Comparative evaluation of amnion chorion membranes versus platelet rich fibrin membranes in extraction and ridge preservation using the open membrane technique – A pilot randomized clinical trial

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ABSTRACT

Purpose: Amnion-chorion membranes (ACM) and platelet rich fibrin (PRF) membranes have been reported to have inherent biological properties that can favorably influence wound healing, regenerative and patient related outcomes. The aim of this study was to compare the effectiveness of these membranes in ridge preservation, when used in an open membrane technique, with a pilot randomized clinical trial for the primary outcomes of wound closure rates and perceived post operative pain and secondary outcomes of ridge dimensional. **Materials and Methods:** A double masked randomized controlled clinical trial was used to compare the two membranes in premolar sites undergoing extraction and ridge preservation procedures. Ridge dimensions were measured pre surgery and four months post healing with help of a CBCT and radiographic stent with fiduciary markers. Wound closure tissue closure was documented and compared prospectively using intra oral scanning and digital measurements. Perceived pain was compared with help of Visual analog scale which was administered through a web-based survey that the subjects filled out starting from day 1 till day 16 post-surgery. **Results:** Radiographic bone level changes and intra oral wound healing rates were not significantly different between the two groups. Statistically significant results were observed in the VAS scores (i.e., perceived pain) with the ACM group subjects reporting significantly less VAS scores in the mornings as well as over 24hr periods. **Conclusion:** Within the limits of the study, both ACM and PRF membranes are effective in ridge dimensional when used with open membrane technique. Wound closure rates are comparable irrespective of the type of membrane. Use of ACM may reduce self-reported perceived pain compared to PRF as evidenced by lower postoperative VAS scores with ACM.

Keywords: amnion-chorion, platelet rich fibrin, cone beam computed tomography, ridge preservation, intra oral scan, wound healing

INTRODUCTION

Dental implant therapy is increasingly becoming the treatment of choice for tooth replacement. From 1999-2000 approximately 0.7% of US adults missing teeth had at least one implant, which had increased to 5.7% by 2016. Latest projections estimate that by 2026 roughly 17% of US adults will have sought and received dental implants.[1]

One of the crucial obstacles to placing dental implants are edentulous sites that are deficient inadequate ridge dimensions. Several factors, including difficulty of extraction, technique used, skill levels of provider, disuse atrophy etc., can contribute to that. Even when extractions are performed with minimal trauma ridge dimensional loss is a predictable sequalae. After tooth loss, residual alveolar bone is rapidly re-contoured with an average volume loss of about 25% within the first year, and can range from 40-50% of width loss within the first 3 years[2]. This is often at the expense of the facial dimension where within the first four months, an average of 2mm of vertical bone height loss and an average of 4-5mm horizontal ridge width loss have been reported. Anterior teeth with thin phenotype can undergo drastic bone loss with a reported average vertical loss of 7.5mm in the central aspect of the remodeling socket[3].

The benefits of ridge preservation have been previously demonstrated with the help of high level evidence including randomized clinical trials. Yet, there is lack of reliable information on frequency of clinical use of these procedures. Indirect evidence suggests that this treatment modality is not being typically offered to all patients, with ridge preservation constituting only 29% of procedures that use bone substitute materials in dentistry[4].

While this can be attributed to a myriad of reasons, a chief driver is that this procedure is not accessible to a typical general dental practitioner. Reconstructing and regenerating lost ridge through guided bone regeneration requires greater levels of surgical skill, yet results in an averages width gain of about 2.9mm and a highly unpredictable vertical dimensional gain[5]. Loss of keratinized gingiva after spontaneous healing post dental extraction further complicates successful reconstructive outcomes[2].

A number of case reports have demonstrated that that resorbable membranes like amnion chorion membranes and platelet rich fibrin as well as non-resorbable membranes like dense Poly-Tetrafluro-ethylene (d-PTFE) can be used successfully in ridge preservation using the 'open membrane technique'[6]. Open membrane technique involves the use of barrier membrane that is intentionally left exposed to the oral cavity. This approach simplifies ridge preservation procedures as it overcomes the need to obtain primary closure which requires specialized flap management skills and knowledge. An additional advantage that has been attributed to the open membrane technique is that it is much more effective in preserving in keratinized tissue obviating the need for additional soft tissue phenotype modification procedures to enhance dental implant long term success. While several membranes can be used with this technique, including relatively inert barrier membranes such as collagen, dPTFE etc.,; empirically biologically active membranes like Platelet Rich Fibrin (PRF) and Amnion Chorion Membranes (ACM) can enhance clinical outcomes.[7,8] However, this assertion is not supported by clinical trials.

Here we report the outcomes of a pilot double masked randomized clinical trial that comparatively evaluated the use of ACM (Bioxclude, Snoasis Medical Inc.) to PRF (H-PRF, Bio-PRF) membranes when used in open membrane technique ridge preservations. Primary outcomes that we report here at wound healing rates and self-reported perceived pain evaluated with visual analog scale. Secondary outcomes we report are horizontal and vertical ridge dimensional changes.

MATERIALS AND METHODS

Trial Design

The Institutional Review Board of Oregon Health Sciences University (OHSU IRB) approved the study (STUDY00020324) on 10/14/2022. Study enrollment began at the end of October 2022 and this arm closed to enrollment in September 2023. Study was registered on clinicaltrails.gov (protocol ID: STUDY00020324). All included subjects were given detailed explanations of the study protocols and consent forms were executed prior to beginning. A double blind randomized clinical trial design was used.

Eligibility and Exclusion Criteria

Criteria for inclusion were subjects in need of extraction(s) for a non-molar tooth due to caries, fractures, restorative problems, endodontic complications (e.g. instrument fracture) and orthodontic or prosthetic reasons. Male or female, of any ethnicity $\geq$ 18 years of age with a health status of ASA I or ASA II, as classified by the American Academy of Anesthesiologists. Subjects were to have no evidence of any active moderate or severe periodontitis, must be available for multiple follow-up visits for the duration of the study. Presence of at least one adjacent tooth at the extraction site was required as was adequate oral hygiene (bleeding on probing <20%; modified O'Leary Plaque index <20%). Pre-surgical clinical and CBCT evidence of presence intact buccal and lingual plate >1mm thickness (i.e., socket with intact bony walls) and the difference between the most coronal aspect of mid buccal and most coronal aspect of mid lingual alveolar walls should not be greater than 2mm. Exclusion criteria included inability or anticipated failure to maintain adequate oral hygiene; women who report being pregnant, breast feeding, or intend to become pregnant. Participants with unstable systemic diseases or with a compromised immune system (e.g., uncontrolled diabetes- HbA1c > 7.0%) or unstable bleeding disorders; subjects with active infectious diseases (e.g., hepatitis, tuberculosis, HIV, etc.); mental disabilities that may hinder participation; taking steroid medications or undergoing immunosuppressive

therapy; cancer therapy, and/or radiation to the oral cavity within last 6 months; medications or conditions contraindicated for bone regeneration(e.g., methotrexate, RANK-l inhibitors, Anti-TNF-alpha medications, corticosteroids, history of bisphosphonate use, or cyclosporin-A); existence of bone metabolic disease; participants refusing the use of allograft or autograft materials; tooth loss caused by severe periodontal disease; and presence of acute periapical lesion.

Study Setting/Location

Screening, recruitment, and all related procedures were performed at the clinics of Oregon Health Sciences University School of Dentistry (OHSU SOD) Graduate Periodontics Residency Program by 2nd and 3rd year residents. A total of 8 subjects were treated with an even allocation between recipients of ACM and PRF membranes.

Extraction and Ridge Preservation

All subjects had blood drawn and PRF fabricated to blind them to their allocation. For the purposes of this study, the available centrifuge was the horizontal model produced by Bio-PRF using the H-PRF protocol proposed by Miron et al. of 700g for 8 minutes[9] . The PRF membranes were fabricated using a PRF tray. At this time the resident was informed discreetly of the allocation (described later). Minimum trauma extractions were performed via sulcular incisions circumferentially around the tooth to be extracted, gentle elevation, and forceps delivery with care to not damage the buccal or lingual plates of bone. In cases where adequate mobility was not attainable for a minimally traumatic extraction, a vertical extractor (Benex CBE00) was used to remove the tooth. In the ACM allocated group, a 70% mineralized 30% demineralized cortical allograft hydrated with sterile saline was placed in the socket and covered by the amnion chorion membrane tucked gently under the free gingiva, then secured with 4-0 PTFE sutures via an inverse figure 8 design. In the PRF group the allograft was hydrated with the plasma exudate obtained from the membrane fabrication, and placed in the socket was covered with a PRF membrane and secured with the same suture design. See figure 1. No antibiotics were prescribed,

and subjects were advised to use saltwater rinsing and over the counter medication if needed. Subjects were then evaluated at 2-4 days, 6-8 days, 13-15 days, 20-22 days, 27-29 days, and finally at 100-108 days.

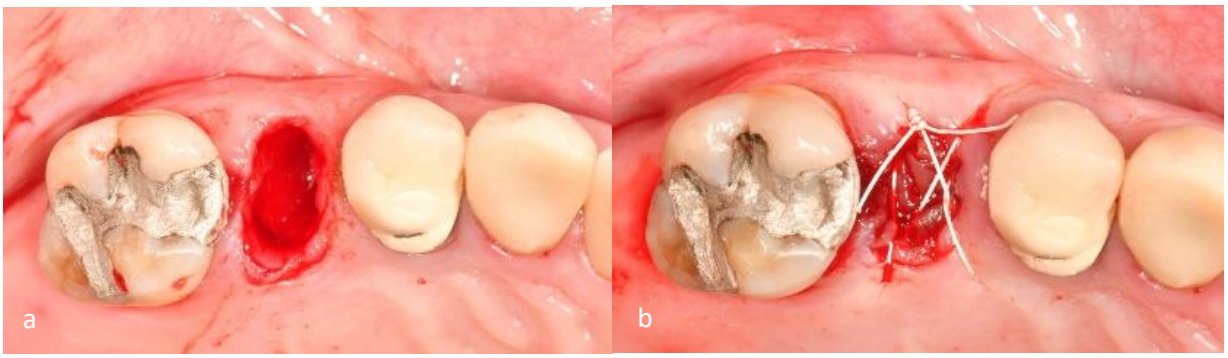


Figure 1. Clinical photograph of surgical protocol. Image (a) showing an extraction site prior to grafting. (b) Image showing the grafting procedure open described in this study of allograft and exposed membrane.

Study Procedures and Clinical Measurements

Subjects were randomly allocated to one of the treatment modalities using randomization scheme generated using the randomization function using spreadsheet software (Microsoft excel, MS corporation, Seattle, WA). Subjects were masked to the treatment allocation they received. To ensure integrity of masking all subjects had their blood drawn and the PRF prepared. On the day of the surgery, a secure envelope containing the allocation was given to the surgeon by the study coordinator ensuring that the allocation is not revealed to the subject. Subjects were deidentified using a unique ID and this information was stored in a spreadsheet that was accessible only to study PI (SP) and Study coordinator (LL). All subject related data were associated with the unique ID and any information containing identifiable information of the subject in the data was removed. All study measurements (image-based measurements and VAS scores) were done by one evaluator (LH) who was masked to the subject identity and accessed the data associated with the unique ID. The evaluator (LH) was part of some of the

study surgeries and/or assisted them, however LH was masked to the subject identity at the time of the data measurements and analysis.

Wound Closure Rates

3Shape Trios digital scans were taken the day of surgery immediately post extraction and at each subsequent post operative visit. Using the ruler feature in the software, measurements were made from a point on the buccal most gingival border of the extraction wound to the lingual most border. See figure 2. The measurements were recorded and graphed until the wound opening was no longer discernable. Given the timing of the post-operative visits however, the actual day of closure cannot be known with certainty. Hence the rate was determined using the day of surgery as the minuend and the last measurable open wound day as the subtrahend. Choosing these two points minimizes possible erroneous estimation. The difference was then divided by the actual number of days lapsed for each subject resulting in a quotient that represents the daily rate of epithelial migration over the extraction wound.

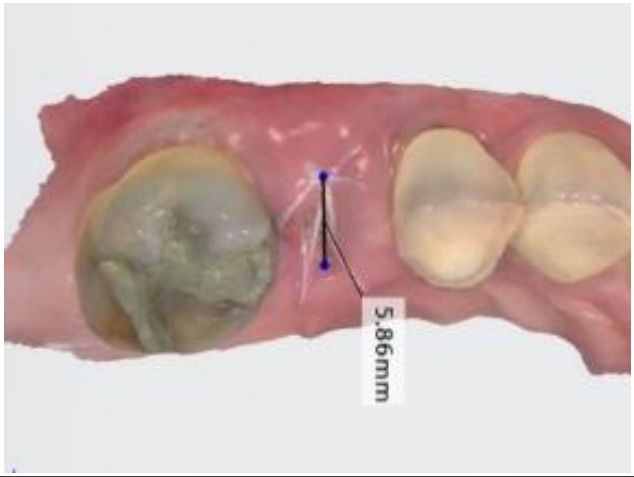


Figure 2. Wound closure measurements. Image of an intra oral scan used for measurement of the wound size.

Radiographic assessment of ridge dimensional loss:

The cone beam computed tomography (CBCT) images were captured using a Carestream CS81003D set at 150 voxel size and 90kV with a preoperative sectional arch field of view and a postoperative full arch

field of view. Acrylic radiographic stents with radiolucent fiduciary markers were fabricated and used in both CBCT images. This stent, the 'SivRoy' stent, has hollow tunnels running horizontally that appear radiolucent on radiographic sections. These radiolucent areas were used as a radiographic reference to align images taken temporally and allows for reliable and reproducible measurement of difference in temporal dimensional changes.

CBCT's images were taken prior to the study surgical procedure and again at 110-120 days after surgery. The markers in the SivRoy stent were used to line up the oblique view before and after cross sections in the Carestream viewing software, and then again to merge the section images. Five sections were created per site, one in the center of the tooth along the coronal-apical axis, and two consecutive sections 1mm apart on both the mesial and on the distal. The slices are denoted as C = center, M1 = 1mm mesial from center, M2 = 2mm mesial from center, D1 = 1 mm mesial from center, and D2 = 2mm mesial from center. See figure 3.

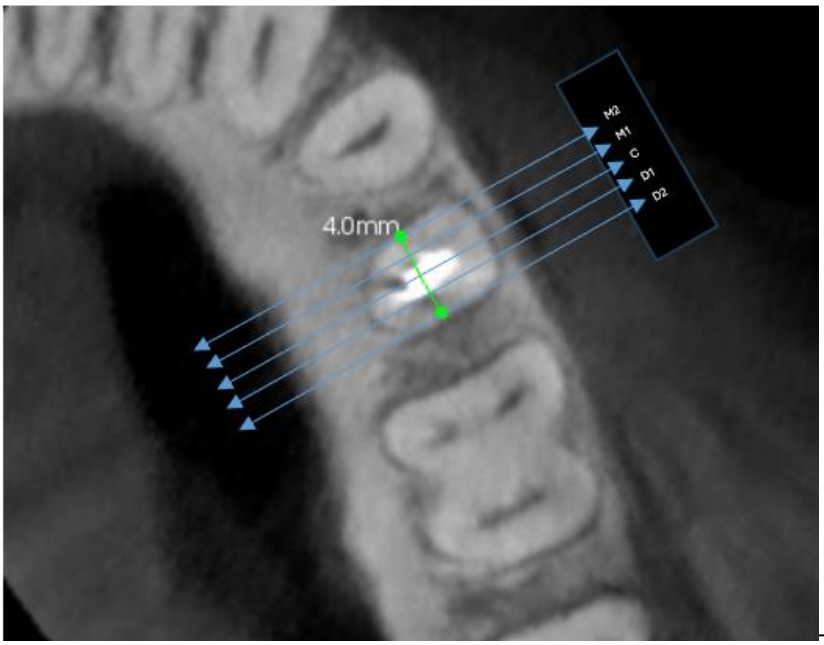


Figure 3. Radiographic analysis. CBCT cross sections analyzed at the center of the tooth to be extracted, and 2 measurements 1mm apart both mesial and distal to the center section. These have been labeled as C=center M1=1mm mesial to C, M2=2mm mesial to C, D1=1mm distal to C, D2=2mm distal to C.

The cross section from before the extraction was measured from the top of the crest on the buccal and the lingua aspects marking the highest points of the plates of bone and creating a measured line with which to later calibrate the measuring software. To be able to overlay the images and compare the dimensions of bone, the post-surgical image was adjusted in its transparency, artistic effect, and color in Microsoft PowerPoint to allow visual differentiation. See Figure 3. The merged images were uploaded into ImageJ software. The measurement line previously created was used to calibrate the ImageJ measuring tool. To standardize the method of assessing bone changes, 90-degree lines extending apically were made again from the measurement line, and the distance from the original buccal and lingual points to the bone long the 90-degree line was measured. Where the 90-degree line did not intersect with the bone due to the angulation of the alveolar ridge, a line parallel to the initial measurement line was made where the cortical plate bone on the post-surgical images were highest. The point where this second line intersected with the 90-degree line became the measuring point. Horizontal bone loss was measured where visible from the outline of the buccal plate on the per extraction image to the buccal plate on the post extraction image along a line also parallel to the original measurement line. See figure 4.

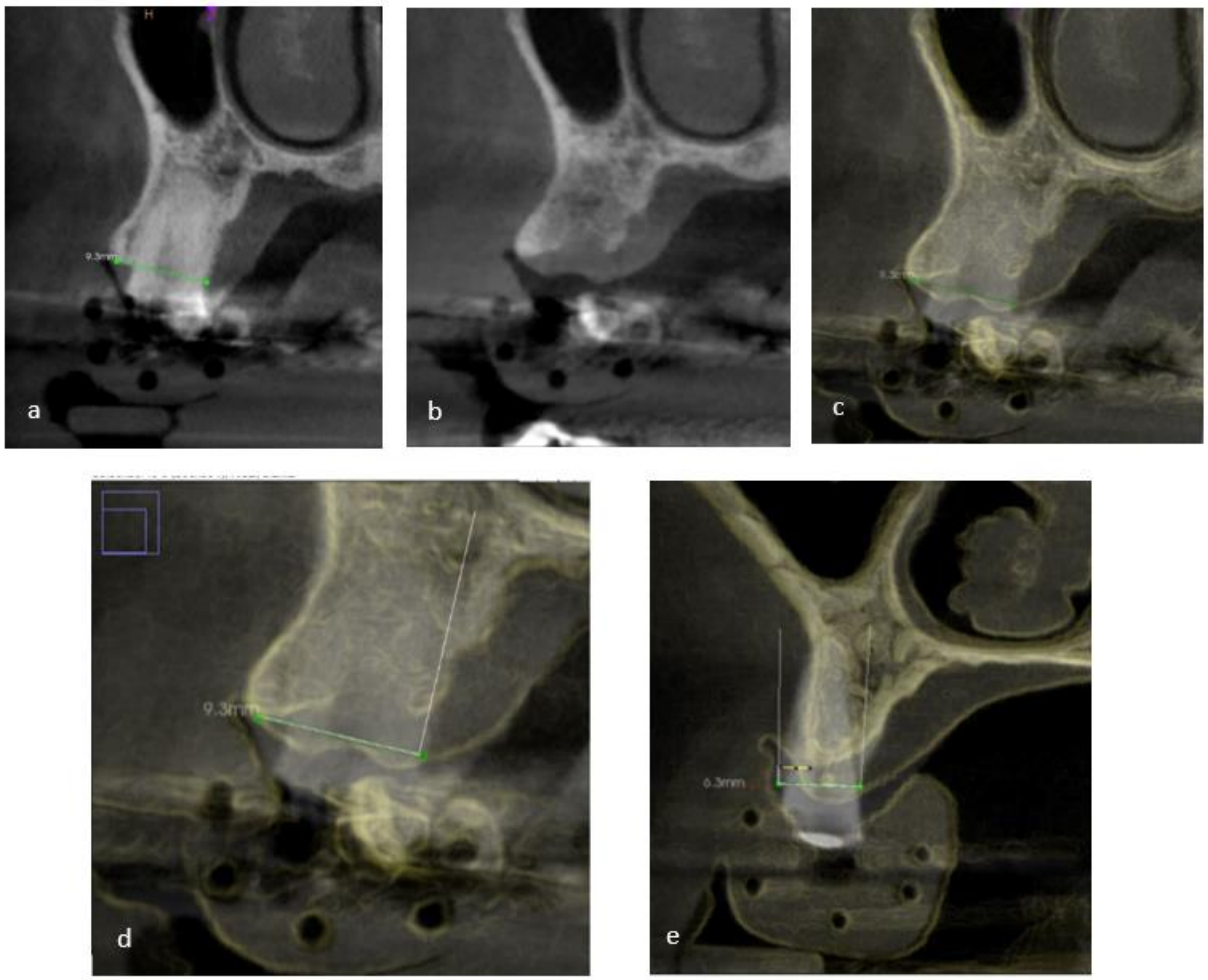


Figure 4. Radiographic analysis strategy. CBCT sections used for measurements. (a) an example of an initial cross section marking the highest points of the buccal and palatal plates of bone. (b) an example of the same site 120 days post extraction. (c) image b made transparent and overlayed over image a. (d) measurements made from the initial markings at a 90-degree angle to measure the change in bone height. (e) a separate image where horizontal bone loss was appreciated, the measurement of the buccal height and width change using a line parallel to the 90-degree line from the initial buccal point to the new highest point of the buccal plate.

Visual Analog Scale

A digital visual analog scale (VAS - pain scale form) survey (Supplementary material) was sent to the participants via email with instructions to record pain and discomfort levels. Subjects were enrolled in the software system around the 2nd visit (pre-surgery) to confirm the subjects' information, then

received surveys starting the 3rd visit (day of surgery) and every day after the until the 16th post-operative day. The subjects were asked to rate their highest and lowest rates of pain on a scale of 1-10 with 1 being no pain and 10 the worst imaginable.

A Priori power analysis:

Sample size determinations were done using the data from the study by Thakkar et al[10]. An assumption was made that the standard deviations of the differences between treatments would be similar to their data i.e., 0.7035 for the change in horizontal ridge width. With a sample size of 21- sites per group, the study was expected to have 80% power to detect a difference of 0.61 mm (observed in Thakkar et al study) between treatments for the change in horizontal ridge width. The sample size calculations assumed two-sided tests with 5% significance level.

Posthoc Power analysis:

Posthoc power analysis was estimated using the data from one of the primary outcomes i.e., subject reported AM VAS scores. The mean and SD of this attribute along with its respective sample size for both groups (PRF – 4 and ACM -3) was inputted into an online post hoc power calculator (Clincalc.com). Through this method the posthoc power of this study is estimated at 51%. For subject reported highest AM VAS scores the posthoc power is estimated at 55.3%. To have 80% power for these parameters the minimum sample size required is estimated at 12 per group.

Statistical analysis:

Descriptive statistics including mean, standard deviations were calculated for all measurements. All data points were normally distributed. The two groups were statistically compared using the two-sample t-test with unequal variations and two tailed distributions. The statistical interpretation of the data is limited given the pilot nature of this study.

Results

Study Demographics

Thirteen eligible subjects were recruited and consented. One declined to participate after consenting and was exited from the study. Three subjects were excluded after the consent process as their extraction was done prior to study start date. Nine subjects were allocated into the study, and one subject had to be exited from the study due to failure in blood draw procedure on day of surgery. Thus 4 subjects were allocated to ACM group and 4 subjects in PRF group. All subjects completed all study visits, however, one of the ACM group subjects did not fill out their VAS surveys (See figure 5 - Consort flow diagram).

The distribution of teeth included one maxillary first premolar, six maxillary second premolars, and one mandibular first premolar. The age and gender composition of the ACM group was two males and two females ages 41-70 with an average age 60. The PRF group was composed of one male and three females ages 37-77 with an average age of 62.25. The average age by gender was 56 years for the female subjects and 68.6 years for the male subjects. See Figure 6.

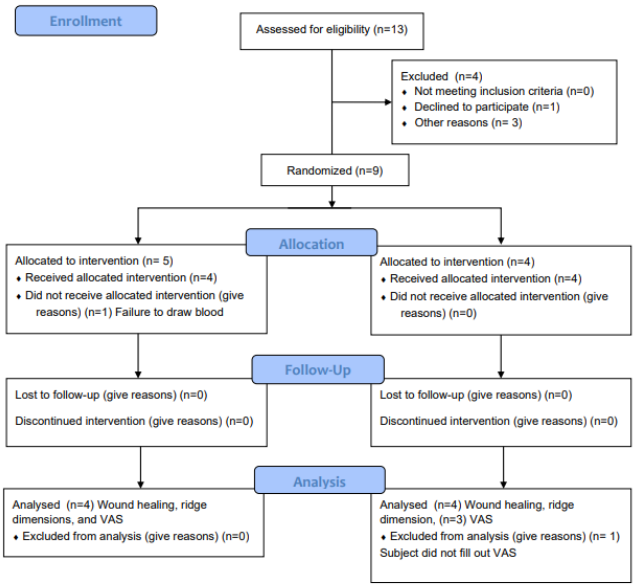


Figure 5. Clinical trial flow diagram. The flowchart describes the number of screened subjects recruited/excluded. It also describes the number of subjects allocated to study interventions, completed study, and number of subjects lost/excluded from analysis.

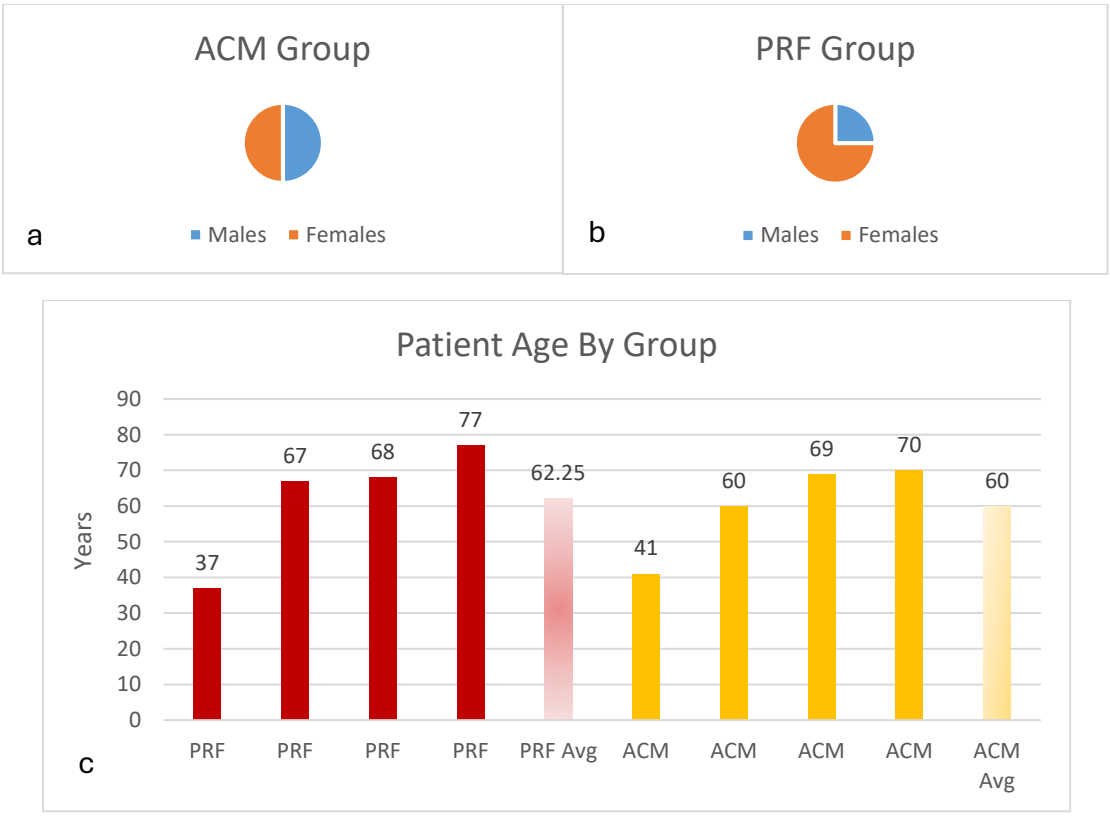


Figure 6. Patient demographics. (a) graph showing the gender distribution of the ACM groups subjects of 2 males and 2 females. (b) graph showing the gender distribution of the PRF group subjects of 3 females and 1 male. (c) the age of each patient in each group as well as the average age of each group.

Wound Closure

Wound closure rates were measured as described previously. Early and overall wound closure rates were estimated as follows. Wound sizes were measured from intra oral scans obtained at every post op visit. Overall wound closure rates were calculated by dividing the initial wound dimension from day of post-surgical visit by the day of post-surgical scan at which there was no discernable opening (See figure 7). ACM group had wound closure rate of 0.34mm/day compared to 0.26mm/day in the PRF subjects. This difference, however, was not statistically significant (p-value of *.132*). Early wound closure rates were determined by dividing difference between initial wound dimension with dimension on 2nd post

operative visit by day of the 2nd post operative visit (days 6-8). The differences (ACM - 0.3125mm/day vs. PRF -0.3325/day) in early wound closure rates were not statistically significant (p = *0.82*) between the two groups. Figure 8 shows the group average early wound closure rates and individual subject wise early wound closure rates.

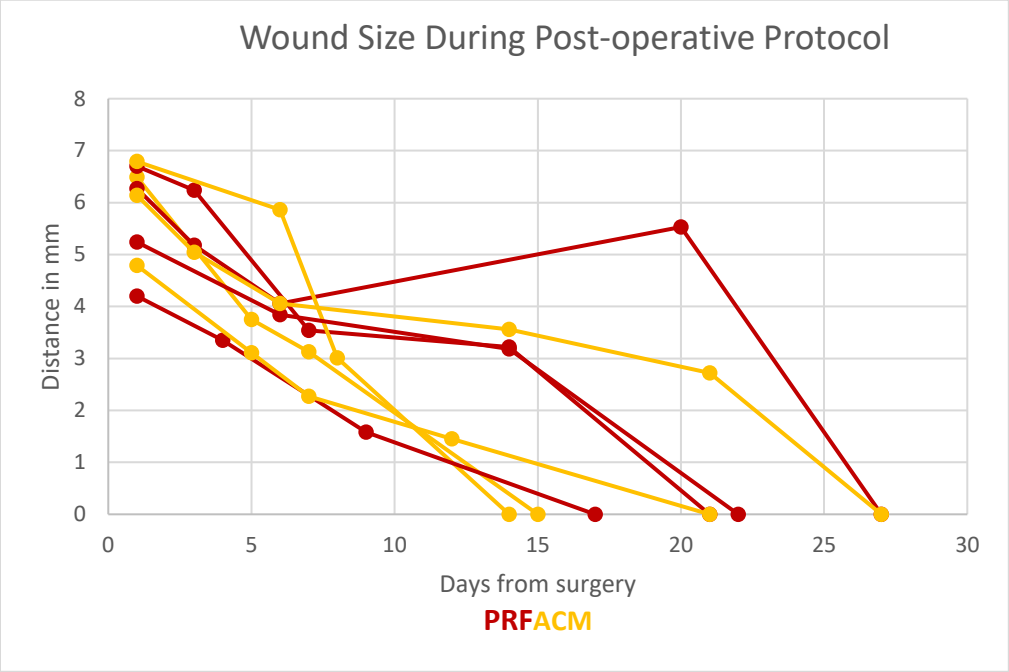


Figure 7. Subject wise Time course of Wound closure. Line graph showing the wound closure measurements by the day from initial surgery. The measurement made on each of the post operative visits denoted by color as belonging to the PRF or ACM group.

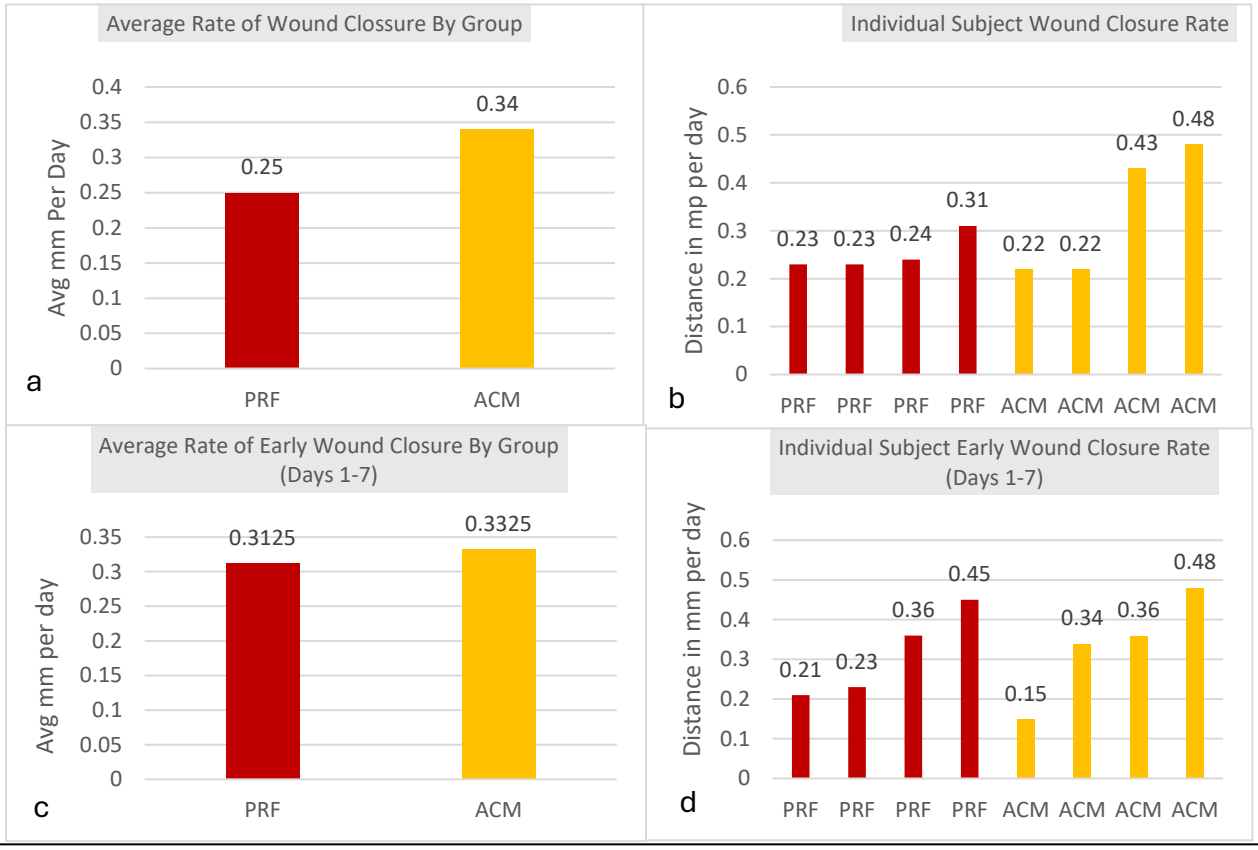
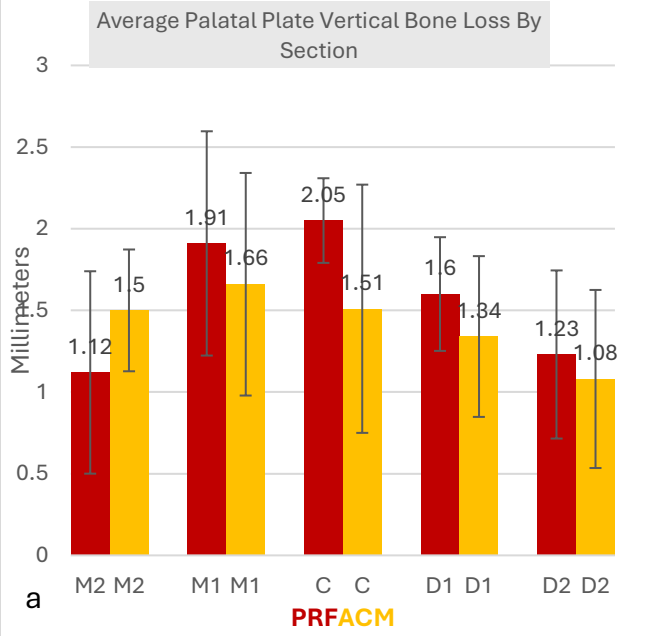


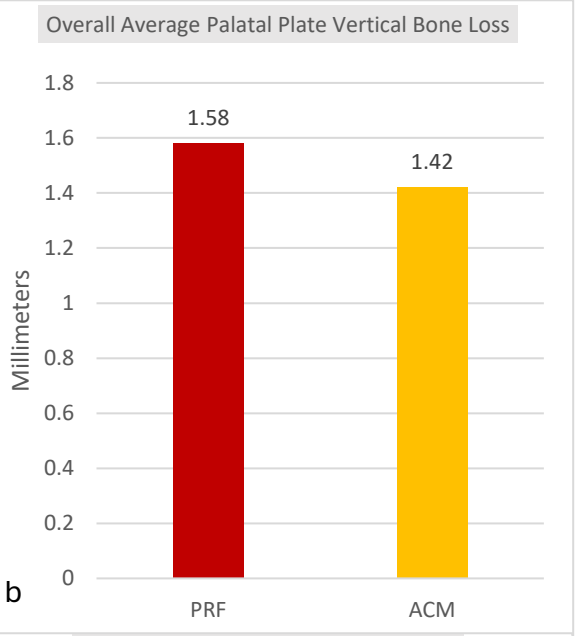
Figure 8. Wound closure rates. wound closure rates determined by the change from the day of surgery to the last measured open day. (a) average wound closure rate of all subjects by group. (b) individual wound closure rate of all subjects by group. (c) average early wound closure rates (days 1-7) by group. (d) individual early wound closure rates (days 1-7) of all subjects by group.

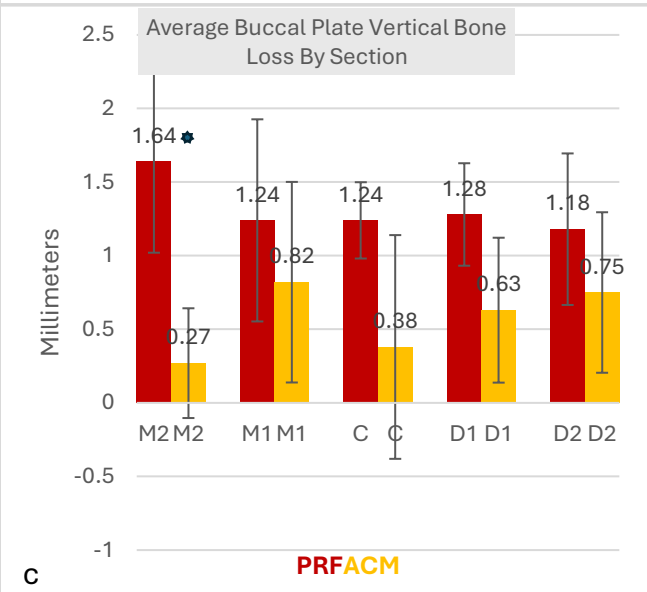
Changes in Bone on CBCT

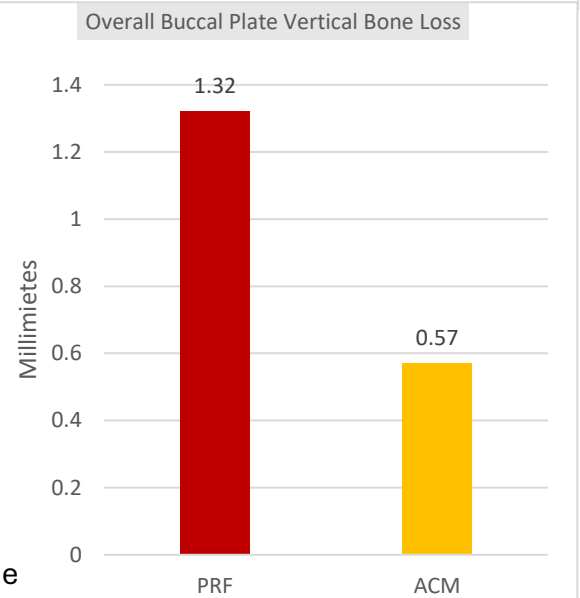
A total of 5 slices were measure for each ridge dimensional measurements, including the following slices, center of extraction socket (C), 1mm mesial from center (M1), 3mm mesial from center (M2), 1mm distal from center (D1) and 3mm from distal from center (D2). Average palatal vertical ridge dimensional loss for the PRF group was 1.58mm and 1.42mm for the ACM subjects. Average buccal vertical ridge dimensional loss for PRF was 1.32mm and 0.57mm for the ACM group. However, the differences between the two groups were not statistically significant. Average Horizontal ridge dimensional loss for PRF group was 0.32mm and 0.13mm for ACM. However, the differences between

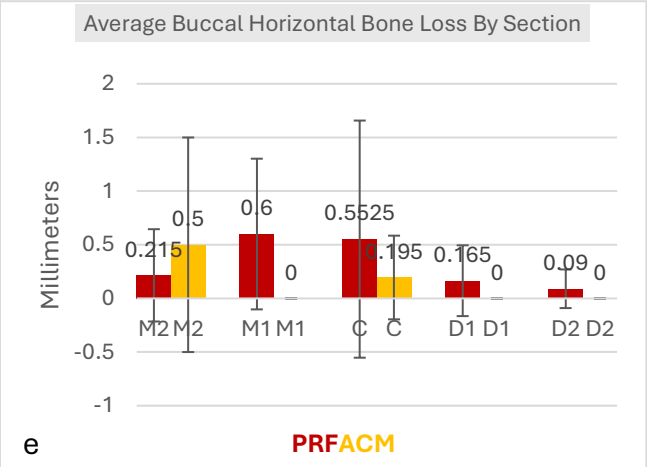
the two groups were not statistically significant. No statistically differences were noted when ridge dimensional levels were compared based on the slice of measurement except for statistically significant difference (p = *0.009)* noted for the buccal vertical ridge dimension loss (1.64mm for PRF vs. 0.27mm for ACM) at the M2 slice.


Average Palatal Plate Vertical Bone Loss By Section
Millimeters
3
2.5
2
1.5
1
0.5
0
1.12
1.5
1.91
1.66
2.05
1.51
1.6
1.34
1.23
1.08
M2 M2
M1 M1
C C
D1 D1
D2 D2
PRFACM
a


Overall Average Palatal Plate Vertical Bone Loss
Millimeters
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1.58
1.42
PRF
ACM
b


Average Buccal Plate Vertical Bone Loss By Section
Millimeters
2.5
2
1.5
1
0.5
0
-0.5
-1
1.64
0.27
1.24
0.82
1.24
0.38
1.28
0.63
1.18
0.75
M2 M2
M1 M1
C C
D1 D1
D2 D2
PRFACM
c


Overall Buccal Plate Vertical Bone Loss
Millimietes
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1.32
0.57
PRF
ACM
e


Average Buccal Horizontal Bone Loss By Section
Millimeters
2
1.5
1
0.5
0
-0.5
-1
0.215
0.5
0.6
0
0.5525
0.195
0.165
0
0.09
0
M2 M2
M1 M1
C C
D1 D1
D2 D2
PRFACM
e

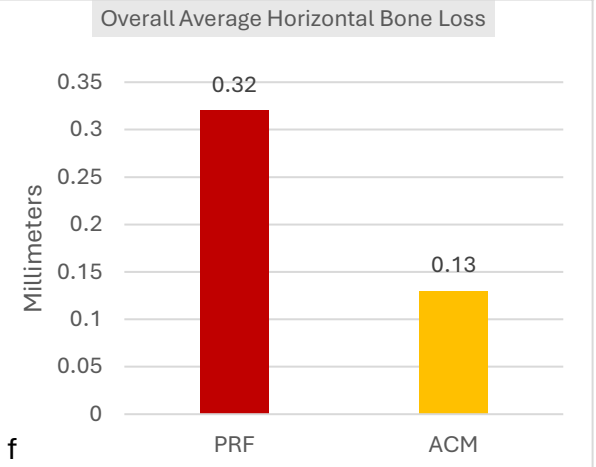

Overall Average Horizontal Bone Loss
Millimeters
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.32
0.13
PRF
ACM
f

Figure 9. Changes in bone dimensions. (* denotes $p < 0.05$). Average bone loss at each measurement point (M2, M1, C, D1, D2) over 110–120-day healing period as well as average total bone loss by group. (a) average palatal vertical bone loss at each measurement site of all subjects by group. (b) average vertical bone loss of all measurement sites cumulative to the palatal by group. (c) average vertical buccal bone loss at each measurement site of all subjects by group. (d) average vertical bone loss of all measurement sites cumulative to the buccal by group. (e) average buccal horizontal bone loss at each measurement site of all subjects by group. (f) average horizontal bone loss of all measurement sites cumulative to the buccal by group.

Visual Analog Scale

VAS Data was available for seven of the eight subjects as one of the subjects did not fill out the daily VAS surveys. ACM subjects reported lower VAS scores (ranging from 0-4) compared to PRF subjects (VAS ranging from 0-6). These differences were statistically significant ($p=0.0002$). See Figure 10.

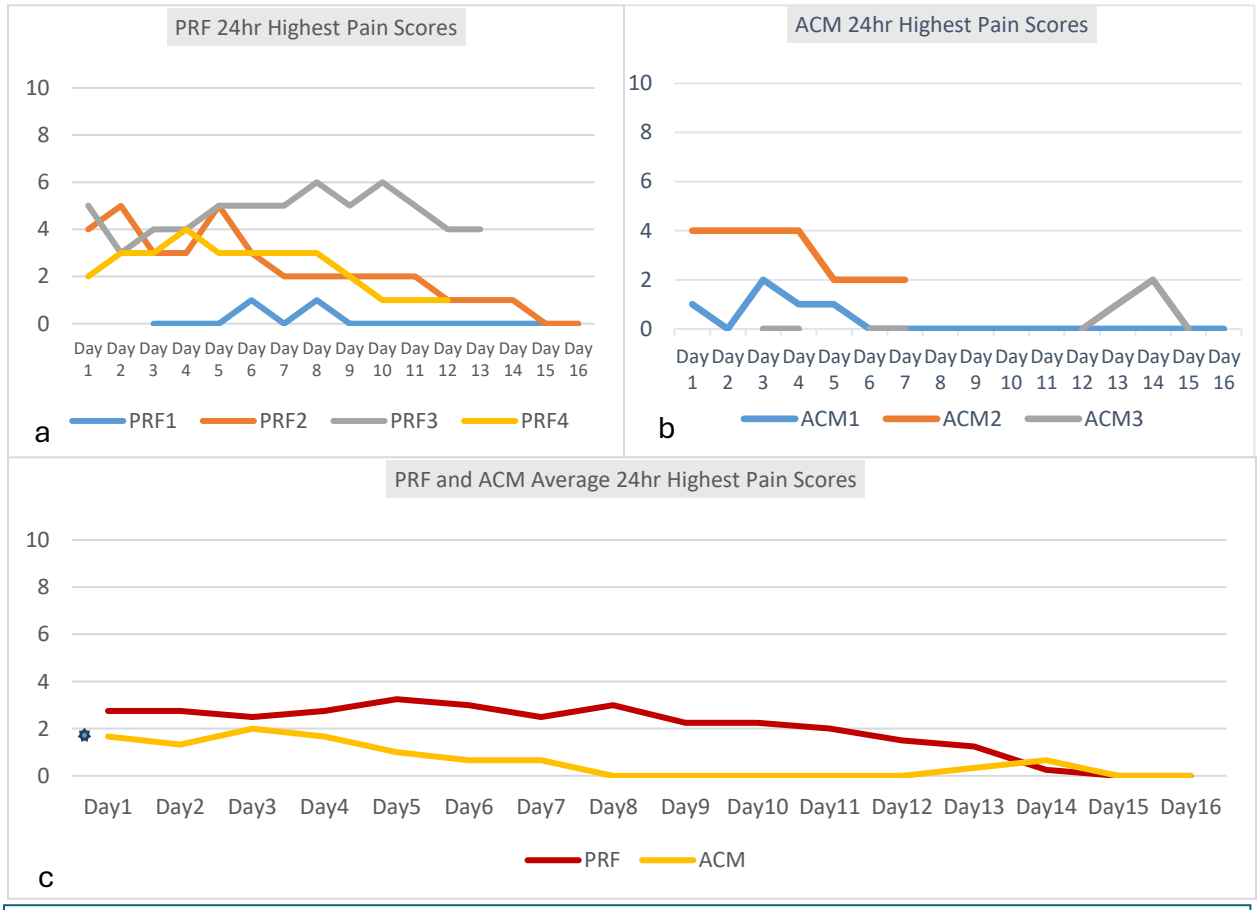


Figure 10. VAS for pain over 24-hour period. (*-denotes $p < 0.05$). VAS results for highest pain level reported in the previous 24 hours on a scale of 1-10. (a) daily pain reporting by each patient in the PRF group. (b) daily pain reporting by each patient in the ACM group. (c) the average of all patient's daily pain reporting by treatment group.

Self-reported perceived pain scores upon waking were also significantly different between the two membrane groups. The PRF group VAS scores ranged from 0-5 with non-zero VAS score that were noted for greater number of days compared to ACM group whose VAS scores from 0-3 and with much lesser number of days with non-zero VAS scores. These differences were statistically significant (P= 0.004). See Figure 11.

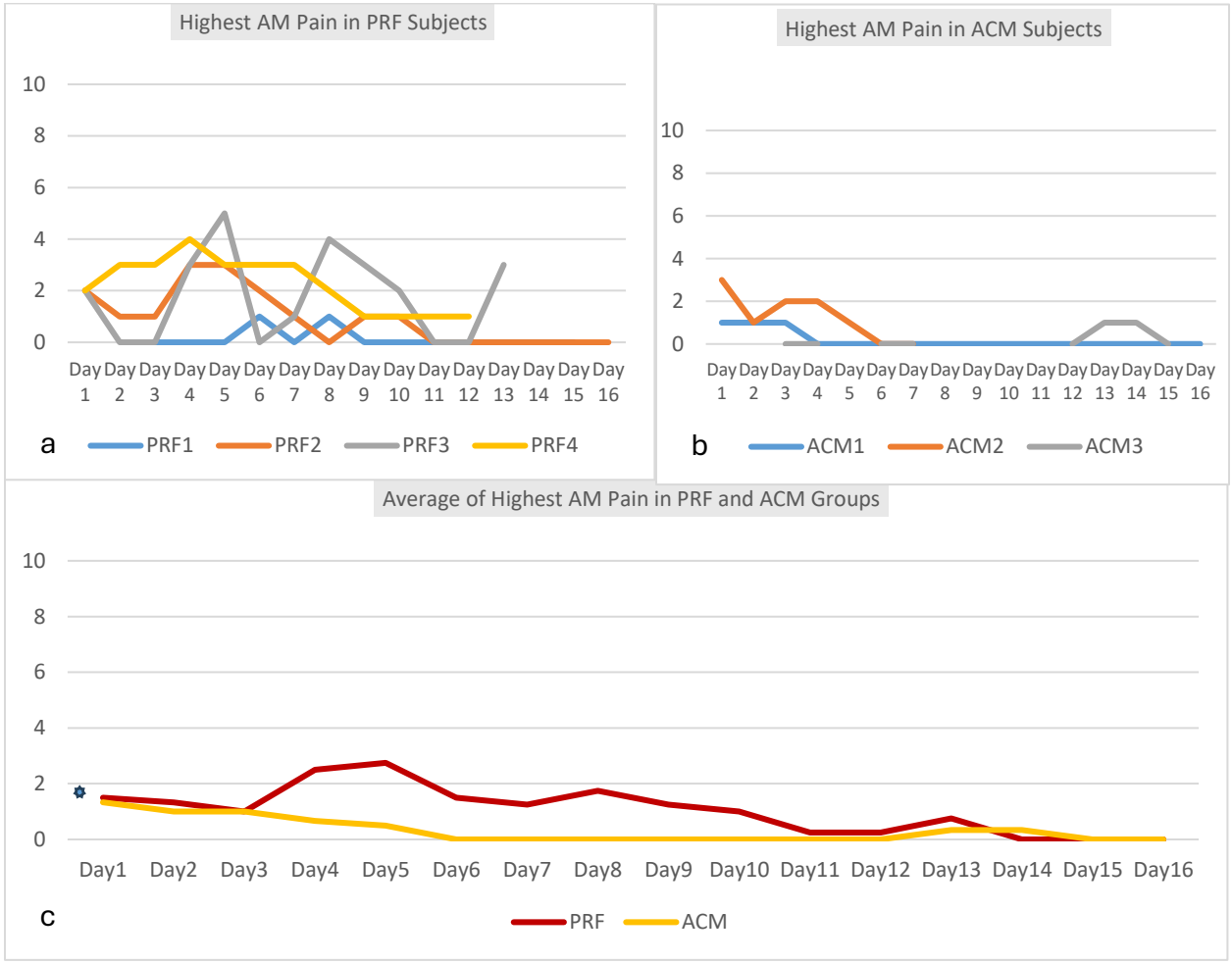


Figure 11. VAS for pain in the morning. (* denotes $p < 0.05$) VAS results for highest pain level reported each morning on a scale of 1-10. (a) daily morning pain reporting by each patient in the PRF group. (b) daily morning pain reporting by each patient in the ACM group. (c) the average of all patient's daily morning pain reporting by treatment group.

To confirm that the pain being reported correlated to the surgical treatment, subjects were asked regarding the side of the mouth in which they felt the pain on each day. To this question 100% of the subjects accurately indicated the same side of which surgery was performed.

DISCUSSION

Placental allografts refer to membranes that are harvested from the amniotic sac. This sac is the physical barrier between the developing fetus and the mother and provides soluble immune factors that protect against uterine infections and temper inflammatory responses that could affect the fetus. It also contains a myriad of growth factors that direct tissue growth and development. The placenta consists of two main membranes, the amnion, and the chorion. The amnion is an internal epithelial layer that lines the amniotic sac facing the fetus. The chorion is the external membrane that faces the uterine tissue. The basement membranes provide barrier function, and the compact and reticular layers provide elastic and tensile strength. The fibroblast and trophoblast layers do not provide much structural support, but they instead contain soluble antimicrobial peptides and growth factors. ACM is composite membrane that is fabricated by separating the amnion and chorion layers and cleansing them through a proprietary cleansing process which preserves the natural protein composition of these layers. The separated layers are than sandwiched together and used in surgical procedures as a barrier membrane. We have previously reported through a randomized clinical trial that that ACM can be used in an open membrane technique and can result in successful ride preservation outcomes when compared to an inert non-resorbable membrane namely d-PTFE. Moreover, the subjects in ACM group reported lower VAS scores compared to the d-PTFE group.

PRF has now been used in regenerative medicine for almost two decades. The original PRF has now been renamed leukocyte PRF [L-PRF], due to its higher leukocyte content, does not contain anticoagulants, hence takes full advantage of the coagulation cascade which is important

for tissue wound healing[11]. L-PRF provides a three-dimensional fibrin matrix that may be used as a scaffold for a variety of procedures including serving the function of a barrier membrane in guided bone regeneration and guided tissue regeneration procedures[12-14]. The original L-PRF protocol has been modified by reducing centrifugation speed (G-force) to allow for increase in leukocytes and consequently higher amounts of growth factors resulting in a product termed A-PRF[15,16]. The latest modification to this protocol is reduction in centrifugation time, which has been shown to increase the number of cells and amounts of growth factor available in the PRF matrix, the resultant product with this protocol is termed as A-PRF+. A-PRF+ is obtained by centrifuging blood without anti-coagulants at 1,300 rpm (200 x g) for 8 minutes[11]. The resultant clot is compressed and fabricated into the A-PRF+ membrane that is then used as a barrier for site preservation. The latest innovation and evolution of the PRF fabrication process is the H-PRF protocol proposed by Miron et al. were the centrifugation is done at 700g for 8 minutes using a horizontal centrifuge[9] resulting in efficient cell separation and uniform distribution of the cells throughout the membrane. Similar to ACM, Platelet rich fibrin (PRF), has been used with varying degrees of success in ridge preservation[17,18] and is shown to have several proteins embedded when it is fabricated. However, it has not been rigorously evaluated for use with the open membrane approach. Here we compared the efficacy of these two biologically active barrier membranes when used as part of the open membrane technique ridge preservation procedure through a pilot randomized clinical trial.

It's well known that ridge preservation minimizes loss of the residual ridge dimension after tooth extraction. Without such grafting, the ridge can lose up to 50% and can compromise the provider's ability to properly restore an edentulous site[19]. The average bone level change of the eight surgeries performed in this report showed comparable but mixed results when compared to previously published data. Iasella found that preserved sockets underwent a reduction in width of 1.2mm which is more bone loss than our results (PRF=0.32mm, ACM=0.13mm), however our study showed greater vertical bone

loss on the buccal plate of 1.32mm in the PRF group and 0.57mm in the ACM group compared to a +1.3mm gain[20]. Jambhekar found in a systematic review of randomized controlled clinical trials found that when the ridge was preserved there was a mean loss of buccolingual width at the ridge of 1.63mm when allografts and membranes were used, along with a buccal wall height from the ridge crest of 0.58mm[21]. Our study showed greater buccolingual width preservation, but more buccal crest height loss as mentioned above. An explanation for this could be attributed to the methodological differences of measuring ridge dimensions between the current study and the previous reports. Variations could also be attributed to the relatively small sample size of our study compared to the other reports.

Intra-oral scanning devices and digital measurement tools have improved the ease and accuracy of calculation wound closure rates. Given that its unsurprising that our results fall within the standard deviation of historical studies on mucosal wound healing rates being around 0.5mm per day[22]. The benefit of using intra oral scanners is that the scale of the images is always the same, unlike when using standard photography that requires calibration to account for this. Given that there was no significant difference between ACM and PRF as pertains While some trends are apparent in the soft tissue closure rates in this study, given the small sample size and lack of statistical significance, no conclusions can be made regarding the efficacy of these membranes for this primary outcome. However, this data would be valuable in powering future studies that aim to measure wound closure rates. The presence of laminins in the basement membrane of ACM, specifically laminin 5 which supports higher cell adhesion and migration[23] can possibly enhance wound healing rates when ACM is used. PRF notably doesn't have laminins. Laminins interact with integrins on epithelial cells surfaces initiating a cascade of intracellular actions including on the cytoskeleton facilitating migratory actions significantly compared to controls in vitro. This leads not only to faster migration but also more rapid organization via aggregation.[24]

Subject reported outcomes of pain perception is valuable data that can help dental provider's decision making to provide personalized care plan for their patients. Morbidity, and the anxiety that comes with

the anticipated pain levels are a strong factor in patient case acceptance.[25] Both ACM and PRF have shown decreased discomfort compared to other membranes or control (no membranes). Temmerman et al showed that compared to control sites, use of L-PRF at the time of extraction showed significantly less pain sensation from the day of surgery through day 6 via self-reporting VAS score.[26] Similar results of decreased reported pain as well as incidence of alveolar osteitis were shown by Kumar et al when using PRF in third molar extraction sites.[27] Concurrently, studies have shown ACM to also have positive effects on subject reported discomfort. We have previously shown, through a split mouth randomized clinical trial, that ACM subjects report significantly lower VAS scores compared to dPTFE subjects and subjects becoming pain free sooner.[7] This study showed that ACM appeared to show a significantly lower reporting of pain in the morning as well as pain throughout the day. One of the PRF subjects had an adverse event (potential infection) at the third post-operative visit and was given a regimen of amoxicillin. Given the sample size, this should be considered in the evaluation of the data.

We utilized a novel standardization tool for this study named the SivRoy radiographic stent. Conceived by Dr. Sivaraman Prakasam (Siv) and Dr. LeRoy Horton (Roy), with input from Dr. Panagiota Stathopoulou, this methacrylate-based resin stent is an inexpensive 3D printed radiographic aid with reproducible radiolucent fiduciary markers, surrounded by mildly radiopaque material, (radiolucent tunnels) that can assist in duplicating planes and cross sections across CBCT images of the same subject at different time periods. This avoids the challenges when other strategies are used, for example, scatter associated with metal markers or masking when radiopaque materials are used. See Figure 12.

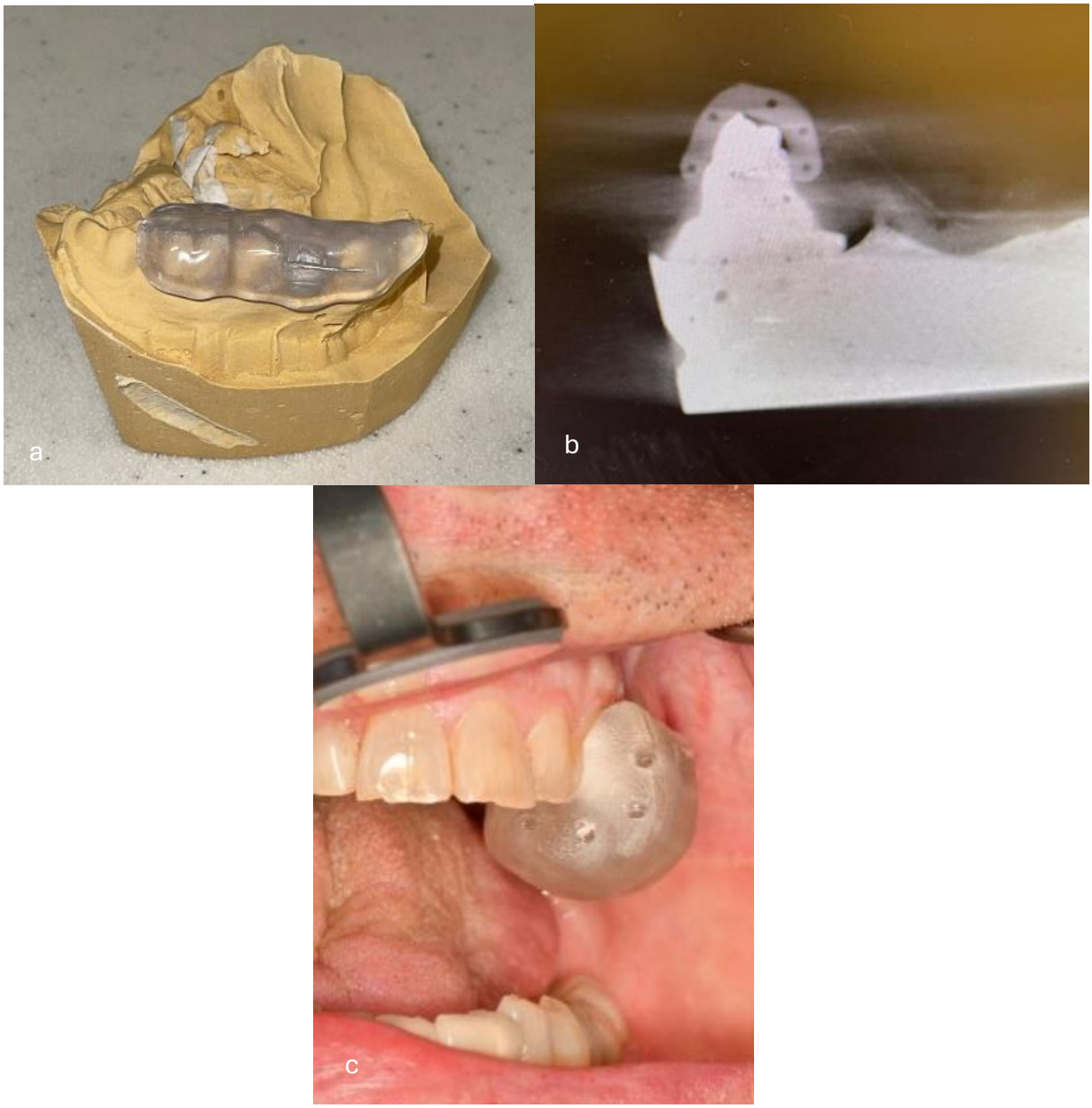


Figure 12. (a) the “SivRoy” stent prototype on stone model. (b) CBCT of the stent on a stone model exhibiting the slight radio-opaqueness of the acrylic material contrasted against the marked radiolucent tunnels that can be used for positional indexing across separate radiographic images. (c) example of a stent positioned on a patient’s dentition on the extraction site.

CONCLUSIONS

ACM and PRF appear to have similar effects on the other outcomes evaluated i.e., soft tissue closure rates and ridge dimensional changes. Within the limits of this study, ACM appears to be beneficial in reducing self-reported perceived pain of ACM group study participants when compared to the PRF group study participants. However, this conclusion should be interpreted in light of the very small sample size of the study. Nevertheless, this observation is consistent with previous reports that ACM appears to have beneficial effects of reducing perceived post operative pain when used as a barrier membrane for ridge preservation procedures.

Acknowledgments

Deepest gratitude to my Principal Investigator Dr. Sivaraman Prakasam for inviting me to participate on this clinical trial and serving as my main mentor over the course of the research. I would also like to thank my committee members Drs. Yota Stathopoulou, Saulo Sousa Melo, Phillip Marucha, and Harjit Sehgal for their guidance and contributions to the development of this study as well as study coordinators Dr. Li-Jung Lin and Mohammed Rauch. A sincere thank you to all my fellow residents, dental students, and clinic staff whom without this study could not have been completed. Lastly, OHSU as an institution and its support of research is of paramount importance in the furtherance of our profession and the evidence of which it rests on. This study was fully funded by Snoasis Medical Inc. Dr. Richard Miron/Bio-PRF Inc. also contributed via a donation in kind to the OHSU graduate periodontics program of a horizontal centrifuge and set of PRF instruments.

Conflict of Interest

There is no conflict of interest related to the study for any of the listed authors.

FIGURE LEGEND

Figure 1. Clinical photograph of surgical protocol. Image (a) showing an extraction site prior to grafting. (b) Image showing the grafting procedure open described in this study of allograft and exposed membrane.

Figure 2. Wound closure measurements. Image of a Trios intra oral scan used for measurement of the wound size.

Figure 3. Radiographic analysis. CBCT cross sections analyzed at the center of the tooth to be extracted, and 2 measurements 1mm apart both mesial and distal to the center section. These have been labeled as C=center M1=1mm mesial to C, M2=2mm mesial to C, D1=1mm distal to C, D2=2mm distal to C.

Figure 4. Radiographic analysis strategy**.** CBCT sections used for measurements. (a) an example of an initial cross section marking the highest points of the buccal and palatal plates of bone. (b) an example of the same site 120 days post extraction. (c) image b made transparent and overlayed over image a. (d) measurements made from the initial markings at a 90 degree angle to measure the change in bone height. (e) a separate image where horizontal bone loss was appreciated, the measurement of the buccal height and width change using a line parallel to the 90 degree line from the initial buccal point to the new highest point of the buccal plate.

Figure 5. Clinical trial flow diagram. The flowchart describes the number of screened subjects recruited/excluded. It also describes the number of subjects allocated to study interventions, completed study, and number of subjects lost/excluded from analysis.

Figure 6. Patient demographics. (a) graph showing the gender distribution of the ACM groups subjects of 2 males and 2 females. (b) graph showing the gender distribution of the PRF group subjects of 3 females and 1 male. (c) the age of each subject in each groups as well as the average age of each group.

Figure 7. Wound closure tracking. Line graph showing the wound closure measurements by the day from initial surgery. The measurement made on each of the post operative visits denoted by color as belonging to the PRF or ACM group.

Figure 8. Wound closure rates. Wound closure rates determined by the change from the day of surgery to the last measured open day. (a) average wound closure rate of all subjects by group. (b) individual wound closure rate of all subjects by group. (c) average early wound closure rates (days 1-7) by group. (d) individual early wound closure rates (days 1-7) of all subjects by group.

Figure 9. Changes in bone dimensions. . (* denotes $p < 0.05$) Average bone loss at each measurement point (M2, M1, C, D1, D2) over 110–120-day healing period as well as average total bone loss by group. (a) average palatal vertical bone loss at each measurement site of all subjects by group. (b) average vertical bone loss of all measurement sites cumulative to the palatal by group. (c) average vertical buccal bone loss at each measurement site of all subjects by group. (d) average vertical bone loss of all measurement sites cumulative to the buccal by group. (e) average buccal horizontal bone loss at each measurement site of all subjects by group. (f) average horizontal bone loss of all measurement sites cumulative to the buccal by group.

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FIGURES

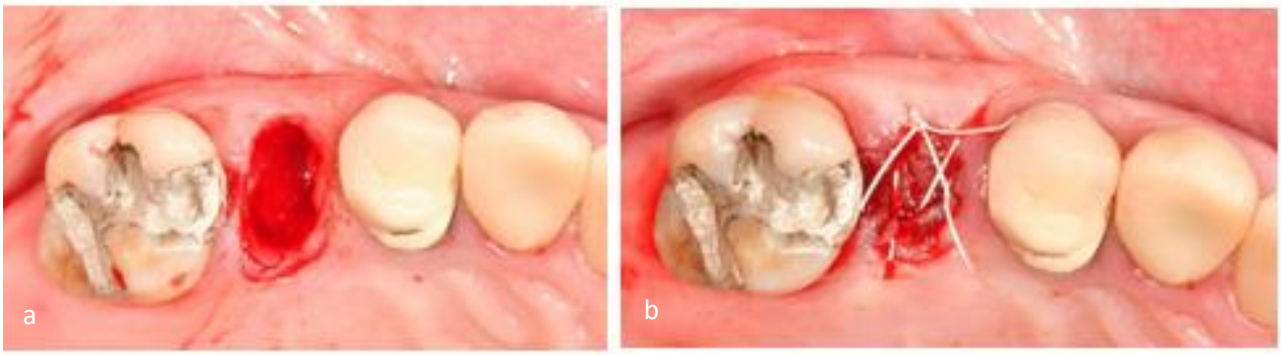
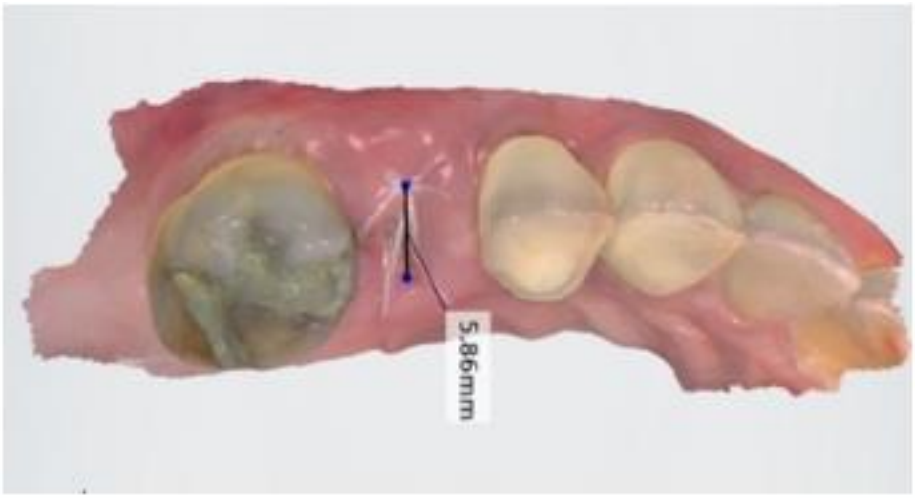


Figure 1



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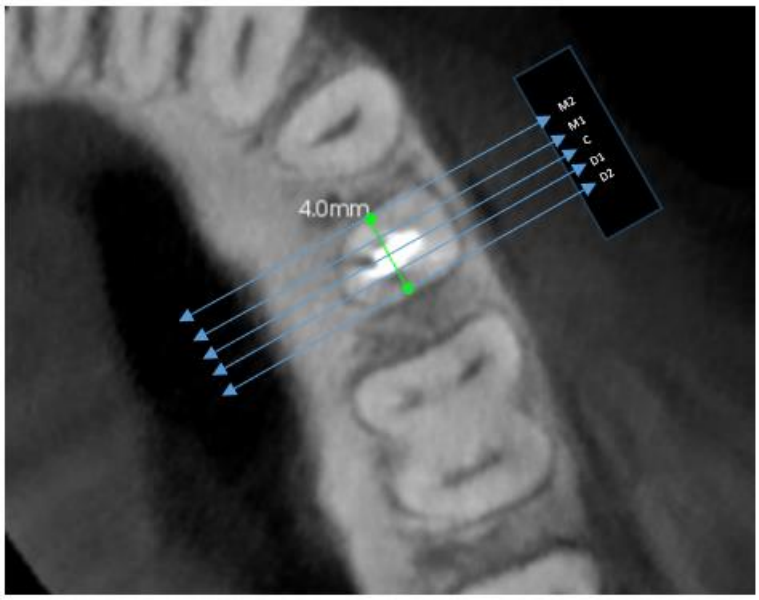


Figure 3

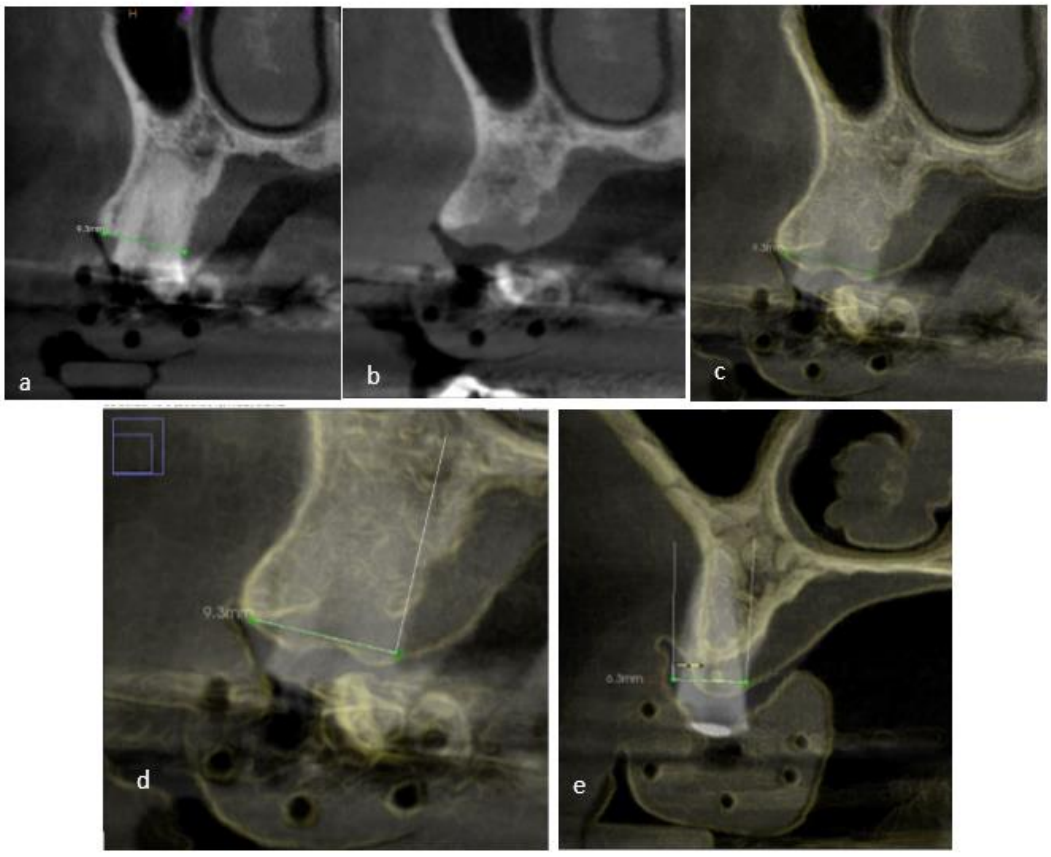


Figure 4

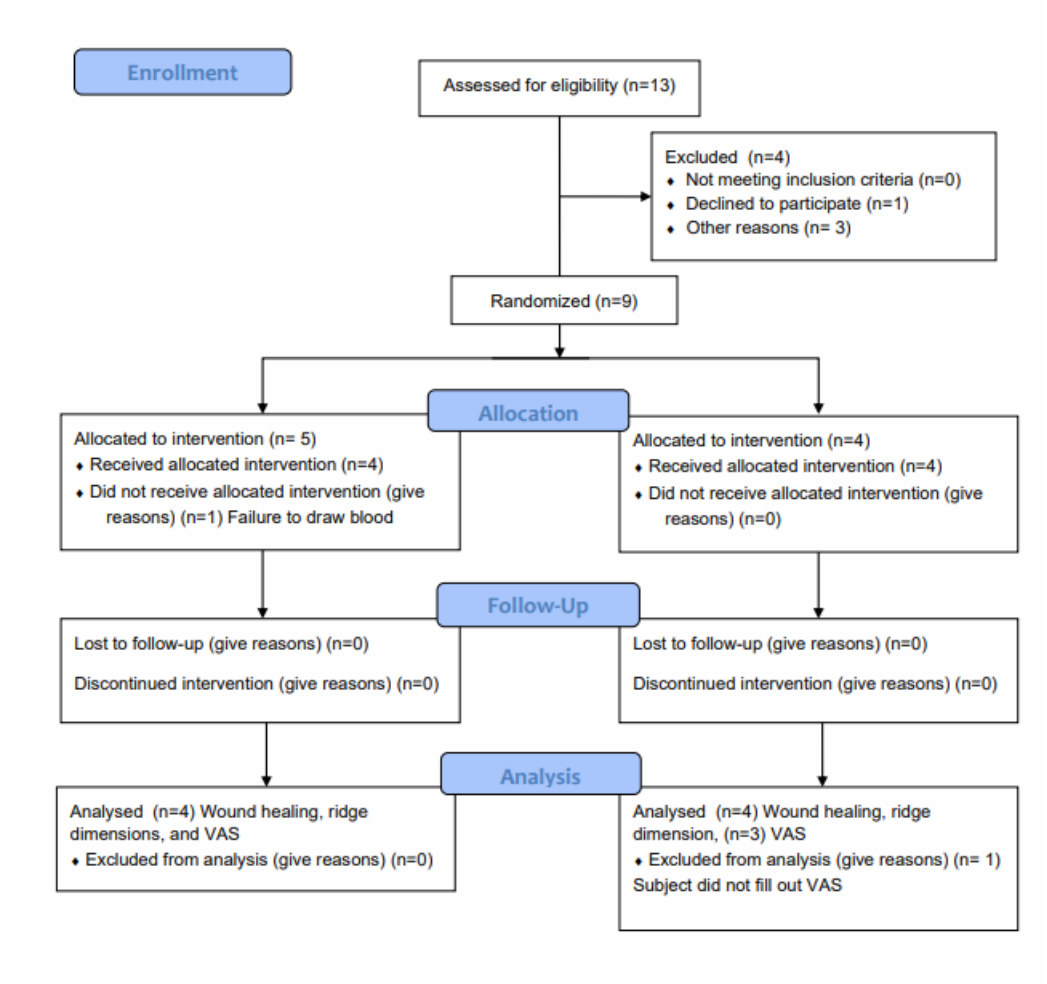

Enrollment
Assessed for eligibility (n=13)
Excluded (n=4)
Not meeting inclusion criteria (n=0)
Declined to participate (n=1)
Other reasons (n= 3)
Randomized (n=9)
Allocation
Allocated to intervention (n= 5)
Received allocated intervention (n=4)
Did not receive allocated intervention (give reasons) (n=1) Failure to draw blood
Allocated to intervention (n=4)
Received allocated intervention (n=4)
Did not receive allocated intervention (give reasons) (n=0)
Follow-Up
Lost to follow-up (give reasons) (n=0)
Discontinued intervention (give reasons) (n=0)
Lost to follow-up (give reasons) (n=0)
Discontinued intervention (give reasons) (n=0)
Analysis
Analysed (n=4) Wound healing, ridge dimensions, and VAS
Excluded from analysis (give reasons) (n=0)
Analysed (n=4) Wound healing, ridge dimension, (n=3) VAS
Excluded from analysis (give reasons) (n= 1)
Subject did not fill out VAS

Figure 5

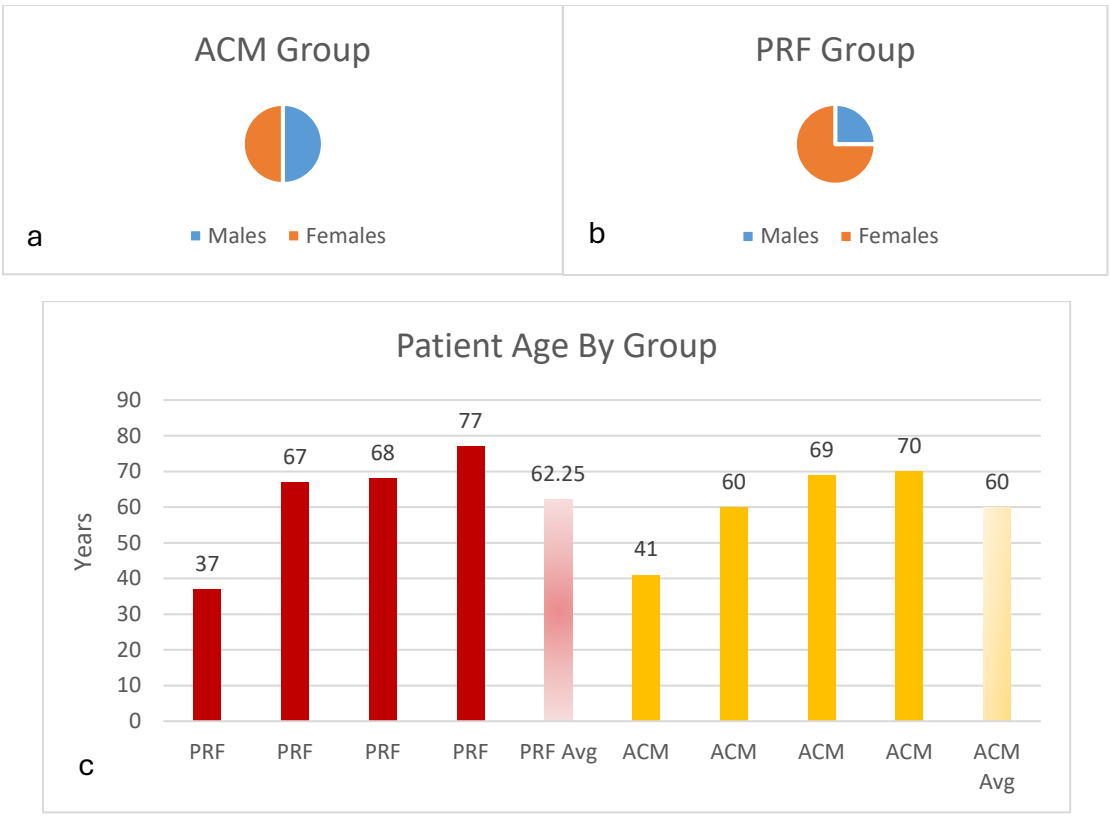

ACM Group
Males
Females
a
PRF Group
Males
Females
b
Patient Age By Group
Years
90
80
70
60
50
40
30
20
10
0
37
67
68
77
62.25
41
60
69
70
60
PRF
PRF
PRF
PRF
PRF Avg
ACM
ACM
ACM
ACM
ACM Avg
c

Figure 6

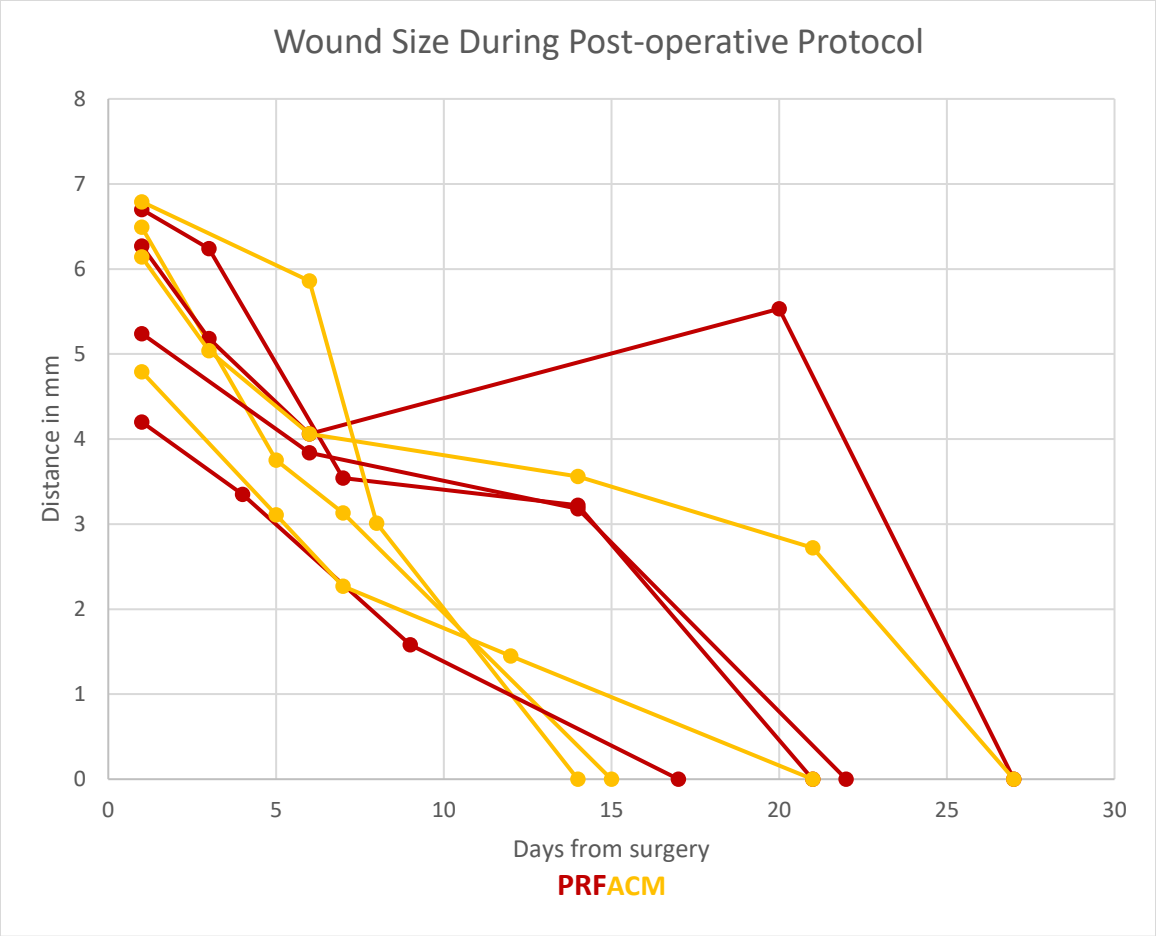

Wound Size During Post-operative Protocol
Distance in mm
0
1
2
3
4
5
6
7
8
0
5
10
15
20
25
30
Days from surgery
PRF
ACM

Figure 7

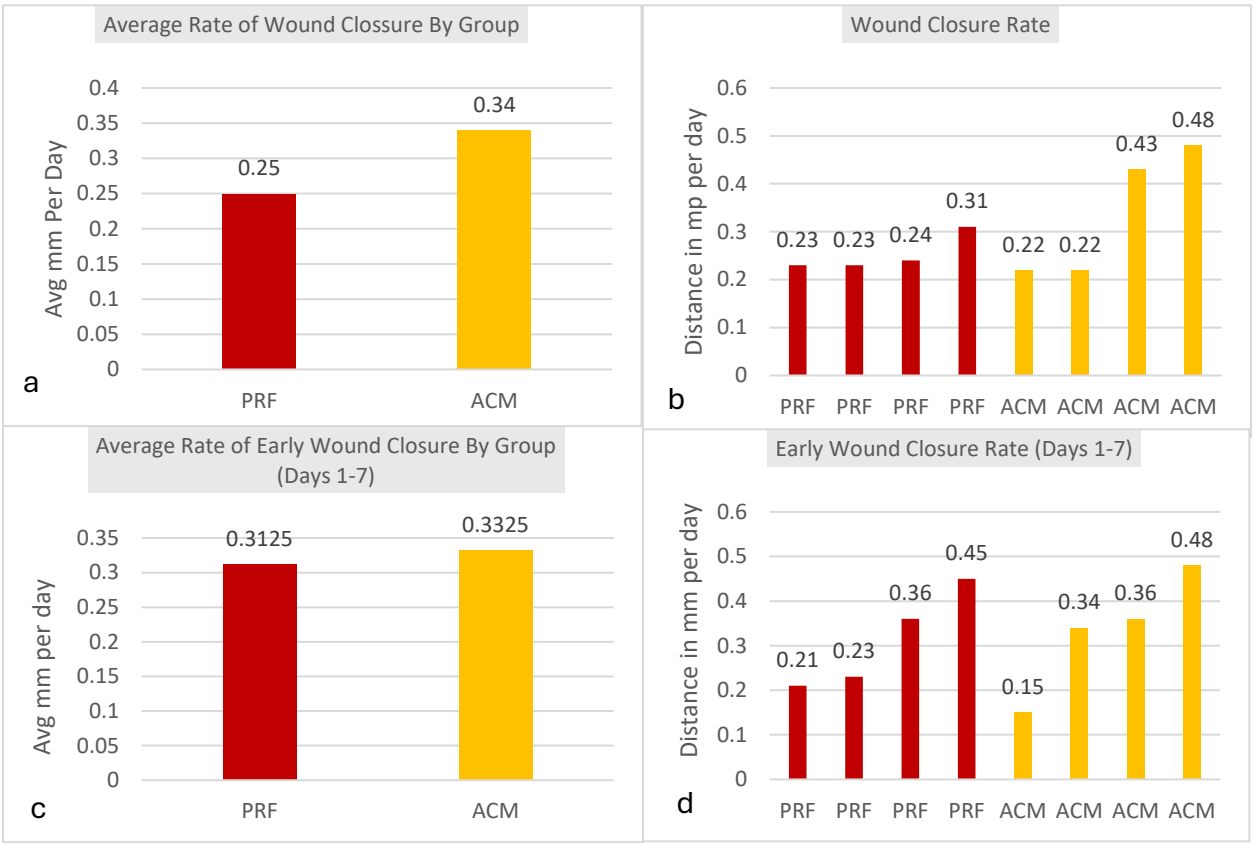
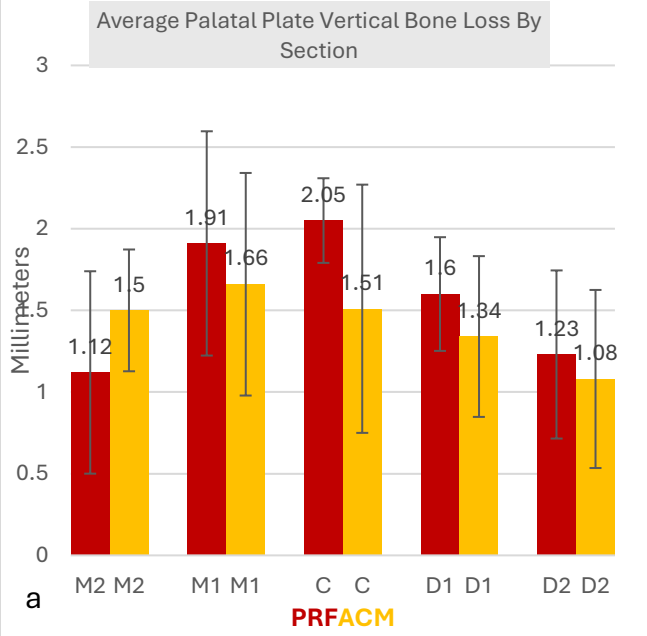
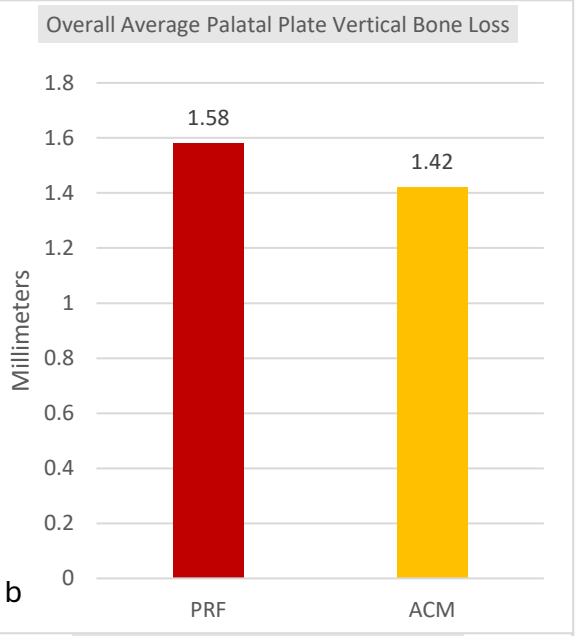
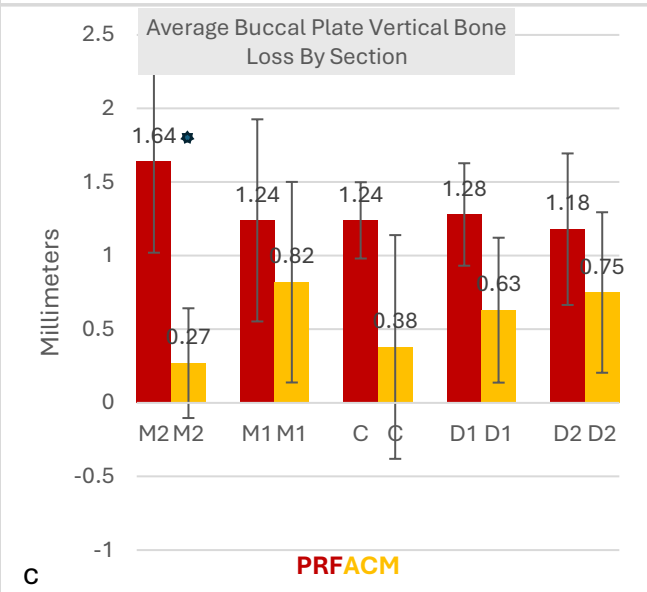
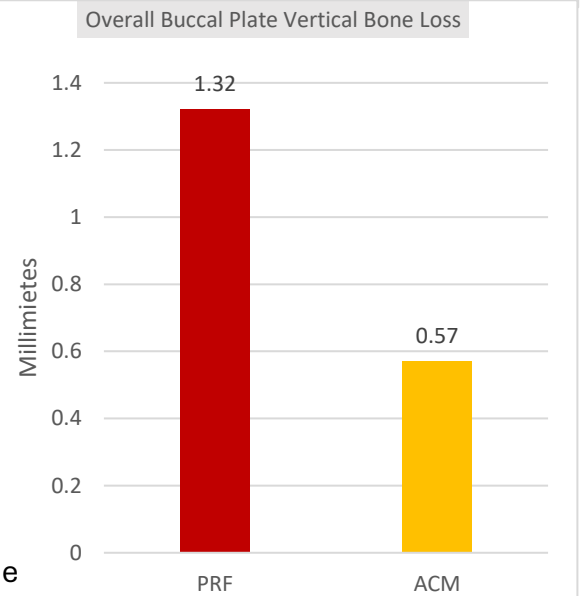

Average Rate of Wound Clossure By Group
Avg mm Per Day
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.25
0.34
PRF
ACM
a
Wound Closure Rate
Distance in mp per day
0.6
0.5
0.4
0.3
0.2
0.1
0
0.23 0.23 0.24 0.31 0.22 0.22 0.43 0.48
PRF PRF PRF PRF ACM ACM ACM ACM
b
Average Rate of Early Wound Closure By Group (Days 1-7)
Avg mm per day
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.3125
0.3325
PRF
ACM
c
Early Wound Closure Rate (Days 1-7)
Distance in mm per day
0.6
0.5
0.4
0.3
0.2
0.1
0
0.21 0.23 0.36 0.45 0.15 0.34 0.36 0.48
PRF PRF PRF PRF ACM ACM ACM ACM
d

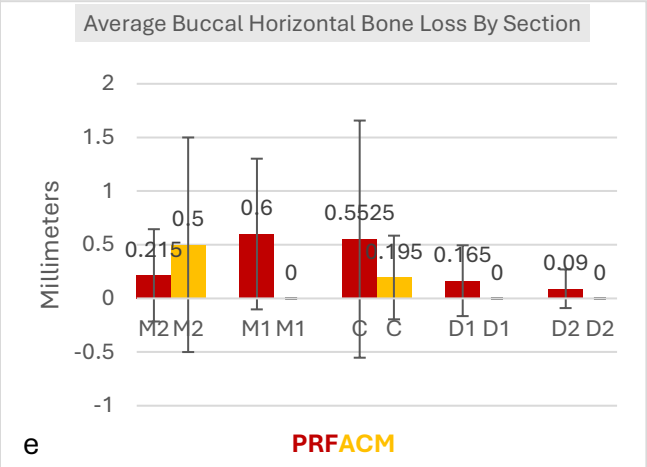
Figure 8


Average Palatal Plate Vertical Bone Loss By Section
Millimeters
3
2.5
2
1.5
1
0.5
0
1.12
1.5
1.91
1.66
2.05
1.51
1.6
1.34
1.23
1.08
M2 M2
M1 M1
C C
D1 D1
D2 D2
PRFACM
a


Overall Average Palatal Plate Vertical Bone Loss
Millimeters
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1.58
1.42
PRF
ACM
b


Average Buccal Plate Vertical Bone Loss By Section
Millimeters
2.5
2
1.5
1
0.5
0
-0.5
-1
1.64
0.27
1.24
0.82
1.24
0.38
1.28
0.63
1.18
0.75
M2 M2
M1 M1
C C
D1 D1
D2 D2
PRFACM
c


Overall Buccal Plate Vertical Bone Loss
Millimietes
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1.32
0.57
PRF
ACM
e


Average Buccal Horizontal Bone Loss By Section
Millimeters
2
1.5
1
0.5
0
-0.5
-1
0.215
0.5
0.6
0
0.5525
0.195
0.165
0
0.09
0
M2 M2
M1 M1
C C
D1 D1
D2 D2
PRFACM
e

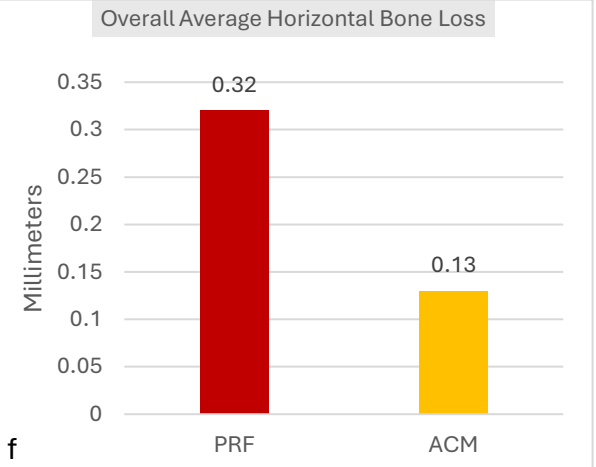

Overall Average Horizontal Bone Loss
Millimeters
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.32
0.13
PRF
ACM
f

Figure 9

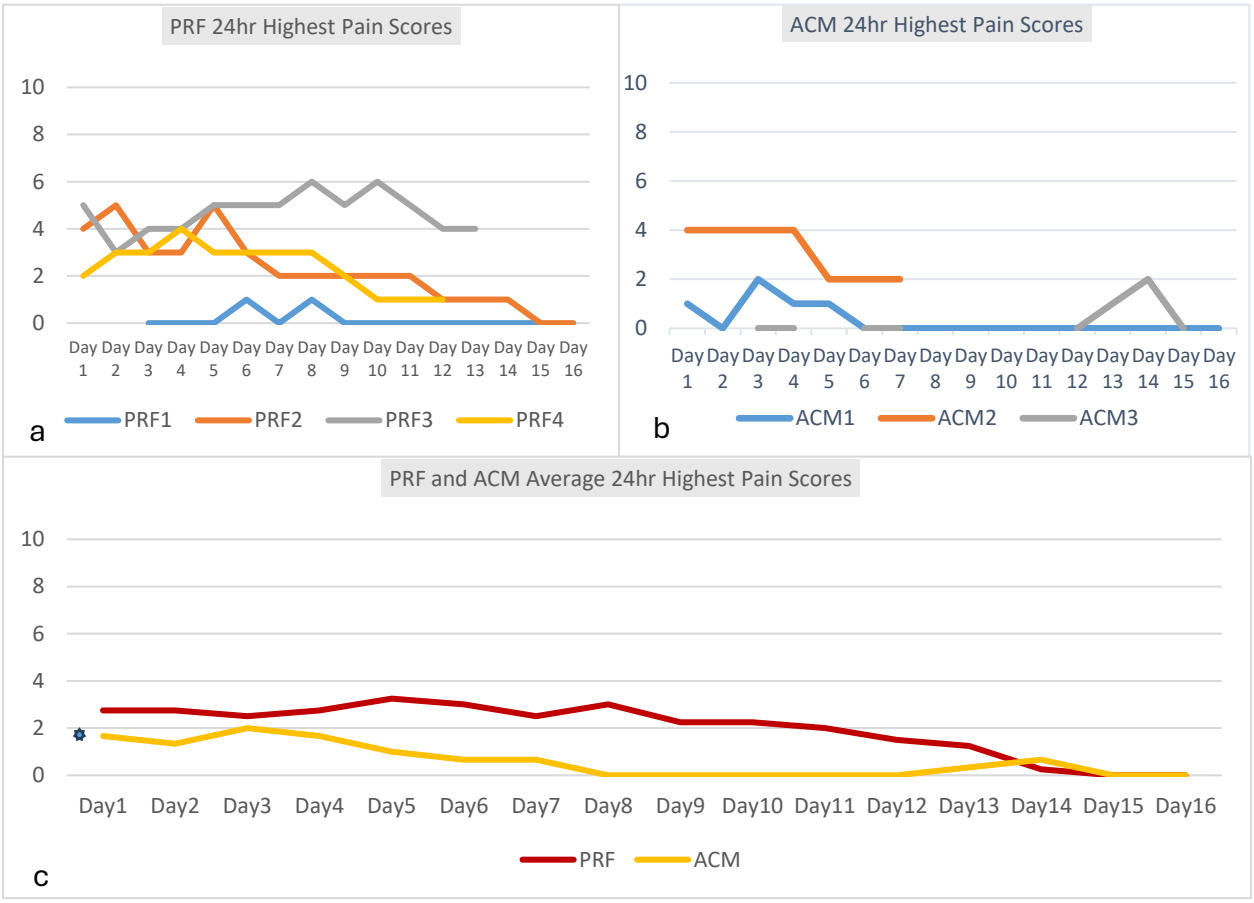


Figure 10

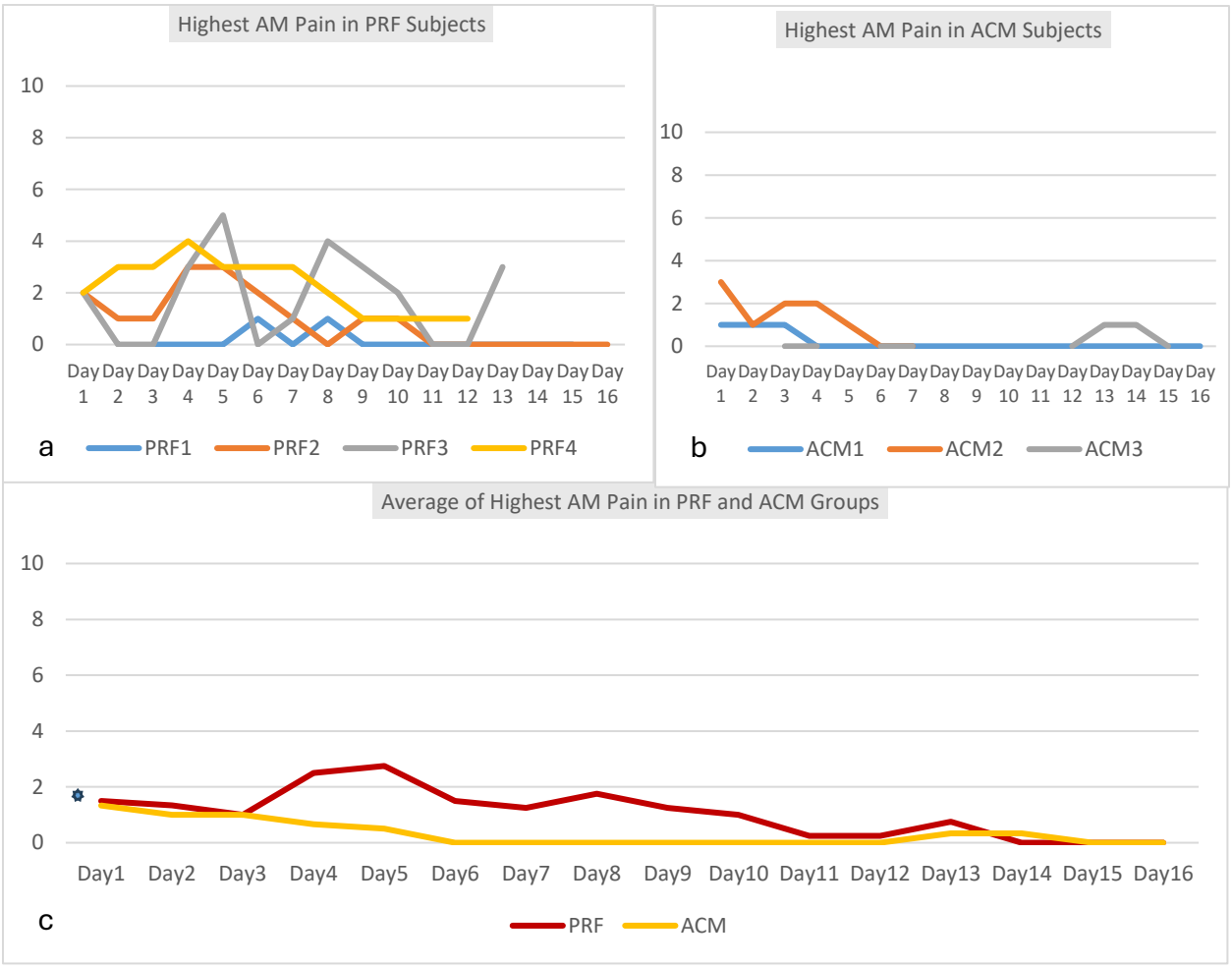

Highest AM Pain in PRF Subjects
10
8
6
4
2
0
Day 1 Day 2 Day 3 Day 4 Day 5 Day 6 Day 7 Day 8 Day 9 Day 10 Day 11 Day 12 Day 13 Day 14 Day 15 Day 16
a
PRF1
PRF2
PRF3
PRF4
Highest AM Pain in ACM Subjects
10
8
6
4
2
0
Day 1 Day 2 Day 3 Day 4 Day 5 Day 6 Day 7 Day 8 Day 9 Day 10 Day 11 Day 12 Day 13 Day 14 Day 15 Day 16
b
ACM1
ACM2
ACM3
Average of Highest AM Pain in PRF and ACM Groups
10
8
6
4
2
0
Day1 Day2 Day3 Day4 Day5 Day6 Day7 Day8 Day9 Day10 Day11 Day12 Day13 Day14 Day15 Day16
c
PRF
ACM

Figure 11

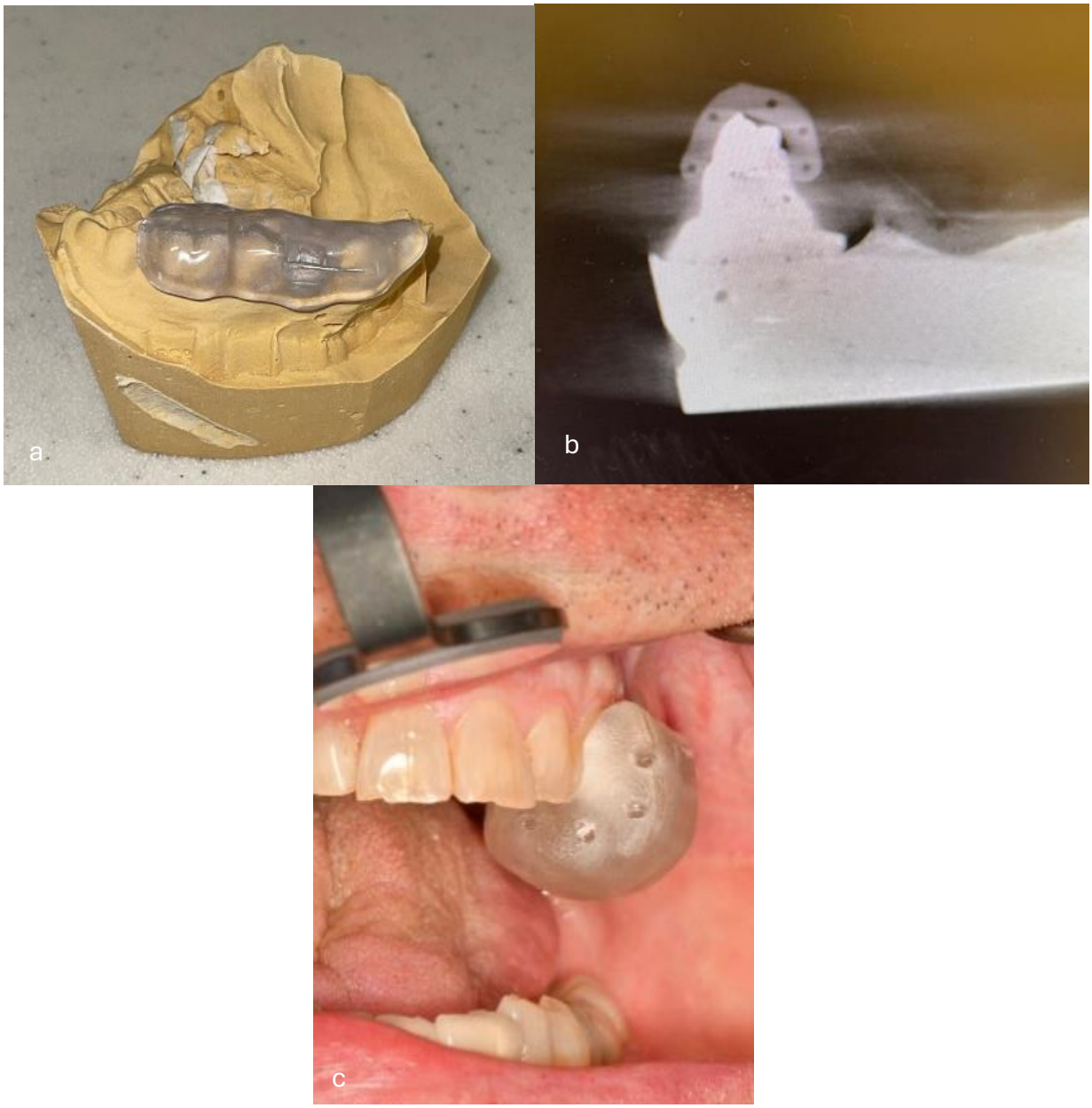


Figure 12

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