TITLE

Histological evaluation of alveolar ridge preservation using MInerOss® versus Osteogen plug® bone graft techniques: clinical study analysis part II.

NCT Number: Unassigned

Date: February 8, 2019

ABSTRACT

Background: The aim of this histological study was to compare the percentage of hard tissue (bone) after 12 -weeks to 20 weeks post-ridge augmentation surgery after using two different ridge preservation procedures.

Methods: Eleven extraction sites were randomly allocated to either group using a computer-generated randomization assignment software. The groups were: group CCBC, received cortico-cancellous bone chips (CCBC) mix and a dense polytetrafluoroethylene (dPTFE) barrier membrane; and group CPCAC, received type I bovine *Achilles* tendon collagen plug with bioactive resorbable calcium apatite crystals -OsteoGen- (CPCAC). The histomorphometry analyses were done using a computer-based image analysis system (IMAGE-J 1.4, National Institute of Health, Bethesda, MD, USA) to calculate pixel area of bone tissue and remaining bone graft material. The histomorphometry data were analyzed by the *Student t* test to compare the data from both groups. In addition, histological quantitative evaluation was conducted for evidence of presence of foreign body reaction in both groups. Statistical significance was evaluated at $p < 0.05$.

Results: There was no statistical significance when comparing percentage of surface area of bone for both experimental groups, CCBC and CPCAC. However, there was evidence of foreign body reaction in group CPCAC. Conclusion: The sockets that received CPCAC showed less bone formation compared to the CCBC group. However, there was no statistically significant difference between the experimental groups. In addition, group CPCAC showed more evidence of foreign body reaction when compared to group CCBC.

Keywords: ridge preservation, histomorphometry. Bone graft(s), alveolar bone, osteocyte(s), osteoblast(s), osteoclast(s), collagen(s).

Introduction

Ridge dimensions preservation after tooth extraction is critical to success of dental implant placement.[1] Ridge preservation procedures are also necessary for esthetic purposes. Even if an implant is not planned to be placed in the site, the healed site will be more aesthetic for the final restorative plan.[2]

Following tooth extraction, the alveolar ridge undergoes resorption and exhibits a wide range of dimensional changes.[3, 4] The soft and hard tissue healing within the extraction sockets following tooth removal was studied by histologic and clinical evaluations.[4, 5]In this study, healing of the extraction socket involved a series of events, including the formation of a coagulum that was replaced with a connective tissue matrix: after 1 month, woven bone filled the extraction socket; a cortical ridge, including woven and lamellar bone, was observed after 3 months. Subsequently, the woven bone was gradually replaced with lamellar bone and marrow.[6] According to Tan et al. (2012), extraction without ridge preservation can result in a mean height loss of 1.24 mm and mean width reduction of 3.79 mm. [7] If the ridge is severely resorbed, the dimension loss may prevent implant placement in the ideal restoratively driven position.

Also, Avila-Ortiz et al. (2014), [1] demonstrated that ridge preservation procedures show beneficial effect on reducing resorption by an average of 1.89 mm in the buccal-lingual width and 2.07 mm in the mid-buccal height when compared to no ridge preservation. Anatomic concerns of the maxillary sinus and the mandibular nerve make implant placement precarious at times, limiting the general dentist's desire to place implants in compromised situations. Therefore, considering

that more than 45 million teeth are extracted annually in the United States, socket grafting must become a routine procedure. [8]

Wound healing can be described as a race between various cells, like pluripotent, osteogenic, epithelial, fibroblasts cells to the healing site. The epithelium invagination can compromise the use of the socket grafting technique, so the graft material must be shielded from the epithelial advancement. The socket preservation procedures are centered on the migration of pluripotent and osteogenic cells from the periosteum and alveolar bone to the extraction site while at the same time excluding epithelial cells and fibroblasts from infiltrating and potentially disrupting new bone formation.[2, 9] This tissue exclusion process is accomplished using resorbable or non-resorbable barrier membranes. it is critical that either resorbable or non-resorbable membrane extend at least 2.0 mm beyond the facial and lingual defect to avoid premature bone exposure. The non-resorbable barrier membrane can be removed 4-6 weeks after placement, but it requires an extra patient visit. If the membrane is prematurely lost, the prognosis becomes compromised due to invagination of the epithelium cells into the grafted site or infection. In addition, a resorbable membrane must be a long-lasting resorbable membrane to contain the bone graft particulates in the extraction site.[1]

To achieve these goals a variety of materials and techniques are commonly used including autogenous tissues, allografts, alloplasts, and xenografts. Allograft and Xenograft materials have gained recognition because they do not require a second surgical site and have slower resorption rates when compared to some materials and hence can maintain dimensional stability of the ridge. [1, 10] Freeze-dried bone allograft (FDBA) is an alternative to alloplasts. The performance of the FDBA is similarly to autogenous grafts, which are the gold standard for bone graft, and has out-

performed alloplastic materials regarding dimensional stability and new bone formation. [11, 12] Benefits of FDBA also include low-cost, unlimited supply, and lack of a secondary surgical site. [11] FDBA has is osteoconductive, and therefore serves as a scaffold to permit capillary proliferation and migration of host osteoprogenitor cells to initiate the process of new bone formation, In this process, host cells will slowly resorb the residual graft particles as new bone is forming.[13] [14] Ideally, a graft material would provide enough resistance to resorption to maintain dimensional stability while simultaneously permitting adequate proliferation of blood vessels and migration of host cells for new bone formation. [11]

Cortical and cancellous FDBA are available for use in ridge preservation procedures. The healing process after grafting procedures with cortical bone is different compared with cancellous bone. [11] Cortical allografts have been indicated to have slower rate of resorption by the host compared with cancellous allografts. [15] Others have found a cancellous allograft to be beneficial over a cortical allograft in that the lower density of cancellous bone offers a greater surface area for more rapid revascularization and cell proliferation, then providing faster healing.[16] However, an animal study showed no difference in healing patterns and new bone formation when comparing cortical with cancellous grafts. [17] In human study Eskow & Mealey (2014)[18] showed similar new bone formation when comparing cortical and cancellous FDBA in ridge preservation. Also Demetter, Calahan & Mealey (2017)[11] compared a 50%:50% of cortico:cancellous allograft to 100% cortical allograft and 100% cancellous allograft when used in alveolar ridge preservation in non-molar teeth sites. The histomorphometric analysis showed no significant differences among groups regarding percentage of vital bone or CT.

Placing a collagen-based clotting material, also called collagen plug, such as a 100% collagen plug, is not considered a simple procedure and won't preserve the ridge in the edentulous site due to it resorbing in less than 2 weeks and not providing sufficient scaffolding for new bone formation. [2]

The OsteoGenR® nonceramic bone graft with bovine Achilles tendon collagen contains crystals inside of the collagen matrix and therefore cannot wash out after placement, as it happens with particulate graft materials. [19, 20] This feature allows the use of this material as a plug, without the need to add a separate membrane over the site. OsteoGen® is highly hydrophilic and has been used as a particulate graft material since 1984. It shows documented clinical success when used for prior to implant placement in periodontal procedures. [20, 21] OsteoGen® strips were cleared by the FDA in 2009 and, more recently, OsteoGen® plugs were introduced to address the grafting needs of ridge preservation procedures.

The OsteoGen® plug is radiolucent, on the radiograph, when placed in the socket after the extraction and as the crystals are resorbed and replaced by host bone, the site gradually becomes radiographically radiopaque. The collagen component promotes keratinized tissue coverage while the graft component promotes new bone formation.[2]

Clinical case studies have been reported in the use of OsteoGen® plugs for ridge preservation.[2] To date, and to the best knowledge of the authors, no histological studies have compared FDBA allograft with OsteoGen® plug in alveolar ridge preservation after tooth extraction. Therefore, the purpose of this study is to compare bone healing after the use of FDBA allograft with a non-resorbable membrane to OsteoGen® plug alone in alveolar ridge preservation procedures. The primary outcome of the current study is to histologically evaluate bone formation in the two groups. The secondary outcome is to evaluate the foreign body reaction on each of the groups.

Materials and Methods

The present histomorphological study is part II of a previously conducted clinical study on wound closure after the use of the same experimental groups described below. The part I of this study had 17 patients. Of those, 11 were used for the present histomorphometry study.

In the present study, the samples were collected from study part I to conduct histomorphometric analysis. This study had 2 experimental groups. The groups were:

Experimental group (CCBC)

Exodontia was conducted as part of the patient's treatment plan. After extraction the sockets were thoroughly curetted, and a clot was left in place. Cortico-cancellous allograft (MinerOss®, BioHorizons, Birmingham, AL, USA) were hydrated with saline solution and compacted into the socket up to the level of the alveolar crest. The cortico-cancellous allograft was selected for this study due to its confirmed bone formation at the treatment site that would receive osseointegrated implants to replace the missing maxillary posterior teeth. Expanded polytetrafluoroethylene (ePTFE) (Cytoplast™ TXT-200 non-resorbable high-density) membrane were mapped, trimmed, and placed on the surgical site. 4-0 ePTFE (Cytoplast) thread were used to conduct a hidden "X" suture.

Experimental group (CPCAC)

Same surgical protocol was conducted as indicated in experimental group CPCAC until exodontia. After exodontia and preparation of the socket, the OsteoGen® plug was compacted into the site.

Sample collection phase

The patients from the study were monitored during the healing phase. At the time of planned implant placement, the samples were collected for histomorphometric analysis. Patients that were submitted to mandibular ridge preservation procedures were scheduled for dental implant placement 3-4 months after the ridge preservation procedure had been conducted. Patients that were submitted to maxillary ridge preservation procedures were scheduled for dental implant placement 5-6 months after the ridge preservation procedure had been conducted. At the implant placement visit, the bone sample was collected from the surgical site by a 3.0 mm by 4.0 mm trephine and subsequently placed into a 10% buffered formalin solution. An osteotomy site was then prepared for the dental implant followed by manufacturer instruction. Flaps were replaced and sutured with 4-0 chromic gut.

Histologic Analysis

The Trephine cores (Figure 1) were submitted for routine formalin-fixed, paraffin-embedded histologic studies. All the specimens were decalcified using Decal solution, then processed, longitudinally sectioned, and stained with hematoxylin and eosin. Then the microscopic slides were scanned and viewed using the software of CaseViewer (2.4.0.119028).

For histomorphometric analysis for each core sample three components including viable bone, residual graft, and connective tissue. Other tissues, such as: vascular tissue, macrophages, and adipose tissue were considered. Viable bone was defined by visualization of osteocytes within lacunae of the mineralized tissue. Residual bone graft was defined as a mineralized tissue with no lacunae. Connective tissue/other was defined as the absence of the above categories and the presence of fibrous connective tissue. Once tracing of components was completed, individual images for each layer were created and converted to binary images (ImageJ, National Institutes of Health, Bethesda, MD). Binary images were then used to calculate the total number of pixels in each image. Using the total number of pixels per component and the total number of pixels in all three images, the percent of total area occupied by vital and residual graft material, and connective tissue/other were calculated.

Statistical analysis

Student paired 't' test was be conducted to evaluate differences between groups. Student 't' test was employed to compare the data in two sets of groups to identify the significance of difference in their means (intergroup). Probability values (p) less than 0.05 will be used considered significant (Table 3).

RESULTS

Fifteen (15) patients were enrolled from the period of July 2019 to January 2021. The patient profile included 7 females and 8 males, with ages ranging from 25 to 75. Two (2) patients were withdrawn due to lack of compliance to the scheduled PO visits. An additional two patients were withdrawn due to the COVID-19 related school closure, leaving a total of 11 patients who completed the study. Of the 11 patients who completed the study, the average age was 55 (range 27 to 82), consisting of 6 females and 5 males (Table 1).

The patients received a VAS pain rating scale to report their pain levels at 1-, 3- 5- and 7-days post-operatively. The patient pain levels evaluated by the VAS pain rating scale showed no statistical significance between groups I and II.

None of the patients developed any complications and PO infections. A total of 11 extraction sites were evaluated, of which 5 patients were in the group CCBC and the remaining patients were in the extraction sites were for group CPCAC.

Histologic studies of the group CCBC (CBCT/PTEF, total 5 cases) samples revealed variable viable lamellar bone exhibiting focal reversal lines and osteoblastic rimming (except one case which showed woven bone) interspersed with fibrous/fibro-vascular connective tissue. Minimal or no inflammation was noted and all the cases except one demonstrated minutes remnants of the graft material (Figure 1). In the group CPCAC (the OsteoGen® plug, total 6 samples) a variable amount of viable bone, either of lamellar or woven type, intermixed with fibro-vascular connective tissue

was noted. Reversal lines and plump osteoblastic rimming were also noted. The remnants of graft material were found in all the cases and 3 out of the 6 cases showed foreign body granulomatous inflammation (Figure 2).

Table 2 shows average of the bone area measurements in (pixels) for each of the experimental sample of groups CCBC and CPCAC. Table 3 shows the percent bone area measurements for each of the experimental sample of groups CCBC and CPCAC. Note a higher average bone presence in the histological sections for group CCBC when compared to group CPCAC. The histomorphometric results showed an average of 35.13% of bone area in group CPCAC when compared to an average of 42.38% group CCBC, but there is no statistically significant difference between the groups.

DISCUSSION

The primary purpose of ridge preservation is to maintain the ridge dimensions to allow for restoratively driven implant placement[22, 23]. Secondly, ridge preservation procedures may provide adequate vital bone for dental implant placement [23]. Xenografts and allografts are the two most used bone graft materials in dentistry. Osteogen® plug is a unique product in that it is a synergy between both bovine type I bovine *Achilles* tendon collagen and non-ceramic resorbable calcium apatite bone graft crystals. To the knowledge of the authors no study compared the Osteogen plug bone formation to the allograft material.

In this histomorphometric study comparing two different ridge preservation techniques, there were no histomorphometric statistically significant differences found between the two experimental groups. Primary outcome variable tested was percentage of bony tissue area present

for both experimental groups. The second outcome variable observed was trace of inflammatory reaction for group CPCAC, which may affect the long-term outcome of implant success.

From the standpoint of implant placement, both experimental groups evaluated in this study showed similar results in a short-term success basis, but long-term analysis is necessary in order to further evaluate the success of future implants place in the sites using both techniques, especially for the technique that included the use of Osteogen® plug, due to it's histological evidence of foreign body reaction in the sites presenting this product.

Also, the maxillary bone type could be deferent from mandibular[24] , so with larger sample group the bone formation may be evaluated in each arch.

The radiograph CBCT can also be used for comparing the dimensional changes before and after grafting in the future studies.

The benefits of using Osteogen® plug is it's simplified clinical application, no needs of second surgery for removal of the membrane, in addition to being cost effective when compared to the technique for group CCBC.

There was a limitation in our study, the sample size became limited due to the COVID-19 pandemic which caused three patients to withdraw from the study.

CONCLUSION

To our knowledge, this is the first study to compare the amount of the bone available between use of Osteogen® plug and the use of MinerOss and Cytoplast membrane for ridge augmentation procedures. The current findings indicate that there was no significant difference for the hard tissue available for placing the implant 3-5 months after grafting.

REFERENCES

1. Avila-Ortiz G, Elangovan S, Kramer KW, Blanchette D, Dawson DV. Effect of alveolar ridge preservation after tooth extraction: a systematic review and meta-analysis. *J Dent Res* 2014;93:950-958.
2. Kosinski T. A Simple and Cost-Effective Socket Preservation Technique. *Dent Today* 2016;35:90, 92, 94-95.
3. Devlin H, Ferguson MW. Alveolar ridge resorption and mandibular atrophy. A review of the role of local and systemic factors. *Br Dent J* 1991;170:101-104.
4. Lekovic V, Kenney EB, Weinlaender M, et al. A bone regenerative approach to alveolar ridge maintenance following tooth extraction. Report of 10 cases. *J Periodontol* 1997;68:563-570.
5. Schropp L, Wenzel A, Kostopoulos L, Karring T. Bone healing and soft tissue contour changes following single-tooth extraction: a clinical and radiographic 12-month prospective study. *Int J Periodontics Restorative Dent* 2003;23:313-323.
6. Cardaropoli G, Araujo M, Lindhe J. Dynamics of bone tissue formation in tooth extraction sites. An experimental study in dogs. *J Clin Periodontol* 2003;30:809-818.
7. Tan WL, Wong TL, Wong MC, Lang NP. A systematic review of post-extractional alveolar hard and soft tissue dimensional changes in humans. *Clin Oral Implants Res* 2012;23 Suppl 5:1-21.
8. Zhao R, Yang R, Cooper PR, Khurshid Z, Shavandi A, Ratnayake J. Bone Grafts and Substitutes in Dentistry: A Review of Current Trends and Developments. *Molecules* 2021;26.
9. Becker W, Becker BE. Guided tissue regeneration for implants placed into extraction sockets and for implant dehiscences: surgical techniques and case report. *Int J Periodontics Restorative Dent* 1990;10:376-391.
10. Corbella S, Taschieri S, Francetti L, Weinstein R, Del Fabbro M. Histomorphometric Results After Postextraction Socket Healing with Different Biomaterials: A Systematic Review of the Literature and Meta-Analysis. *Int J Oral Maxillofac Implants* 2017;32:1001-1017.
11. Demetter RS, Calahan BG, Mealey BL. Histologic Evaluation of Wound Healing After Ridge Preservation With Cortical, Cancellous, and Combined Cortico-Cancellous Freeze-Dried Bone Allograft: A Randomized Controlled Clinical Trial. *J Periodontol* 2017;88:860-868.
12. Ten Heggeler JM, Slot DE, Van der Weijden GA. Effect of socket preservation therapies following tooth extraction in non-molar regions in humans: a systematic review. *Clin Oral Implants Res* 2011;22:779-788.
13. Burchardt H. The biology of bone graft repair. *Clin Orthop Relat Res* 1983:28-42.
14. Mastrogiacomo M, Muraglia A, Komlev V, et al. Tissue engineering of bone: search for a better scaffold. *Orthod Craniofac Res* 2005;8:277-284.
15. Balabhadra RS, Kim DH, Zhang HY. Anterior cervical fusion using dense cancellous allografts and dynamic plating. *Neurosurgery* 2004;54:1405-1411; discussion 1411-1402.
16. Dux SJ, Ramsey D, Chu EH, Rimnac CM, Hernandez CJ. Alterations in damage processes in dense cancellous bone following gamma-radiation sterilization. *J Biomech* 2010;43:1509-1513.

17. Malinin TI, Carpenter EM, Temple HT. Particulate bone allograft incorporation in regeneration of osseous defects; importance of particle sizes. *Open Orthop J* 2007;1:19-24.
18. Eskow AJ, Mealey BL. Evaluation of healing following tooth extraction with ridge preservation using cortical versus cancellous freeze-dried bone allograft. *J Periodontol* 2014;85:514-524.
19. Valen M, Ganz SD. A synthetic bioactive resorbable graft for predictable implant reconstruction: part one. *J Oral Implantol* 2002;28:167-177.
20. Epstein SR, Valen M. An alternative treatment for the periodontal infrabony defect: a synthetic bioactive resorbable composite graft. *Dent Today* 2006;25:92-97.
21. Corsair A. A clinical evaluation of resorbable hydroxylapatite for the repair of human intra-osseous defects. *J Oral Implantol* 1990;16:125-128.
22. Evian CI, Rosenberg ES, Coslet JG, Corn H. The osteogenic activity of bone removed from healing extraction sockets in humans. *J Periodontol* 1982;53:81-85.
23. Dahlin C, Linde A, Gottlow J, Nyman S. Healing of bone defects by guided tissue regeneration. *Plast Reconstr Surg* 1988;81:672-676.
24. Devlin H, Horner K, Ledgerton D. A comparison of maxillary and mandibular bone mineral densities. *J Prosthet Dent* 1998;79:323-327.