

**Volumetric changes of soft and hard tissues following alveolar ridge preservation: Freeze-dried bone allograft vs. L-PRF clot covered with d-PTFE membrane.**

**ID: B2017:125 NCT03331185**

Background

One of the most important factors that allows for predictable prosthetically driven implant placement is adequate bone volume at the proposed implant site. The main purpose of alveolar ridge preservation is to maintain the dimensions of the alveolar ridge, to allow for bone formation, and reduce the rate of resorption during the healing phase following tooth extraction. The histological changes observed during the healing of a post extraction socket have been described in detail in multiple histologic studies<sup>1-4</sup>: The following sequence of events has been described in the literature:

- Initial clot formation
- Within 4-7 days the clot is gradually replaced by granulation tissue and subsequent angiogenesis initiated by endothelial cells.
- Connective tissue gradually replaces the granulation tissue within 2 weeks.
- The base of the socket undergoes initial osteoid calcification between days 7-10 with trabecular bone fill of two thirds of the socket by 6 weeks. Osteoblastic activity is at its most pronounced at 4-6 weeks. The process slows down at week 8.
- Epithelial coverage of the socket starts at the 4<sup>th</sup> day and is complete after 4-5 weeks. Bone fill is complete after 16 weeks.

Depending on the amount of destruction due to pathology or trauma, the extraction socket can be classified using Elian's simplified socket classification:<sup>5</sup>

- Type I: The facial soft tissue and buccal plate of bone are at normal levels in relation to the cemento-enamel junction of the pre-extracted tooth and remain intact postextraction.
- Type II: Facial soft tissue is present but the buccal plate is partially missing following extraction of the tooth.
- Type III: The facial soft tissue and the buccal plate of bone are both markedly reduced after tooth extraction.

Extraction sockets with no adjunctive grafting at the time of extraction, will present twelve months post-operatively with approximately 1mm vertical bone loss and 50% width reduction of the alveolus. A recent study reported that in the esthetic zone, patients with a buccal wall of less than 1mm, presented with a median vertical bone loss of 62.3%, 8 weeks following an extraction<sup>6</sup>. When compared to extraction alone, alveolar ridge preservation has a statistically significant effect in limiting the loss of alveolar ridge height and width<sup>7-10</sup>. Several modalities and combinations of augmentation materials have been used successfully in alveolar ridge preservation<sup>9,10</sup>. Some of the modalities employed are various combinations of autogenous bone, xenografts, allografts and growth factors such as Platelet Rich Plasma (PRP), Leukocytic Platelet Rich Fibrin (L-PRF) and Recombinant Human Bone Morphogenic Protein 2 (rh-BMP2), all of which were combined with absorbable or non-absorbable membranes<sup>4,11</sup>. The clinical application and benefit of these grafting materials have been researched at length in the

literature<sup>9,10</sup>. Multiple studies have shown, that when used for alveolar ridge preservation, L-PRF reduced the amount of post operative pain, bone resorption and healing time in extraction sites<sup>12-15</sup>. Leukocytic Platelet Rich Fibrin (L-PRF) is a concentrate of platelets and leukocytes within a fibrin clot that is derived from the patient's own blood sample, when centrifuged at 2700rpm for 12 minutes. L-PRF has been shown to accelerate wound healing by stimulating angiogenesis and neutrophil migration as well as harness stem cells and growth factors<sup>16</sup>. One study found that L-PRF presented with similar results when indirectly compared to bone substitutes<sup>17</sup>. However, when systematically analyzing the literature, regarding different types of grafting materials, no material has produced superior results over the others when comparing changes in ridge height and width<sup>7,9</sup>.

Hard tissue volumetric changes also affect the presence and location of keratinized gingiva<sup>3,9,18</sup>. As mentioned previously, alveolar ridge preservation is performed in an effort to maintain adequate ridge dimension for implant placement and the remaining amount of keratinized tissue at the prospective implant site, may have implications for future implant survival. There is no consensus in the current literature regarding the optimal amount of keratinized tissue required to maintain long-term peri-implant soft tissue health. Some studies have shown that the lack of keratinized mucosa did not have any adverse effects on soft tissue health and implant survival<sup>21,22</sup>. In contrast, other studies have shown that implants with less than 2mm of keratinized mucosa, presented with increased plaque accumulation, higher rates of inflammation, increased risk of recession and attachment loss<sup>23,24</sup>. Although no correlation was found between implant survival, probing depth, bone loss and the amount of keratinized tissue, an increase in plaque accumulation and peri-implant inflammation, especially in the presence

of poor oral hygiene or limited access, it still remains a concern regarding the long term peri-implant tissue health. There is limited evidence in the literature investigating the amount of keratinized soft tissue available, following ridge preservation with freeze-dried bone allograft (FDBA) and dense polytetrafluoroethylene (d-PTFE) membrane<sup>19,20</sup>. To the best of our knowledge, no studies exist in the current literature evaluating the amount of keratinized soft tissue following ridge preservation with L-PRF and d-PTFE membrane.

Another factor that has not been taken into consideration is informed consent and patient preference. A recent study analyzed patient preferences to dental grafts and demonstrated that the highest rate of refusal of grafts was for allografts (20%) followed by xenografts (15%), due to ethical, personal or religious considerations<sup>25</sup>. Clinicians have been presented with the dilemma where a patient rejects the use of allografts and xenografts allowing only for the use of alloplastic materials or autologous grafts. Harvesting autologous grafts may necessitate the need for a second site surgery, can be seen as unnecessarily invasive and may lead to an increased rate of morbidity. An alloplastic graft is a synthetic bone substitute with osteoconductive properties only and carries no risk of disease transmission<sup>26</sup>. Cost, potential for allergic reaction, and patient preference may negate the use of alloplasts. A more conservative and cost effective approach may be to proceed with venipuncture and subsequent production of a suitable grafting material, L-PRF, from the patient's blood. To the best of our knowledge, when reviewing the literature regarding volumetric changes following alveolar ridge preservation, there were no direct comparative studies evaluating the efficacy of L-PRF to other grafting materials.

### Research question

What is the effect of an L-PRF clot covered with a d-PTFE non-absorbable membrane on the hard and soft tissue volumetric changes in ridge preservation procedures when compared to FDBA covered by a d-PTFE non-absorbable membrane.

### Participants

Patients of the Graduate Periodontics Clinic, College of Dentistry, University of Manitoba that require extraction of either molar or premolar teeth and subsequent ridge preservation from ...2017 to December 2018 and have signed the consent form. Patients will receive initial periodontal examination and treatment if necessary.

### Inclusion Criteria:

- Male or female, 22 years and over.
- Subjects with molars or premolars indicated for extraction.
- Patients that present with a post extraction class I and II socket ( $<30\%$  bone loss on the buccal or lingual plate, measured from the most coronal aspect of intact bone to the most apical aspect of the defect divided by the measurement from the most apical aspect of the defect to the apex of the socket x 100).
- Patients presenting with the need for single extractions.

- Patients with general good health that does not have a condition contra-indicating routine dental treatment, extraction and implant placement.
- Patients that are compliant with the research protocol and methods.
- Patients that have read, understood and signed an informed consent form.

Exclusion Criteria:

- Patients younger than 22 years
- Extraction and ridge preservation indicated for teeth other than premolars and molars.
- Patients that present with a post extraction class III socket (>30% bone loss on the buccal or lingual plate, measured from the most coronal aspect of intact bone to the most apical aspect of the defect divided by the measurement from the most apical aspect of the defect to the apex of the socket x 100)
- Patients deemed eligible for immediate implant placement following extraction and intra-operative assessment by the attending supervisor.
- Patients that present with an oral-antral communication, post extraction.
- Patients that present with the need for multiple, adjacent extractions.
- Patients with coagulation disorders, on corticosteroids, uncontrolled diabetes mellitus, or any systemic disease where periodontal surgery is contraindicated and healing may be compromised.
- Pregnant and nursing women.
- Patients with any contact hypersensitivity to the related materials used in the study.
- Heavy tobacco users, >10 cigarettes per day.
- Patients unwilling to sign consent or follow the protocol of the study.

### Proposed method

The study will take place at the Graduate Periodontics Department, College of Dentistry, University of Manitoba and will be for a total of at least 8 visits over a study period of 4 months.

Randomization will be achieved using computerized randomization scheme. Each participant will be assigned to one of two groups and allocated by means of sealed envelope opened on the day of surgery communicated to the surgeon during the surgery by the independent examiner (JT). Participants will be block-randomized for each of the 5 operators for balance.

Group A (control): Extraction followed by ridge preservation with freeze-dried bone allograft covered with d-PTFE membrane.

Group B (test): Extraction followed by ridge preservation with L-PRF clot covered with d-PTFE membrane. The surgical procedure will be performed by one of all the calibrated periodontics residents (CS, JB, DR, JC, BW). Patients will be followed for 2 weeks post-operatively by the same resident to monitor the healing process and to assess for any complications.

**Visit 1:** Following full examination and treatment planning, patients requiring extraction, socket preservation and implant treatment that meet the inclusion criteria, will be eligible to participate. Once deemed eligible and the patient is interested in the study, the patient will be provided with the complete protocol, consent forms, as well as potential risks and benefits of

the study.

**Visit 2:** Confirmation of all relevant forms are signed. Clinical photographs taken and requisition for standardized CBCT filled out.

**Visit 3:** Review of medical history and changes recorded. Anesthesia will be achieved with either infiltration or block anesthesia depending on site. Anesthetic agent used will be Lidocaine 2% with 1:100 000 epinephrine. Keratinized tissue is measured on the buccal and lingual with UNC 15 probe from the gingival margin to the mucogingival junction. Atraumatic extraction technique is recommended to allow for minimal disturbance of the soft and hard tissue architecture<sup>3,5</sup>. The technique requires initial severing of the cervical gingival fibers with a periosteal elevator, then, once the gingival fibers have been completely severed, the PDL is severed by incrementally advancing the blade apically around the circumference of the root into the PDL space up to two thirds of the root distance towards the apex<sup>27</sup>. In the case of multi-rooted teeth, the crown is amputated with a high-speed hand piece, surgical bur and copious irrigation, taking care not to damage the adjacent soft and hard tissues. Following amputation of the crown, the roots are then sectioned into separate entities followed by the use of the periosteal elevator as described above. Roots can further be mobilized with luxators followed by delivery with extraction forceps, taking care to leave the surrounding soft and hard tissues as intact as possible. Irrigation with saline and curettage of the socket with hand instruments to remove all granulation and infected tissue is performed after extraction. The socket walls are then assessed and the socket width measured with a periodontal probe. If there is >30% bone loss caused by trauma, dehiscence or fenestrations in any of the socket walls, the patient will be exited from the study. Patients exited from the study, depending on the extent of the



defect, will at the time proceed with ridge preservation or guided bone regeneration. For eligible patients, a randomized numbered envelope assigned to them is opened to determine the type of alveolar ridge preservation procedure. Group A: Full thickness mucoperiosteal pouch is created up to ~3mm apical of the bony crest of the socket with a periosteal elevator. The socket is incrementally filled with mineralized cortical freeze-dried bone allograft and condensed. Group B: Full thickness mucoperiosteal pouch is created up to ~3mm apical of the bony crest of the socket with a periosteal elevator followed by venipuncture of the antecubital vein with 21G needle and collection of 4-6 vials (10ml each) of venous blood without any additive or anticoagulant. The vials are centrifuged for 12 minutes at 2700 rpm. Once centrifugation is complete, L-PRF specimens are collected and compressed into L-PRF clots. The socket is gently rinsed with saline and incrementally filled with the clots and condensed. Following socket fill, both groups will have the grafts covered by a dense polytetrafluoroethylene membrane. The membrane is trimmed and adapted with the borders tucked 2-3mm below the mucoperiosteal pouch. The soft tissues and membrane are stabilized with 5/0 PTFE sutures, one horizontal mattress suture and one cross suture, without an attempt at primary closure. The patient is then assessed for hemostasis and provided with written and verbal post-operative instructions. Patients will be instructed to avoid mechanical plaque control of the area and instructed to stay on a soft diet for 7 days post-operatively. Patients will be provided with Chlorhexidine 0.12% to rinse twice daily for 30 seconds during the first week of healing. Participants are encouraged to contact the operator if they have any problem at any time. The patients also receive post-operative medication: Amoxicillin 500mg P.O Q8H for 7 days or in the case of penicillin allergy, Clindamycin 150mg P.O Q6H for 7 days. The patients

will also receive a VAS questionnaire evaluating the post-operative pain 1 and 7 days following surgery as well as a supply of 20 tabs, Ibuprofen 400mg P.O Q6H PRN. Intra-oral photographs will be taken pre-, intra- and post-operatively. A CBCT will be taken within 72 hours of the surgery.

**Visit 4:** 7 days post-op: Sutures will be removed if deemed suitable. The VAS questionnaire will be collected and remaining tablets of pain medication collected and recorded. Oral hygiene instructions will be reviewed and a soft post surgical brush will be provided. Intra-oral photographs of the site will be taken.

**Visit 5:** 14 days post-op: Sutures will be removed if they were not removed during the previous visit. Oral hygiene instructions will be reviewed. Intra-oral photographs of the site will be taken.

**Visit 6:** 6 weeks post op: d-PTFE membrane will be retrieved and discarded using tissue forceps. Upper and lower alginate impressions will be taken for the manufacture of a surgical guide. Oral hygiene instructions will be reviewed. Intra-oral photographs of the site will be taken.

**Visit 7:** 11 weeks post op: Second CBCT will be taken with surgical guide. The image obtained will be used to analyze and compare the ridge dimensions to those obtained at baseline as well as for surgical implant planning.

**Visit 8:** 12 weeks post op: Medical history review. Intra-oral photographs of the site will be taken. Soft tissue measurement with periodontal probe and floss spanned over edentulous site from buccal to lingual mucogingival junction will be performed and recorded. Implant placement will be done as per standard procedure. A 2.5mm diameter trephine drill will be

used to harvest a bone core for histologic analysis. The bone core will be immediately submerged in a solution of 10% neutral buffered formalin. The selection of the implant system will depend on the surgical and restorative needs of each individual case. Osteotomies and implant placement will be done following the manufacturer's protocol. A final peri-apical radiograph will be obtained to verify correct implant position and angulation. If adequate primary stability is achieved and one-stage approach is feasible, a healing abutment will be placed. Depending on the buccal bone and soft tissue thickness, ancillary soft tissue augmentation, bone augmentation or combination of these procedures may be indicated. If a two-stage procedure is indicated, a cover screw will be placed and the implant will be submerged. At the end of the appointment, written and verbal post-operative instructions will be given to the patients. The patient will be followed up and referred to the restorative dentist as per standard procedure.

#### Data Collection

CBCT measurements will be performed by the primary investigator (JB) after extraction (visit 3) and before implant placement (visit 7) with the use of i-Dixel image capturing software. The DICOM files will be exported to 3D analysis software, Invivo by Anatomage, to superimpose the two images using static anatomical landmarks as reference points, and measuring the difference between baseline (Visit 3) and post-healing (Visit 7)<sup>28</sup>. Buccal bone plate thickness would be measured at 1, 3 and 5mm from the bone crest, as well as bucco-lingual width at 1, 3 and 5 mm from the bone crest. Vertical measurements would be measured from the buccal and lingual bone crest to the apex of the socket. KT measurements will be compared to baseline and implant placement visit. Objective and subjective post-operative pain experience

will be compared between the two groups using data from VAS questionnaires and tablet counts of remaining medication.

### Statistical methods

Sample size was calculated assuming an alpha of .05, two tailed test, with a power estimate 0.8 which resulted in sample estimates of 42 subjects. The study will be blinded at the level of data assessment. The primary outcome will be the volumetric changes in soft and hard tissue for the two proposed methods. Secondary outcomes will be reported patient discomfort from the VAS questionnaire between the two groups as well as evaluation of the amount of vital bone formed after the healing phase by means of histologic analysis of bone samples taken from the 2 groups at the time of the implant surgery visit. To compare groups at T1 and T2, independent samples t-tests were used. Equal variances were not assumed and the significance levels were corrected as such. In addition, a non-parametric MannWhitney U test was also used. To test for differences at time 1 vs time 2, within and between groups, a linear mixed model was used with restricted maximum likelihood (*REML*) approach. Spearman correlations reported to indicate relationship between Plate (T1) and Width (T2) measurements for 1, 3 and 5mm.

### Risks

Extraction and alveolar ridge preservation may have a risk of membrane exposure, post-operative bleeding, infection, swelling, pain and discomfort from the surgical site for both groups.

### Potential benefits

If L-PRF is proved to be equal or better in terms of alveolar ridge preservation, resulting in faster healing time and less discomfort, it would be of benefit to the patient due to a more comfortable and faster post operative healing phase, reduced costs compared to the purchase of grafting materials, less risk of graft rejection as well as providing the patient with a viable option should personal preference dictate that no synthetic, xeno- or allografts be used.

### Consent Process

Patients of the Graduate Periodontics Clinic that require extraction, ridge preservation and subsequent implant placement, will be asked personally to volunteer. Those showing interest will be provided with a summary of the research study including a consent form to read and sign should they wish to partake.

### Data security

All data will be recorded based on the participant's number assigned once the consent form is signed. Each patient's name and contact information will be kept locked in a secure place at the Graduate Periodontics Clinic. The results of the study may be published or presented in public forums; however the participants' name will not be used or revealed.

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