Time 0) and immediately after tooth extraction (Time 1; Baseline) (n=8)

Vmb-relative ridge height at mesiobuccal; vb-relative ridge height at buccal; vdb-relative ridge height at distobuccal; vml-relative ridge height at mesiolingual; vl-relative ridge height at lingual; vdl-relative ridge height at distolingual; smb-soft tissue level at mesiobuccal; sb-soft tissue level at buccal; smd-soft tissue level at distobuccal; sml-soft tissue level at mesiolingual; sl-soft tissue level at lingual; sdl-soft tissue level at distolingual.

	VARIABLE	Time 0 Mean(SE)	Time 1 Mean(SE)	Differences	p-value
Test	vmb	6.0(0.87)	6.4 (0.81)	-0.4286	0.6485
	vb	4.9(0.88)	5.4 (0.69)	-0.5714	0.5874
	vdb	5.0(0.44)	5.1 (0.40)	-0.1429	0.8554
	vml	4.9(0.40)	5.6 (0.43)	-0.7143	0.3108
	vl	1.7(0.07)	1.7 (0.05)	-0.08397	0.4233
	Vdl	6.1(0.86)	6.0 (0.87)	0.1429	0.8845
	smb	4.4(0.95)	3.7 (0.75)	0.7143	0.4115
	sb	2.9(0.26)	2.6 (0.43)	0.2857	0.6877
	sdb	1.2(0.10)	1.2 (0.10)	-0.0411	0.8269
	sml	1.3(0.07)	1.2 (0.14)	0.1569	0.2686
	sl	1.3(0.08)	1.3 (0.10)	-0.06714	0.6143
	sdl	4.6(0.97)	4.4 (0.75)	0.1429	0.8726

Table 6. Relative ridge height and soft tissue levels in the test group prior to (Time 0) and immediately after tooth extraction (Time 1) (n=7) Vmb-relative ridge height at mesiobuccal; vb-relative ridge height at buccal; vdb-relative ridge height at distobuccal; vml-relative ridge height at mesiolingual; vl-relative ridge height at lingual; vdl-relative ridge height at distolingual; smb-soft tissue level at mesiobuccal; sb-soft tissue level at buccal; smd-soft tissue level at distobuccal; sml-soft tissue level at mesiolingual; sl-soft tissue level at lingual; sdl-soft tissue level at distolingual.

Group	Variable	Baseline Mean (SE)	Week 16 Mean (SE)	Differences	P-Value
Control	vmb	5.1 (0.4)	6.5 (0.5)	-1.43	**0.037**
	Vb	4.4 (0.5)	6.5 (0.8)	-2.06	**0.031**
	Vdb	5.7 (0.4)	7.0 (0.5)	-1.25	0.054
	Vml	5.0 (0.3)	6.0 (0.4)	-0.93	0.069
	Vl	4.7 (0.3)	6.7 (0.4)	-2.00	**0.001**
	Vdl	4.8 (0.4)	6.7 (0.6)	-1.87	**0.013**
	Smb	3.1 (0.4)	2.6 (0.5)	0.50	0.421
	sb	2.8 (0.4)	2.3 (0.5)	0.43	0.484
	sdb	3.7 (0.3)	3.1 (0.5)	0.62	0.285
	sml	3.1 (0.2)	2.6 (0.3)	0.43	0.224
	sl	2.7 (0.3)	2.8 (0.4)	-0.12	0.787
	sdl	3.3 (0.4)	3.1 (0.5)	0.18	0.783

Table 7: Relative ridge height and soft tissue levels in the control group at baseline (immediately after tooth extraction; Time 1) and 16 weeks after tooth extraction (n=8) Vmb-relative ridge height at mesiobuccal; vb-relative ridge height at buccal; vdb-relative ridge height at distobuccal; vml-relative ridge height at mesiolingual; vl-relative ridge height at lingual; vdl-relative ridge height at distolingual; smb-soft tissue level at mesiobuccal; sb-soft tissue level at buccal; smd-soft tissue level at distobuccal; sml-soft tissue level at mesiolingual; sl-soft tissue level at lingual; sdl-soft tissue level at distolingual. Bold values in the column of “P-Value” indicate statistical significance.

	Variable	Baseline Mean (SE)	Week 16 Mean (SE)	Differences	P-Value
Test	vmb	6.2 (0.4)	6.1 (0.6)	0.07	0.921
	Vb	5.1 (0.6)	6.8 (0.8)	-1.71	0.091
	Vdb	5.0 (0.4)	5.7 (0.5)	-0.64	0.347
	Vml	5.2 (0.3)	5.0 (0.4)	0.21	0.692
	Vl	5.5 (0.3)	6.0 (0.4)	-0.5	0.333
	Vdl	6.0 (0.4)	5.0 (0.6)	1.07	0.176
	Smb	4.0 (0.4)	2.9 (0.5)	1.14	0.093
	sb	2.7 (0.4)	3.1 (0.5)	-0.42	0.521
	sdb	3.3 (0.4)	2.5 (0.5)	0.78	0.209
	sml	3.6 (0.2)	2.7 (0.3)	0.92	**0.018**
	sl	3.7 (0.3)	2.5 (0.4)	1.14	**0.025**
	sdl	4.5 (0.4)	2.8 (0.6)	1.64	**0.028**

Table 8: Relative ridge height and soft tissue levels in the test group at baseline and 16 weeks after tooth extraction (n=7) Vmb-relative ridge height at mesiobuccal; vb-relative ridge height at buccal; vdb-relative ridge height at distobuccal; vml-relative ridge height at mesiolingual; vl-relative ridge height at lingual; vdl-relative ridge height at distolingual; smb-soft tissue level at mesiobuccal; sb-soft tissue level at buccal; smd-soft tissue level at distobuccal; sml-soft tissue level at mesiolingual; sl-soft tissue level at lingual; sdl-soft tissue level at distolingual. Bold values in the column of "P-Value" indicate statistical significance.

		Baseline			
		Control	Test	Differences	P-Value
Control versus Test at Baseline	vmb	5.1 (0.4)	6.2 (0.4)	-1.15	**0.048**
	Vb	4.4 (0.5)	5.1 (0.6)	-0.70	0.374
	Vdb	5.7 (0.4)	5.0 (0.4)	0.67	0.211
	Vml	5.0 (0.3)	5.2 (0.3)	-0.15	0.722
	Vl	4.7 (0.3)	5.5 (0.3)	-0.75	0.070
	Vdl	4.8 (0.4)	6.0 (0.4)	-1.19	0.059
	Smb	3.1 (0.4)	4.0 (0.4)	-0.94	0.079
	sb	2.8 (0.4)	2.7 (0.4)	0.09	0.852
	sdb	3.7 (0.3)	3.3 (0.4)	0.39	0.425
	sml	3.1 (0.2)	3.6 (0.2)	-0.58	0.059
	sl	2.7 (0.3)	3.7 (0.3)	-0.96	**0.017**
	sdl	3.3 (0.4)	4.5 (0.4)	-1.18	**0.044**

Table 9: Relative ridge height and soft tissue levels in the test (n=7) and control (n=8) groups at baseline (Time 1). Vmb-relative ridge height at mesiobuccal; vb-relative ridge height at buccal; vdb-relative ridge height at distobuccal; vml-relative ridge height at mesiolingual; vl-relative ridge height at lingual; vdl-relative ridge height at distolingual; smb-soft tissue level at mesiobuccal; sb-soft tissue level at buccal; smd-soft tissue level at distobuccal; sml-soft tissue level at mesiolingual; sl-soft tissue level at lingual; sdl-soft tissue level at distolingual. Bold values in the column of “P-Value” indicate statistical significance.

		Week 16			
		Control	Test	Differences	P-Value
Control versus Test at Week 16	vmb	6.5 (0.5)	6.1 (0.6)	0.35	0.657
	Vb	6.5 (0.8)	6.8 (0.8)	-0.35	0.749
	Vdb	7.0 (0.5)	5.7 (0.5)	1.28	0.096
	Vml	6.0 (0.4)	5.0 (0.4)	1	0.103
	Vl	6.7 (0.4)	6.0 (0.4)	0.75	0.196
	Vdl	6.7 (0.6)	5.0 (0.6)	1.75	0.051
	Smb	2.6 (0.5)	2.9 (0.5)	-0.30	0.685
	sb	2.3 (0.5)	3.1 (0.5)	-0.76	0.306
	sdb	3.1 (0.5)	2.5 (0.5)	0.55	0.426
	sml	2.6 (0.3)	2.7 (0.3)	-0.08	0.834
	sl	2.8 (0.4)	2.5 (0.4)	0.30	0.584
	sdl	3.1 (0.5)	2.8 (0.6)	0.26	0.742

Table 10: Relative ridge height and soft tissue levels in the test (n=7) and control (n=8) groups at 16 weeks after tooth extraction. Vmb-relative ridge height at mesiobuccal; vb-relative ridge height at buccal; vdb-relative ridge height at distobuccal; vml-relative ridge height at mesiolingual; vl-relative ridge height at lingual; vdl-relative ridge height at distolingual; smb-soft tissue level at mesiobuccal; sb-soft tissue level at buccal; smd-soft tissue level at distobuccal; sml-soft tissue level at mesiolingual; sl-soft tissue level at lingual; sdl-soft tissue level at distolingual. Bold values in the column of “P-Value” indicate statistical significance.

	Slope comparison Control vs Test			
Variable	Estimate	P-Value	%95 CI	
vmb	0.09	0.194	-0.05	0.24
Vb	0.02	0.816	-0.17	0.21
Vdb	0.03	0.549	-0.09	0.16
Vml	0.07	0.103	-0.01	0.16
Vl	0.09	0.092	-0.01	0.20
Vdl	0.18	**0.020**	0.03	0.33
Smb	0.04	0.504	-0.08	0.16
sb	-0.05	0.363	-0.17	0.06
sdb	0.01	0.869	-0.11	0.13
sml	0.03	0.399	-0.04	0.10
sl	0.07	0.103	-0.01	0.17
sdl	0.09	0.218	-0.06	0.24

Table 11: The comparison of slopes generated between baseline and 16 weeks after tooth extraction in control (n=8) and test (n=7) groups. Vmb-relative ridge height at mesiobuccal; vb-relative ridge height at buccal; vdb-relative ridge height at distobuccal; vml-relative ridge height at mesiolingual; vl-relative ridge height at lingual; vdl-relative ridge height at distolingual; smb-soft tissue level at mesiobuccal; sb-soft tissue level at buccal; smd-soft tissue level at distobuccal; sml-soft tissue level at mesiolingual; sl-soft tissue level at lingual; sdl-soft tissue level at distolingual. Bold values in the column of “P-Value” indicate statistical significance.

Group	Variable	Baseline Mean (SE)	Week 16 Mean(SE)	Differences	P-Value
Control	Vm	5.1 (0.33)	6.3 (0.31)	-1.1875	**0.0373**
	Vd	5.5 (0.31)	6.9 (0.47)	-1.375	**0.0464**
	Smp	3.3 (0.21)	2.6 (0.26)	0.625	0.1994
	Sdp	3.6 (0.38)	3.1 (0.45)	0.4375	0.446
Test	Vm	6.0 (0.58)	5.6 (0.34)	0.4286	0.4653
	Vd	5.6 (0.56)	5.4 (0.57)	0.2143	0.7629
	Smp	3.6 (0.52)	2.8 (0.37)	0.75	0.1514
	Sdp	3.9 (0.48)	2.7 (0.31)	1.2143	0.055

Table 12. Relative ridge height and soft tissue levels at baseline (Time 1) and 16 weeks after tooth extraction in the control and test groups when only the mesial and distal sites of the socket were examined. Vm-relative ridge height at mesial sites, Vd-relative ridge height at distal sites, Smp-soft tissue level at mesial sites, Sdp-soft tissue level at distal sites. Bold values in the column of "P-Value" indicate statistical significance.

Group	Variable	Baseline Mean (SE)	Week 16 Mean(SE)	Differences	P-Value
Control	V	5.1 (0.3)	6.6 (0.37)	-1.5	**0.0063**
	S	3.2 (0.24)	2.8 (0.37)	0.4167	0.3828
Test	V	5.7 (0.43)	5.8 (0.49)	-0.07143	0.8983
	S	3.6 (0.41)	2.8 (0.36)	0.7738	0.1332

Table 13. Relative ridge height and soft tissue levels at baseline (Time 1) and 16 weeks after tooth extraction in the control and test groups when all 6 sites of the socket were averaged into one value. V-relative ridge height for all six points combined, S-soft tissue level for all six points combined. Bold values in the column of "P-Value" indicate statistical significance.

	Test (n=5)	Control(n=7)
	Variable 1	*Variable 2*
Mean	825.5457833	925.6350829
Variance	122256.1891	87761.44995
Observations	5	7
Hypothesized Mean Difference	0	
Df	8	
t Stat	-0.52041976	
P(T<=t) one-tail	0.308431461	
t Critical one-tail	1.859548038	
P(T<=t) two-tail	0.616862923	
t Critical two-tail	2.306004135	

Table 14: CBCT histogram Student's T-test

	Test (n=5)	Control(n=7)
Mean	2357.228061	2587.574684
Variance	111435.6537	182584.8989
Observations	5	7
Hypothesized Mean Difference	0	
Df	10	
t Stat	-1.047347302	
P(T<=t) one-tail	0.159796924	
t Critical one-tail	1.812461123	
P(T<=t) two-tail	0.319593848	
t Critical two-tail	2.228138852	

Table 15: micro-CT histogram Student's T-test (2-tailed)

	Test (n=7)	Control (n=8)
Max pain 0-10 scale (mean)	1.93 (±1.52)	2.13 (±2.75)
Duration of pain in days (mean)	3.5 (±2.92)	1.75 (±2.71)
Duration of analgesic used (days)	1.86 (±1.36)	1.00 (±1.60)*
Membrane Exposure (average mm)		
Initial	6.86 (±1.96)	n/a
1 week	4.86 (±1.55)	n/a
3 week	2.43 (±2.66)	n/a
6 week	0 (±0)	n/a

*denotes only group to use narcotic

Table 16: Post-op follow-up questionnaire

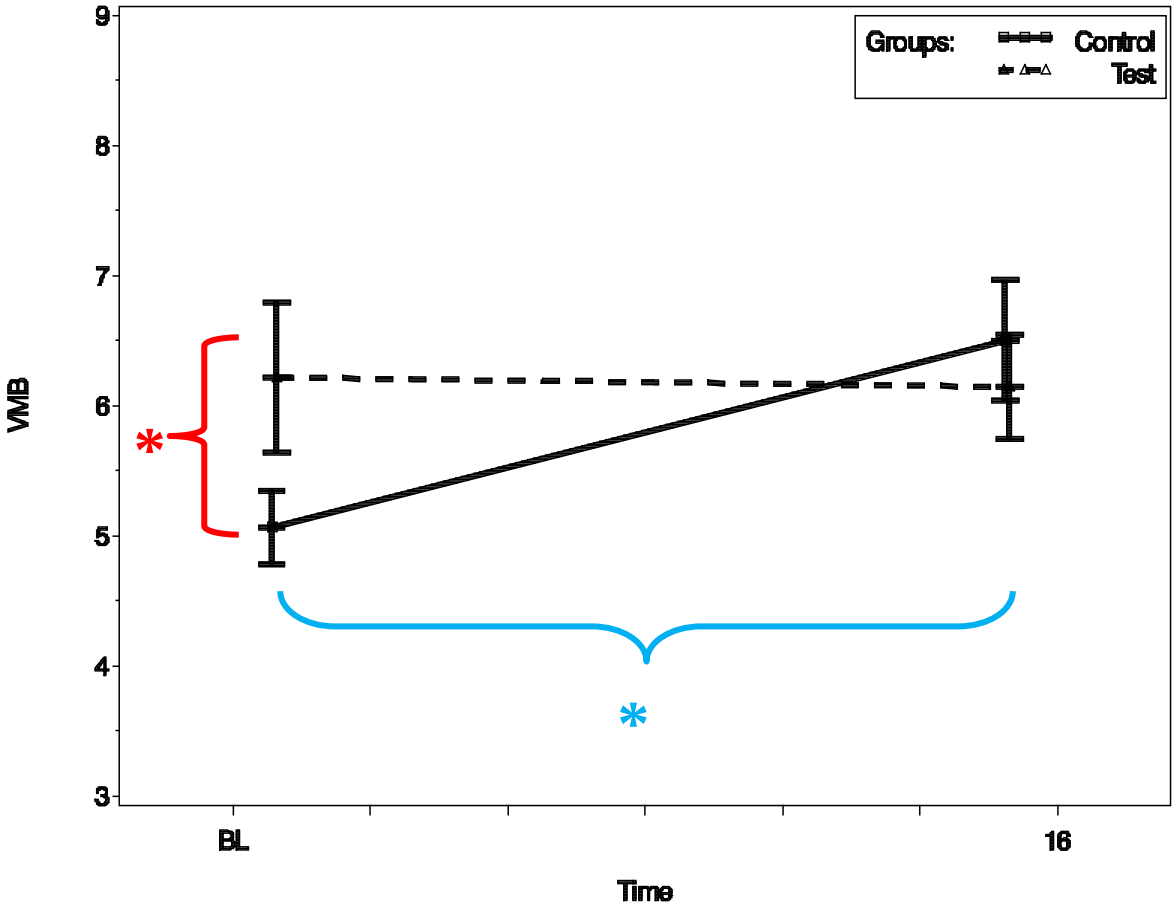


Figure 2: The mean of relative ridge height (mm) in the mesiobuccal aspect of subjects from control and test groups at baseline and 16 weeks after tooth extraction. The loss of vertical bone height was statistically significant difference on the mesiobuccal (p = 0.037) aspect in the control group. There was a significant difference between control and test groups at baseline (* p = 0.048).** VMB- relative ridge height in the mesiobuccal aspect; BL-baseline; 16-16 weeks.

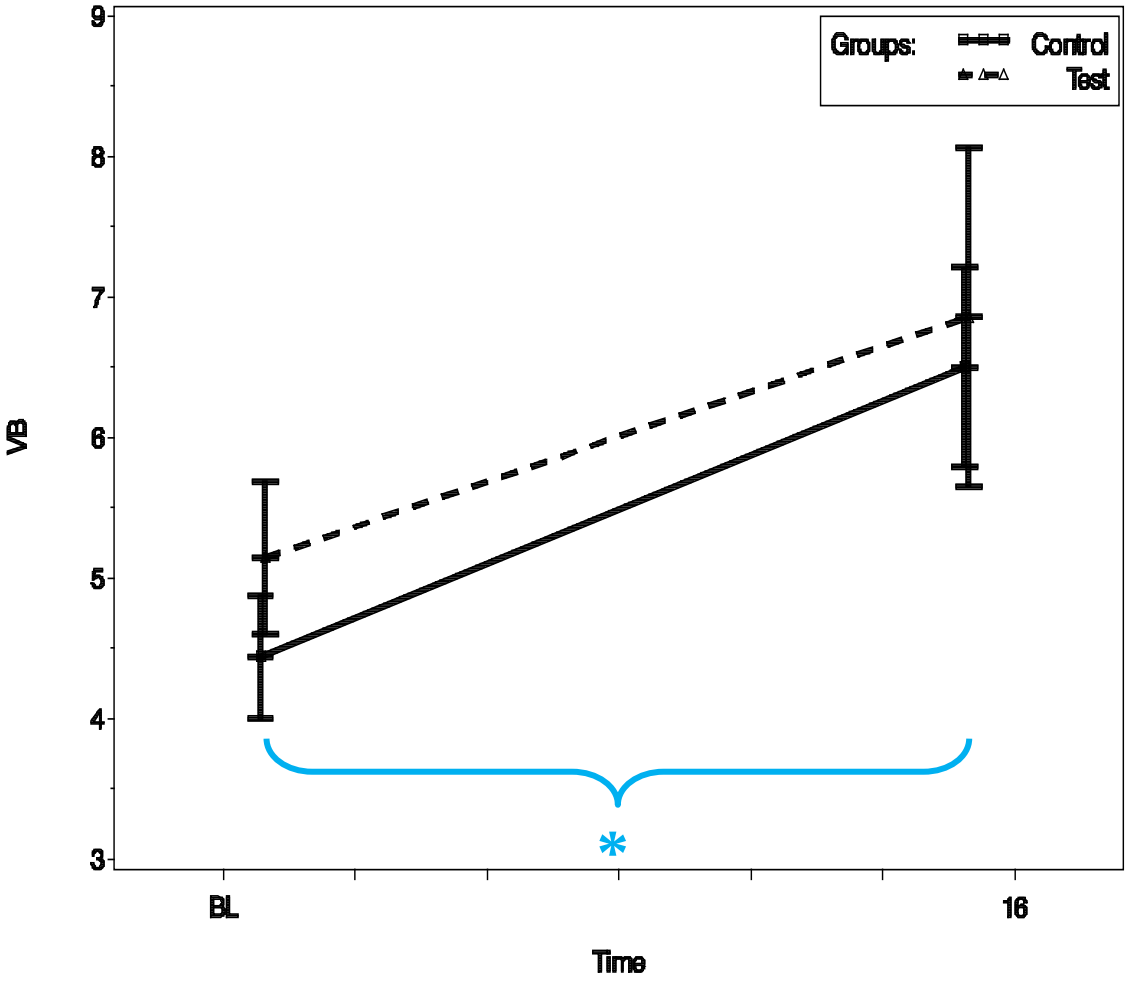


Figure 3: The mean of relative ridge height (mm) in the midbuccal aspect of subjects from control and test groups at baseline and 16 weeks after tooth extraction. The loss of vertical bone height was statistically significant difference on the midbuccal (p = 0.031) aspect in the control group.**
VB- relative ridge height in the midbuccal aspect; BL-baseline; 16-16 weeks.

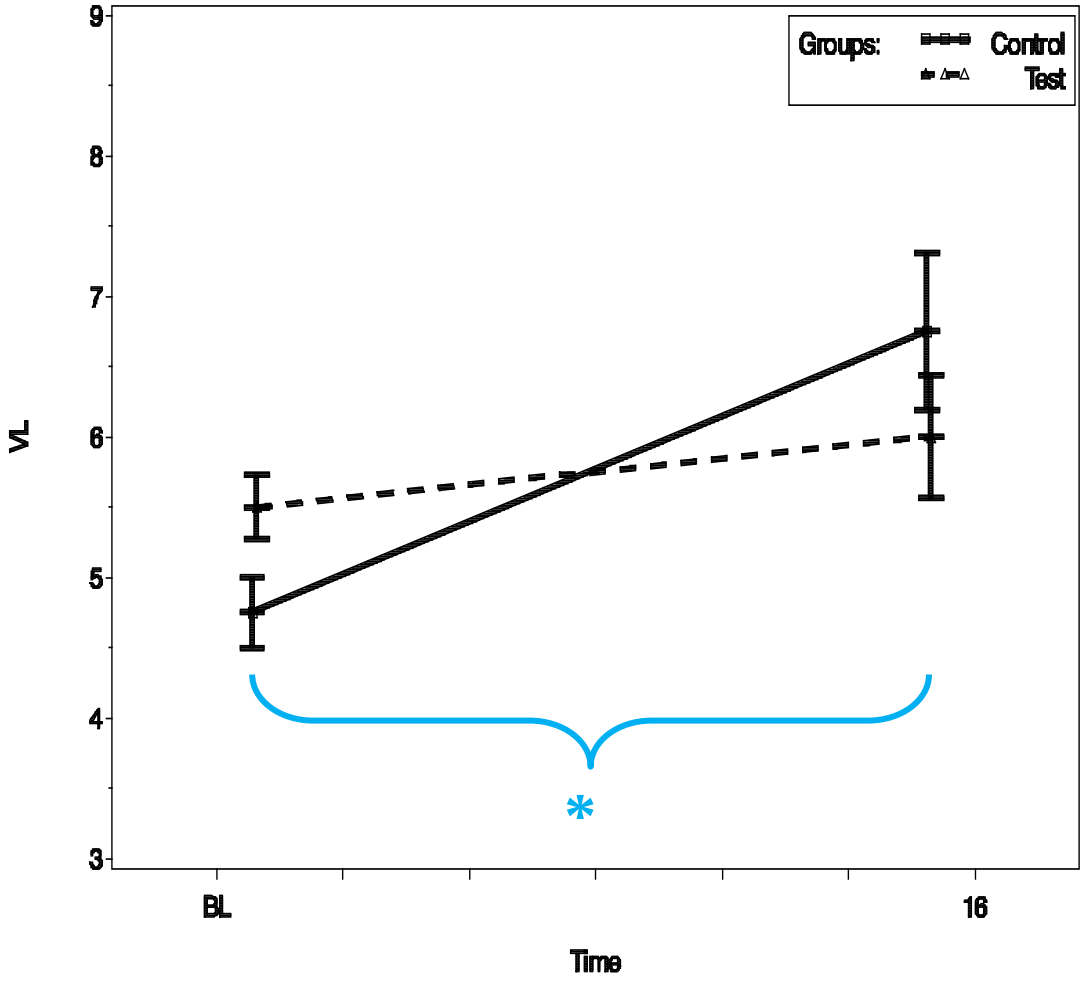


Figure 4: The mean of relative ridge height (mm) in the midlingual aspect of subjects from control and test groups at baseline and 16 weeks after tooth extraction. The loss of vertical bone height was statistically significant difference on the midlingual (p = 0.001) aspect in the control group.**
VL- relative ridge height in the midlingual aspect; ; BL-baseline; 16-16 weeks.

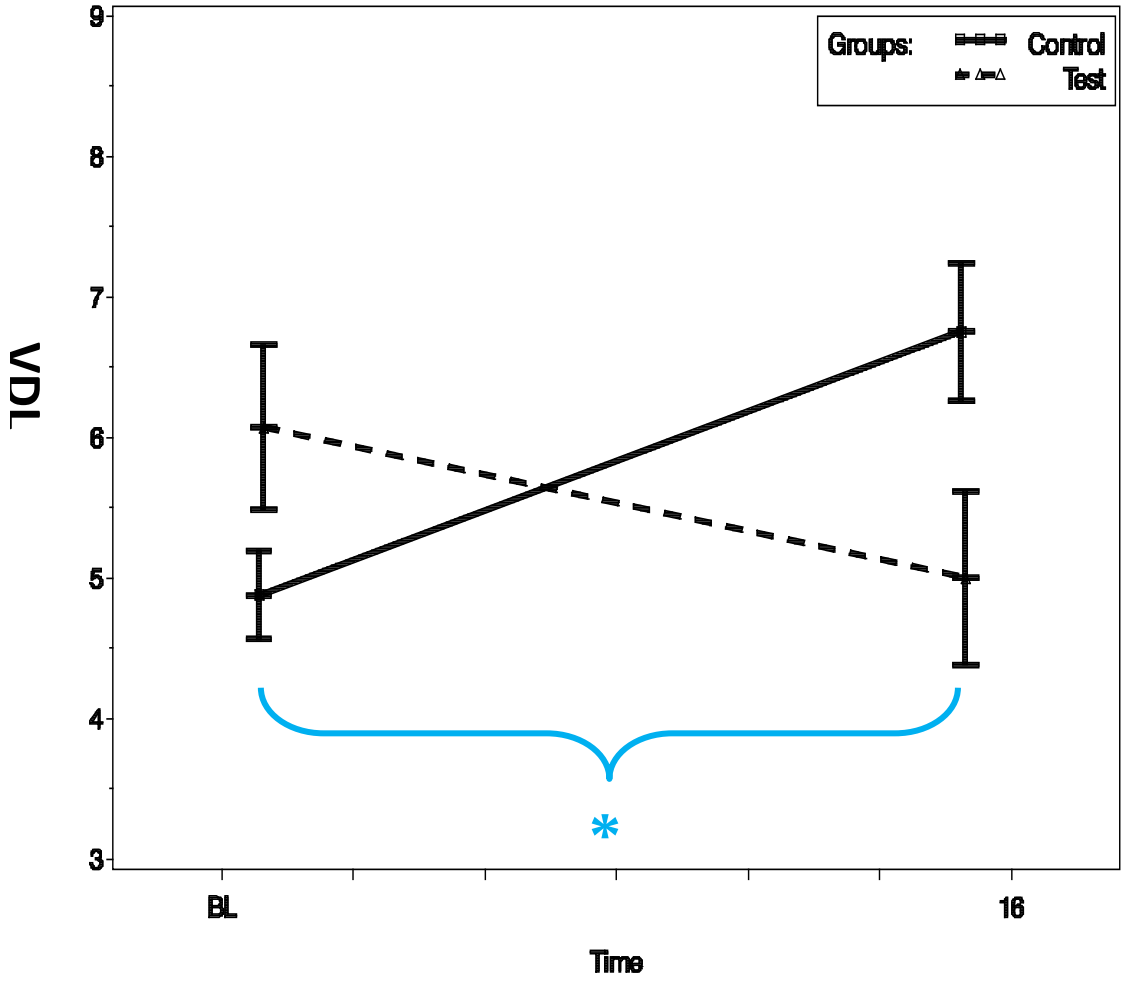


Figure 5: The mean of relative ridge height (mm) in the distolingual aspect of subjects from control and test groups at baseline and 16 weeks after tooth extraction. The loss of vertical bone height was statistically significant difference on the distolingual (p = 0.013) aspect in the control group.**
VDL- relative ridge height in the distolingual aspect; BL-baseline; 16-16 weeks.

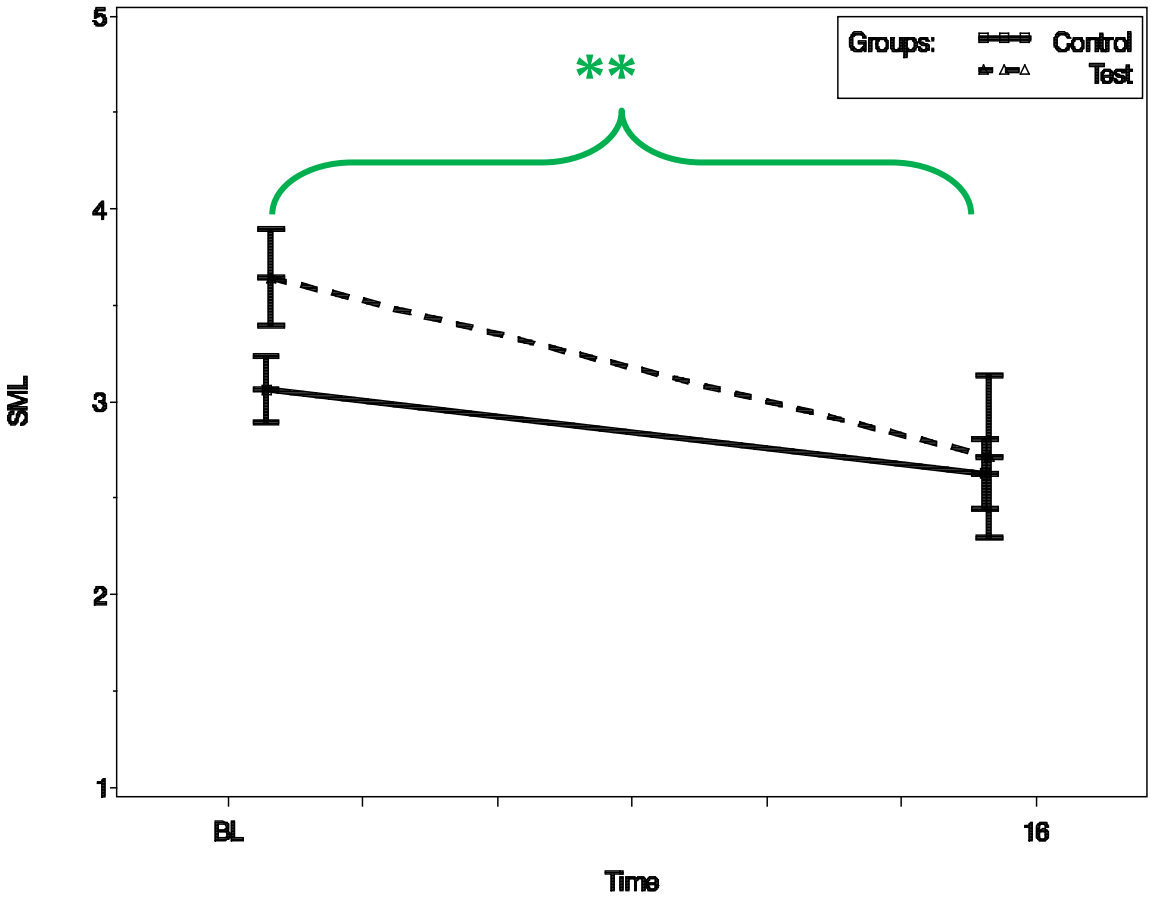


Figure 6: The mean of soft tissue level (mm) in the mesiolingual aspect of subjects from control and test groups at baseline and 16 weeks after tooth extraction. The loss of soft tissue level was statistically significant difference on the mesiolingual (p = 0.018) aspect in the test group.**
SML-soft tissue level in the mesiolingual aspect; BL-baseline; 16-16 weeks.

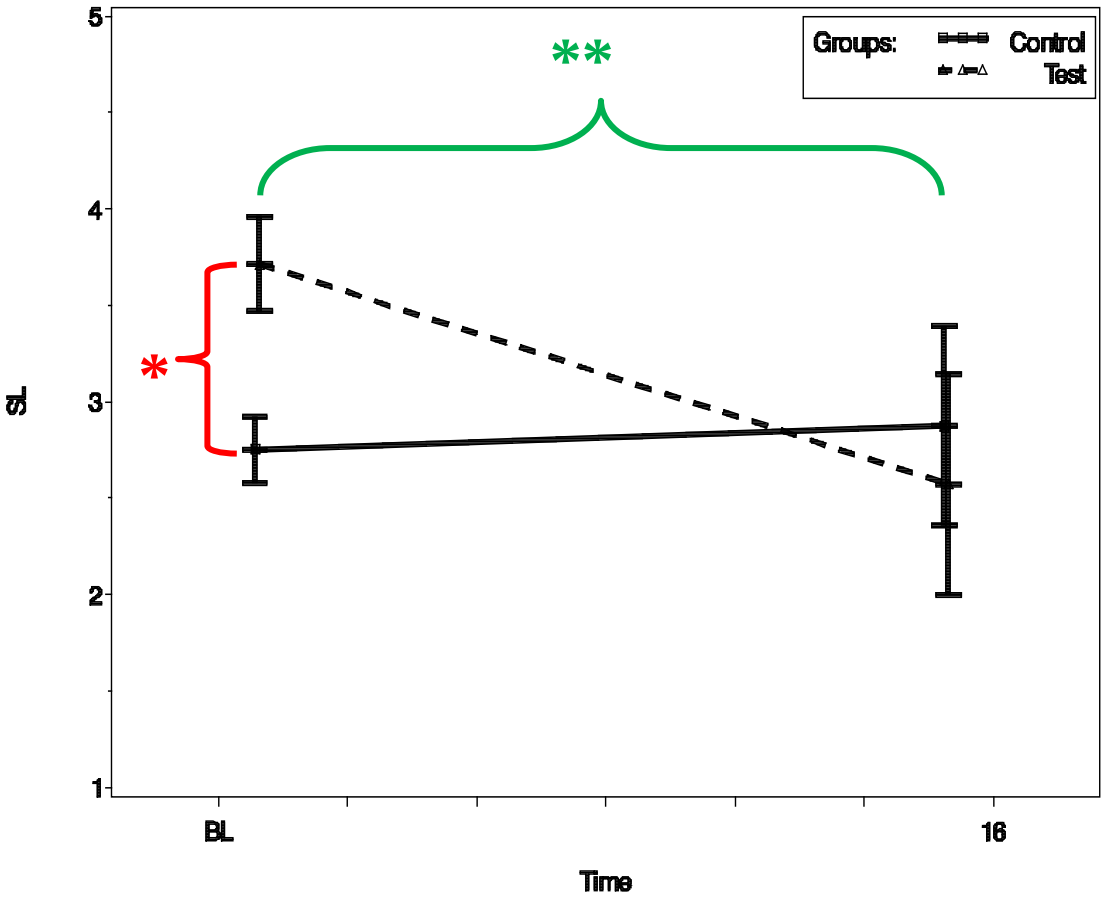


Figure 7: The mean of soft tissue level (mm) in the midlingual aspect of subjects from control and test groups at baseline and 16 weeks after tooth extraction. The loss of soft tissue level was statistically significant difference on the midlingual (p = 0.025) aspect in the test group. There was a significant difference between control and test groups at baseline (* p = 0.017).** SL-soft tissue level in the midlingual aspect; BL-baseline; 16-16 weeks.

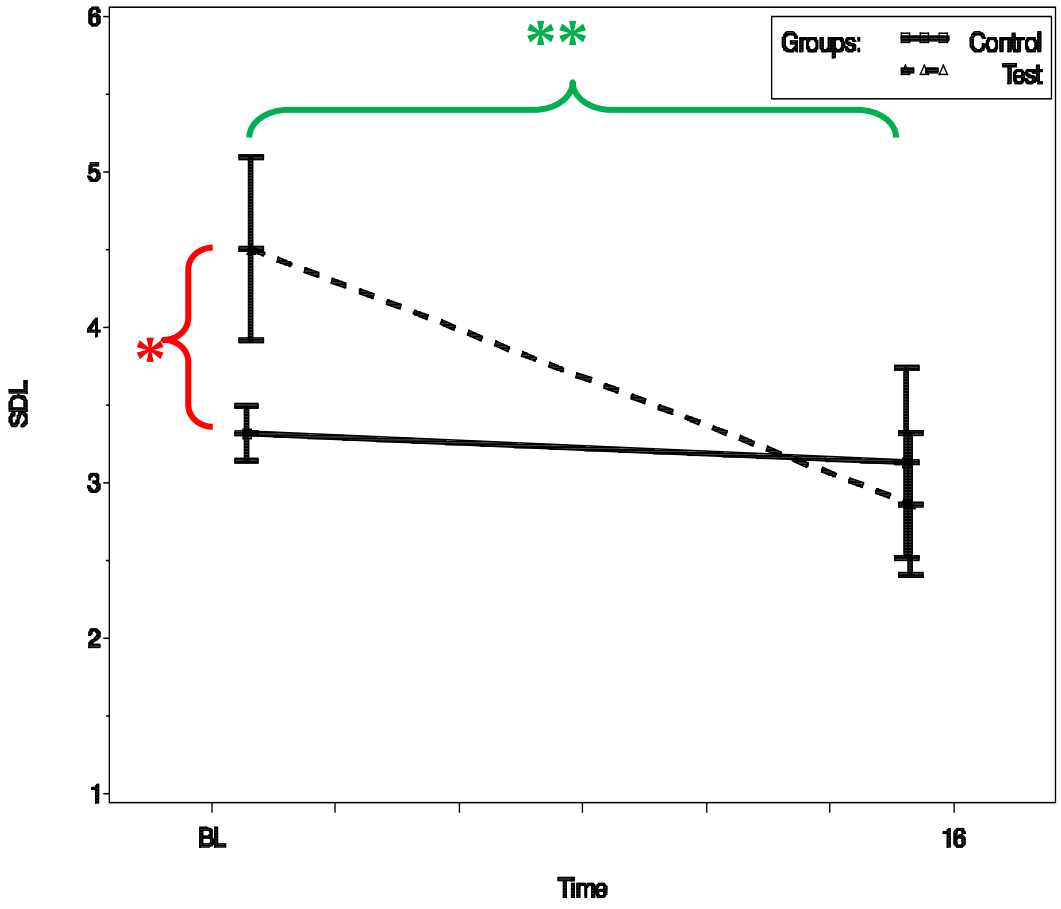


Figure 8: The mean of soft tissue level (mm) in the distolingual aspect of subjects from control and test groups at baseline and 16 weeks after tooth extraction. The loss of soft tissue level was statistically significant difference on the distolingual (p = 0.028) aspect in the test group. There was a significant difference between control and test groups at baseline (* p = 0.044).**SDL-soft tissue level in the distolingual aspect; BL-baseline; 16-16 weeks.

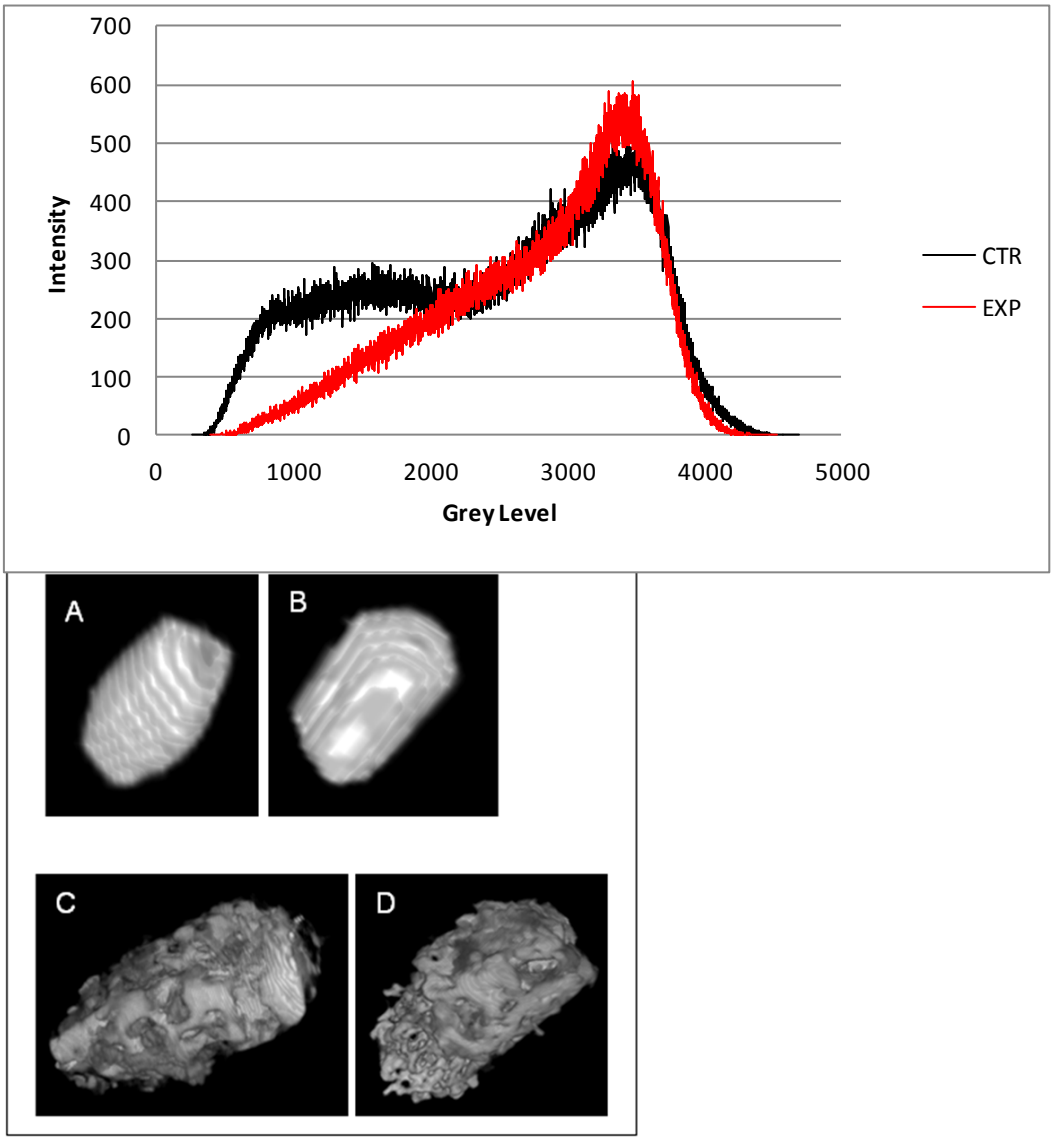
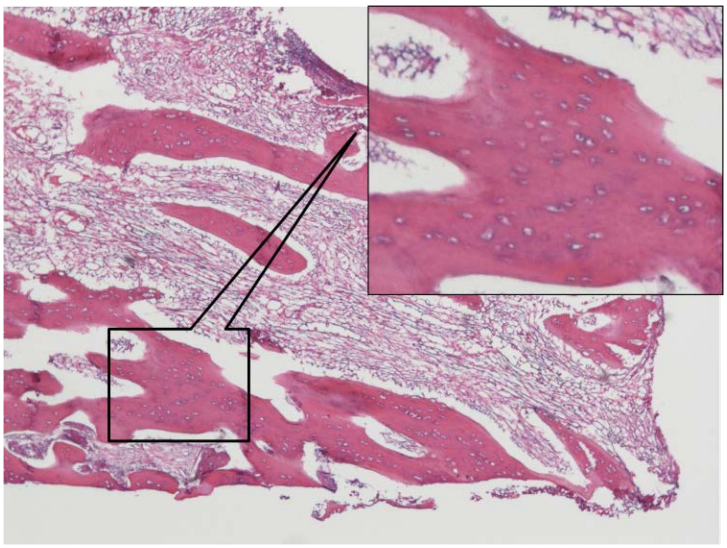


Figure 9: Representative of a micro-CT histogram (upper panel) from patients in control (CTR) and test (EXP) groups. The representative histogram showed similar plots and peak values between the control and test groups. CBCT and micro CT images (lower panel): A) Representative of CBCT image from control patient, B) Representative of CBCT image from test patient, C) Representative of micro-CT image from control patient, D) Representative of micro-CT image from test patient.

A



B

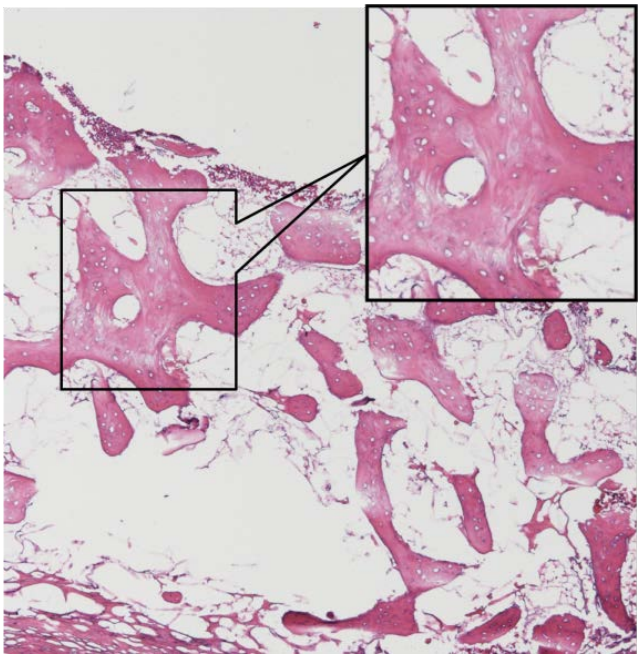


Figure 10: Photomicrographs of representative bone core section examined by H & E stain (original magnification 100x; inset 200x). A: Control group. B: Test group.

Chapter 4: Discussion

The overall aim of this study was two-fold: to determine if using a membrane alone can serve as a reliable alternative for ridge preservation following extractions, and to assess if there is a difference in the bone quality at the time of implant placement roughly four months after extraction when a membrane is used. Our results show that the control group has significantly more vertical bone loss than the test group. However, there were no differences radiographically and histologically between the control and test groups in regards of bone quality.

Due to the limited number of patients there was no significance difference found in any of the population, with the mean age being identical between groups and fairly similar distribution between jaws. The standard deviation being 14 for both groups shows that there was a pretty large spread as far as age. This is important to note because bone density has been noted to decrease with increasing age[21]. With the mean age being over 50 years old it would be interesting to look at young populations and compare the ages and bone densities to see if an age appropriate treatment model would be a valid option. There were 5 maxilla and 2 mandible in the test group when jaw location was examined. Again, because of the lack of subjects included into the study there was no significance here, however, Misch et al. have shown that there is a difference associated with bone

density and jaw distribution[29]. If comparing the control versus the test group there was a very similar distribution, 5 maxillary for both and 3 mandibular in the control and 2 in the test. These distributions, although different intra-group yet, give a very balance view based on the current model.

The clinical results in relative bone height are difficult to interpret on several levels and very clear on others when considering the 6 different sites of the extraction sockets. For instance, the significance found at baseline on the mesiobuccal site when measuring the vertical distance from the stent to bone ($p = 0.048$) can easily be explained by stent fabrication and intrinsic variation associated with the approach employed. The goal was to have the stent at the level of the gingival tissue, which was accomplished. However, as one can easily ascertain, there is inheritable variation; be it from inflamed gingival, to anatomical variation of biological width. Garguilo et al. reported an average of 2.73 mm from gingival margin to alveolar crest (2.07 excluding sulcus depth)[22]. In this report they also found that the connective tissue is the most stable structure, and this varied from 0.69 up to 1.49 mm. So 1 mm may be expected of variation in the most consistent portion. Add into that equation the fact that the sulcus and epithelial attachment, which is even more variable than the connective tissue, then it is not difficult to see why there can be slight differences in the design of the stent. What the stent does promote is an excellent way to monitor change over time. Thus the intragroup analysis shows much promise in terms of

analytical import. There was a significant vertical bone loss at the mesiobuccal, buccal, lingual and distolingual sites in the control group 16 weeks after tooth extraction. Moreover, there was not one site that was found to have gained any height after the extraction, whereas in the experimental group 3 sites had some amount of bone gain. In a Cochrane review of non-molar teeth undergoing extraction by Ten Heggeler et al., teeth that did not have socket preservation revealed 0.4 mm to 3.9 mm of height loss.[23] The group undergoing socket preservation with freeze-dried bone allograft (FDBA) reported the best results with an average of overall gains in 1.1 mm.[23] In our study, the test group cannot claim to have an overall gain in height for all 6 site measurements. However, when only the mesial and distal sites of the extraction socket were examined, the control group has significantly more bone loss than the test group. This difference became starker when all 6 site measurements were grouped to give one value for each extraction socket; the control group had a significant loss of 1.5 mm in vertical bone height with a p-value of 0.0063. In contrast, the test group did not have any significant amount of vertical bone loss. Furthermore, there was a steeper slope, or more change, associated with the control group when comparing the slope from the test group. These results suggest that the control group has significantly more vertical bone loss 16 weeks after tooth extraction than the test group.

When comparing the soft tissue levels prior to extraction and immediately after extraction, there were no statistically significant differences in either control or test group. To our best knowledge, this is the first ever documentation in regards to soft tissue levels before and immediate after tooth extraction. When grouping the soft tissue level measurements into either mesial and distal sites or into one value, there were no statistically significant differences in the change of soft tissue levels between control and test groups, suggesting that the soft tissue remains more constant throughout the 16-week healing period in both groups.

While there are many generally accepted ARP techniques being utilized, what is quickly becoming the "gold standard' by many clinicians is particulate graft and membrane placement. Iasella et al. examined this technique and found very favorable results[8]. While they were not able to completely reduce the buccal-lingual resorption following extraction with graft and resorbable membrane, they were indeed able to eliminate vertical bone loss and even gain bone height. Although our study did not examine the horizontal bone loss, the stent-bone device used in this study to evaluate the vertical bone levels was comparable to those methods in other studies. The inclusion of using a membrane in this study did show a statistically significant difference from a naturally healing socket in the mesial and distal aspects of the socket. The finding of an average of 1.5 mm vertical bone loss in control group and not in test group supports the concept of socket preservation therapy in patients with a tooth extraction[23].

However, more studies of larger sample sizes will be required to confirm the result. Keeping this in mind it may be prudent that in areas where vertical bone might be of an issue (ie. premolars directly above the mental nerve), use of a membrane with particulate allograft may be in the patient's best interest, despite the increase in material cost. All teeth included into this study were premolars, with no difference between the numbers of maxillary or mandibular teeth recruited. While a larger number would be preferable, by limiting the type of teeth included into this study, the number of variables was limited even further.

The removal of 6 patients from the study after the extractions were carried out, 4 from the test group and 2 from the control group which was equal to 33% of the overall subjects recruited. This is roughly equal to the results that Lekovic et al.[24] found with their study of using an ePTFE membrane alone. Three out of 10 subjects they recruited were removed early due to membrane exposure that would not heal. Those 3 sites removed early from the study showed similar results as the control sites (extraction alone). Five out of the 7 sites that received ePTFE membranes has statistically significant less bone loss when compared to the control group, indicating that ePTFE may contribute to regenerative results in extraction sites.[24] The use of a resorbable membrane rather than the ePTFE membrane was another reason for choosing the PLA membrane in this study. The present study shows that all sites, regardless of test or control, healed over with

soft tissue before 6 weeks. This is in contrast to 3 sites continuing to be exposed for 3 months in the study of Lekovic et al.[24]

Evaluating the follow-up questionnaires showed no statistically significant differences. The test group had nearly twice the self-reported pain duration, however the maximum amount of pain was quite similar. In opposition to this, the control group held the only two patients that needed opoid analgesic to control their level of pain. Pain is a very personal and subjective measurement. Each person has a different idea of what they will expect, along with a different tolerance for pain. Due to such low numbers it is quite possible the data could be skewed simply by this bias.

Follow-up measurements of the size of the membrane exposed shows consistently that by 6 weeks there was no membrane left exposed to the oral cavity. Whether the membrane was still intact and covered by soft tissue, hydrolyzed already, or simply displaced by the growth of epithelium underneath the membrane is impossible to determine and was not within the aim of this protocol to evaluate.

A limitation of this study includes a large bias in regards to expected outcomes based on the inclusion criteria. By ensuring the walls of the extraction socket with 1 mm or more in thickness, there was little to be expected in the way of change in vertical bone height. Nevertheless, controls lost 1.5 mm of vertical bone height. By not including the extraction sockets with walls that are known to

be most likely to experience significant changes (e.g. thin or nonexistent walls), it was pre-determined to have less dramatic results than if such sockets had been included in the study.

Conclusion is difficult to be drawn about the radiographic analysis. With such a small sample size to extrapolate from, the variances for both groups were extremely large. While no differences were detected in regards to the bone quality, more studies of larger sample sizes will be required to confirm this conclusion.

Histologically there was very little difference in the appearance of the specimen slides between control and test groups. All slides showed dense lamellar bone with no inclusion of residual woven bone being present. Due to the handling of the samples, the freeze-thaw effect seen hampered the ability to view intact interfaces between the connective tissue and the bone. Regardless, there was consistency throughout the specimens from both control and test groups. No membrane particle or foreign body reactions were noted in any of the slides being examined from the test group.

Chapter 5: Conclusions

Within the limits of this study, the following conclusions can be made:

1. There were no significant changes in relative ridge height and soft tissue levels prior to and immediately after tooth extraction in either control or test group.
2. There were significant changes of bone height in the control group, after tooth extraction and before implant placement, on the mesiobuccal, midbuccal, midlingual, and distolingual aspects of an extraction tooth. The changes of bone heights remained significant in the control group when only the mesial and distal sites of the extraction tooth were examined or when all 6 measurements were averaged into one value. In contrast, there were no significant changes of bone height in the test group when either all 6 measurements were analyzed separately or grouped into one value, or the mesial and distal sites of the extraction tooth were examined. The results suggest that the use of PLA membrane alone after tooth extraction may prevent post-extraction vertical bone loss.
3. The DBM value from the micro-CT and CBCT analyses were similar between the control and test groups, however, the sample size was too small and a large variance was identified. The results may suggest that the use of PLA membrane after tooth extraction may not improve bone quality compared to control; however, more studies with larger sample size will be needed to confirm this finding.

4. Sampling bias was identified in such a way that only extraction sockets with four intact walls were included in the study, even though there was randomness in the selection of the sample. The sampling bias involved in this study may explain the small amount of vertical height changes in the control group.

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