Received: 25 January 2018 | Accepted: 27 January 2018

DOI: 10.1111/cid.12596

ORIGINAL ARTICLE

WILEY

Biocompatibility and osteoconductivity of PLCL coated and noncoated xenografts: An in vitro and preclinical trial

Yaniv Mayer DMD[1] | Ofir Ginesin DMD MSc[1] | Alaa Khutaba DMD MSc[1] | Eli E. Machtei DMD[1] | Hadar Zigdon Giladi DMD PhD[2]

[1]Department of Periodontics, School of Graduate Dentistry, Rambam Health Care Campus, Haifa, Israel

[2]Lab of Bone Regeneration, Department of Periodontics, School of Graduate Dentistry, Rambam Health Care Campus, Haifa, Israel

Correspondence
Dr Yaniv Mayer, DMD, Department of Periodontics, School of Graduate Dentistry, Rambam Health Care Campus, PO Box 9602, Haifa 31096, Israel.
Email: dr.yaniv.mayer@gmail.com

Abstract

Background: Cells, scaffolds, and growth factors are the key components in bone tissue engineering. Scaffold composition, topography, and architecture influence the amount of regenerated bone in the implantation site. The aims of the study were to compare viability and proliferation of mesenchymal stem cells (MSCs) seeded onto two commercial xenografts: Bio-Oss (BO) and bioactive bone bovine (BB). Next, these materials were compared for histomorphometric bone formation in a socket preservation model in rats.

Materials and Methods: MSCs were seeded onto monolayers of BO or BB granules. Cell viability and proliferation were evaluated after incubation of 0, 2, 20, and 48 h. A total of 24 Sprague Dawley rats underwent unilateral extraction of maxillary molars. Rats were randomly divided into three groups: natural healing (nongrafted socket) or socket preservation with either BO or BB. Rats were sacrificed after 8 weeks, and histomorphometric analysis was done to evaluate bone formation and residual scaffold at the extraction site.

Results: Differences in the metabolic activity of MSCs that were seeded onto BO or BB was observed at 2 h after seeding: the metabolic activity was elevated compared to baseline in the BB ($P = .046$) and not changed in the BO wells ($P = .84$). After 20 h, the metabolic activity of MSCs seeded onto BO was decreasing ($P = .005$), while cell viability was not changed in the BB group ($P = .356$). Intergroup comparison revealed higher metabolic activity of MSCs seeded on BB after 48 h compared with BO ($P = .016$). The in vivo results demonstrated differences in socket healing between the groups: percentage of new bone was higher in the BB compared to BO group (39.1 ± 14.3 vs. $23.7 \pm 10.8\%$, respectively, $P = .096$). Connective tissue portion was higher in the BO group compared with BB (73.7 ± 11.1 vs. $49.6 \pm 13.7\%$, respectively, $P = .018$). Residual grafting martial was higher in the BB (11.34 ± 4.18 vs. $2.62 \pm 1.23\%$, $P = .011$).

Conclusions: The results of this study demonstrating higher vitality and proliferation of MSCs seeded onto BB. Furthermore, following ridge preservation, higher percentage of new bone and lower residual scaffold were found in the BB compared with BO. This enhanced regenerative response might be the result of an enhancement of metabolic activity in cells attached to it. Further research will be needed to understand the precise mechanism.

KEYWORDS

alveolar bone, extraction, mesenchymal stem cells, regeneration, socket preservation, xenograft

1 | INTRODUCTION

Tooth extraction initiates several biological processes that one of the final outcomes is alveolar bone loss.[1,2] The loss occurs on all dimensions of the alveolar bone (ie, buccal, lingual, vertically, and horizontally) and is more prominent in the buccal aspect.[1,2] Changes in bone height may pose difficulties in restoring the function and the aesthetics of the extracted tooth. In order to diminish the rate of the alveolar ridge dimensional changes, several clinical and animal experiments were performed to evaluate the hypothesis that grafting of the socket immediately after tooth removal may represent of an advantages outcome following tooth extraction. There are numerous evidences that socket preservation can reduce the alveolar bone resorption after tooth extraction.[3–5] Multiple materials and methods have been tested with different success rate.[3–5] Today, deproteinized (anorganic) bovine bone is one of the most used biomaterials for socket preservation.[6–9] This extensive use in the different periodontal procedures in general and in socket preservation in particular owed to its large availability and his cost effectiveness compare to other products.

Cells, scaffolds, and growth factors are the key components required to generate engineered tissues. A scaffold can deliver growth factors and supports cell attachment. The material composition, topography, and architecture influence cell behavior and intercellular interactions and overall performance of the scaffolds including the angiogenesis and degradation rate. New xenograft-based material was introduced, with scaffold modification, cover by poly(L-lactide-co-ε-caprolactone) (PLCL) and polysaccharides.[10] The properties of this new xenograft suggest better cell adhesion[10] and as such may provide better outcomes in preserving bone after tooth extraction, compared to the current xenograft materials that are in use. Mesenchymal stem cells (MSCs) contribute to the maintenance of various tissues, especially bone, in adults.[11] Previous animal studies showed that transplantation of MSCs formed ectopic bone and improved healing of bone defects.[12,13]

Hence, the first aim of the present study was to compare the viability and proliferation of MSCs onto two commercial xenografts (with or without PLCL coating). The second aim was to compare the histomorphometric bone formation in a socket preservation model in rats. Our hypothesis is that PLCL coating of xenograft will increase MSCs' adherence to the scaffold in vitro and, therefore, stimulate bone formation in a rat extraction socket model.

2 | MATERIALS AND METHODS

2.1 | In vitro assay

To investigate cell proliferation on the examined scaffolds, MSCs were seeded on a monolayers of (BB) (Alpha Bio's Graft, Bioactive Bone; Industrie Biomediche Insubri SA, Mezzovico-Vira, Switzerland) or (BO) (Bio-Oss; Geistlich Pharma, Wolhusen, Switzerland) that covered the bottom of 96-well plates. Both materials were used with the same granules size (0.25-1 mm granules). Human MSCs were isolated and characterized as previously described.[14] A total of 3×10^3 bone marrow-derived human MSCs were suspended in DMEM (Sigma-Aldrich, Munich, Germany) and dripped (50 μl) over the granules layer. Cell proliferation kit (XTT) (Sigma-Aldrich) was used to quantify cell growth, viability, and proliferation after 0, 2, 20, and 48 h of incubation. The assay was performed twice in triplicates. Absorbance of the wells containing the cells (indicating on cell metabolic activity) and the blank background control wells were investigated at 450–500 nm wave length using a microtiter plate reader (BioTek, Winooski, Vermont).

2.1.1 | Fluorescent assay

Cells were labeled with fluorescent DII stain (D282; Thermo Fisher Scientific, Waltham, Massachusetts) and seeded onto BB or BO scaffolds. Twenty-four hours later, scaffolds without cells and scaffold loaded with cells were observed under a fluorescent microscope (Nikon eclipse TsTR).

2.2 | In vivo assay

The study protocol was approved by the Committee for the Supervision of Animal Experiments at the Faculty of Medicine, Technion, IIT (approval # B126111010).

A total of 24 Sprague-Dawley rats (300 g) were used. The rats were allowed to acclimate for 1 week before the first procedure.

Rats were anesthetized with isoflurane inhalation followed by intramuscular injection of ketamine (Ketaset, Fort Dodge, Iowa) (10 mg/100 g body weight) and xylazine. In addition, local anesthesia (2% lidocaine with epinephrine in the ratio of 1:100 000) was infiltrated to the surgical site (Figure 1A). Two right maxillary molars were extracted (Figure 1B), and the sockets of both molars were connected using a diamond bur with copious saline irrigation. Rats were randomly assigned using a designated allocation chart to one of the following three groups (eight rats in each group):

1. Experimental group (BB): the socket was grafted with coated bovine bone (Figure 1C).
2. Positive control (BO): the socket was grafted with uncoated bovine bone.
3. Negative control (NC): the socket was left to natural healing.

Flaps were coronally positioned to achieve primary closure and sutured using resorbable vicryl 5-0 sutures (Figure 1D). Postoperative treatment included buprenorphine (0.01 mg/kg) subcutaneously for 3 days.

The rats were housed and given water and solid diet until sacrificed. The rats were sacrificed 8 weeks postoperation with CO_2 inhalation. The right maxilla of each rat was retrieved for histological analysis.

2.2.1 | Histological preparation

All specimens were fixed in 4% paraformaldehyde for 2 days, decalcified in 10% EDTA, for 4 weeks, and cut into two halves in the midline. The water within the samples was removed by dehydration; the samples were washed in ethanol baths (in increasing concentrations) in order to remove residual water. This was followed by a hydrophobic clearing agent (Xylol) to remove the alcohol content. After the samples were dehydrated, cleared, and infiltrated with Paraffin wax, they

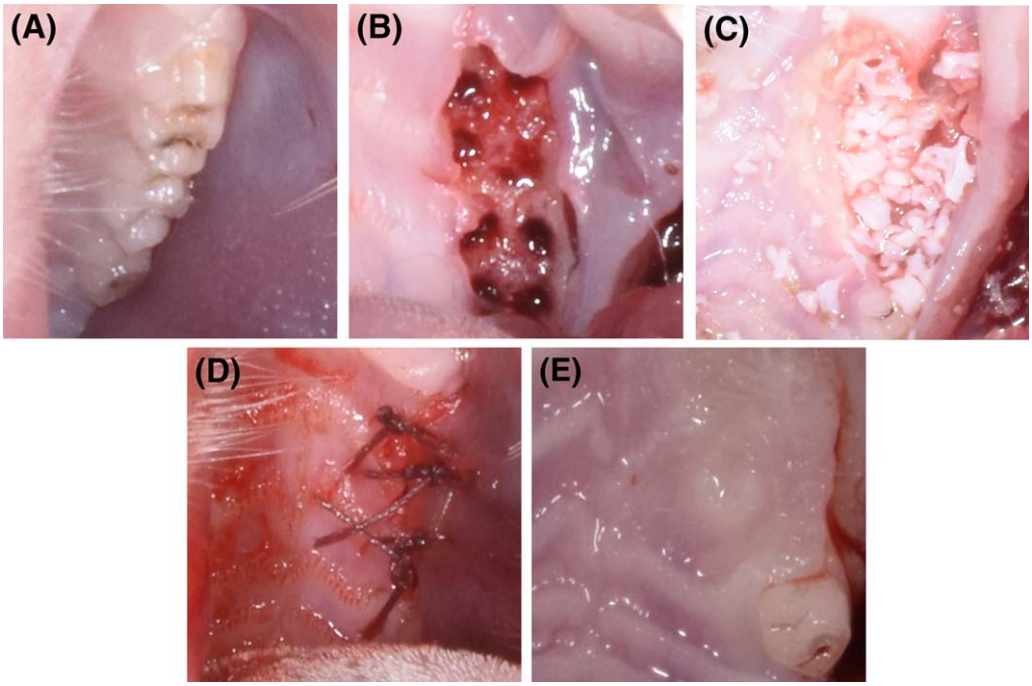

FIGURE 1 Clinical macroscopic view of the surgical procedure and healing. (A) Maxillary molars before extraction. (B) Extraction sockets of M1 + M2. (C) Filling the defect with xenograft. (D) Sutures for primary closure. (E) Healing of extraction and ridge preservation site, 8 weeks after surgical treatment

undergo external embedding. For light microscopy, the samples were sectioned using a steel knife mounted in a microtome (Leica RM 2135, Jung RM 2065; Leica Microsystems, Wetzlar, Germany) to a thickness of 8 μm and the sections were mounted on a glass microscope slides using paraffin section mounting bath (Electron Microscopy Sciences, Hatfield, England). For the determination of bone morphology, the mounted sections were stained with: hematoxylin and eosin and Masson's trichrome.

2.2.2 | Histomorphometric analysis

Histomorphometric evaluation of the socket preservation region was performed from each specimen, under a light microscope (Zeiss Axioskop; Carl Zeiss, Jena, Germany). Images were analyzed using software (ImageJ; National Institutes of Health, Bethesda, MD). The following values were measured: (1) total bone area, (2) connective tissue, and (3) residual bone graft. The measurements were expressed as percentages of the total sample area.

2.2.3 | Statistical analysis

To compare between the group regarding the metabolic activity and histomorphometric parameters, one-way analysis of variance was used. A *P*-value of $<.05$ was selected to determine statistical significance.

3 | RESULTS

3.1 | In vitro

Two hours after MSCs seeding onto the scaffold, the metabolic activity was not changed (compared to baseline) in the BO wells (0.277 ± 0.06, $P = .84$). However, in the BB, the metabolic activity was elevated significantly (0.359 ± 0.06, $P = .046$). A similar trend was observed after 20 h: the metabolic activity of MSCs seeded onto BO was decreasing significantly (0.144 ± 0.01, $P = .005$), while cell viability was not changed in the BB group (0.289 ± 0.1, $P = .356$). Forty-eight hours following incubation, metabolic activity continued to decrease in the BO group (0.129 ± 0.01, $P = .03$), whereas cells seeded onto BB presented increased metabolic activity (0.329 ± 0.08, $P = .12$). Intergroup comparison revealed higher metabolic activity of MSCs seeded on BB compared to BO after 20 h ($P = .08$) and after 48 h ($P = .016$) (Table 1).

3.1.1 | Fluorescent assay

Cells were dispersed over both scaffolds with a higher cell density on BB (Figure 2A,B)

3.2 | In vivo

No surgical complications were reported. All rats survived to the end of the study. Rats tolerated the surgical procedure and gained weight (Figure 1E). Six extraction sockets healed with secondary intention.

TABLE 1 Metabolic activity (fluorescent units) of bone-marrow derived human MSCs seeded on the scaffolds

Group	2 h	20 h	48 h
BB	0.359 ± 0.06	0.289 ± 0.1	0.329 ± 0.08
BO	0.277 ± 0.06	0.144 ± 0.01	0.129 ± 0.01
P value, BB vs BO at each time point	NS	.08	.016

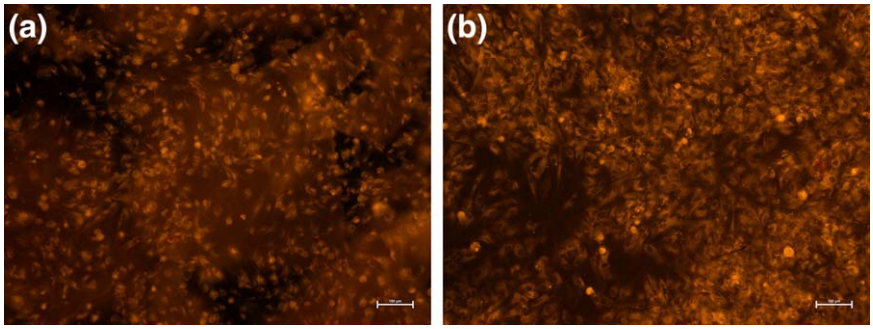

FIGURE 2 Adhesion of MSCs over BO scaffold (A) and BB scaffold (B)

3.2.1 | Histology

Histological sample from BO and BB groups presented direct contact between the residual bone substitutes and the newly formed bone, suggesting that the both xenografts have osteoconductive properties. The newly formed bone was vital and vascularized (Figure 3A,B).

3.2.2 | Histomorphometric analysis

Results for each type of treatment after 8 weeks of healing are presented in Table 2.

The amount of newly formed bone was 39.06 ± 14.26, 23.7 ± 10.77, and 48.86 ± 12.84% for BB group, BO group, and negative control group, respectively. The statistical comparison regarding the three different treatment modalities is presented in Table 3. Statistically significant difference was found between the negative control and BO group ($P = .021$), and borderline statistically significance was found between BB and BO group ($P = .096$). No statistical difference was found between BB and control ($P = .291$).

The amount of residual graft was significantly higher in the BO compared with BB groups (11.34 ± 4.17 vs. 2.62 ± 1.23%, respectively, $P = .011$).

The percentage of the connective tissue was 49.6 ± 13.67, 73.68 ± 11.05, and 51.14 ± 12.84 in the BB group, BO group, and negative control group, respectively. Comparison between the groups revealed statistically significant difference between BO and control ($P = .012$) and between BB and BO ($P = .018$), and no statistical difference was found between BB and negative control ($P = .86$).

4 | DISCUSSION

In the present study, we compared biocompatibility and osteoconductivity of two commercial xenograft scaffolds. According to the results, xenograft that was reinforced with PLCL showed superior in vitro and in vivo results. MSCs that were seeded on the PLCL reinforced xenograft showed higher viability and proliferation compared with the noncoated xenograft.

Socket preservation has been proven to be effective in reducing the bone loss postextraction.[15] Still studies report on bone resorption on all aspect with a range of 11–22% on vertical dimension and 29–63% on the horizontal dimension depends on the material and method of the preservation.[16] Currently, ideal grafting material is yet to be found. Xenograft is one of the most used materials that is in use for socket preservation, and it is biocompatible and osteoconductive. Two major flaws of the xenograft that are widely used are the poor cell adhesion[17] and different clinical behaviors depending on source.[18] Coating the xenograft scaffold might enhance cell adhesion and further maintain the bone volume after tooth extraction. Our findings further strengthen previous material characterization of PLCL reinforcement that was published.[10] Pertici and colleagues[10] showed that coating xenografts with degradable synthetic [PLCL] and natural (polysaccharides) polymers has increased their mechanical properties. Furthermore, PLCL coating was shown to improve cell adhesion due to improved hydrophilicity.

According to the in vitro results, MSCs adhere better to the PLCL-reinforced xenograft (BB) in comparison to the noncoated xenograft

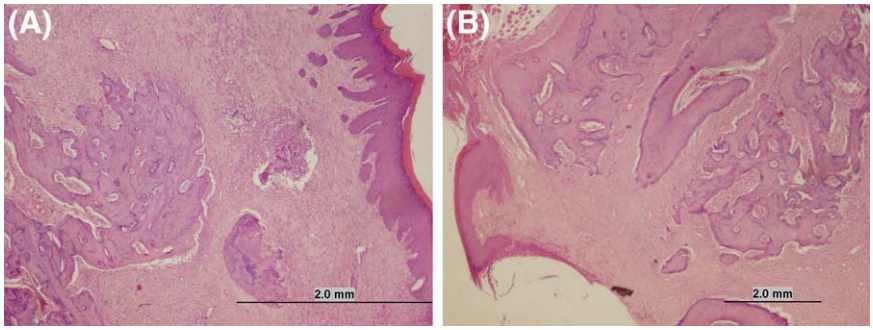

FIGURE 3 Histological sections of sockets grafted with BO scaffold (A) and BB scaffold (B). H&E staining

TABLE 2 Histomorphometric analysis (% total sample area, mean ± SD)

Group	% bone	% connective tissue	% residual graft
BB	39.06 ± 14.26**	49.6 ± 13.67*	11.34 ± 4.17*
BO	23.7 ± 10.77‡,**	73.68 ± 11.05*,‡	2.62 ± 1.23*
Negative control	48.86 ± 12.84‡	51.14 ± 12.84‡	–

*BB-BO $P < .05$.
**BB-BO $P < .1$.
‡BO-NC $P < .05$.

(BO). Furthermore, cells' viability and proliferation were higher when seeded on PLCL-reinforced xenograft. Since MSCs play a critical role in new bone formation,[19] the adherence and viability of MSCs to the scaffold may influence the amount of new bone formed in the implantation site. Previous studies prove the ability of MSCs to stimulate new bone formation in cases of bone loss,[20] and bone regeneration.[21] Indeed, the in vivo and in vitro results were in agreement as higher bone formation was found in the BB group compared to the BO group. Further studies are needed to explore the exact mechanism of bone formation around xenograft with PLCL coating.

Implantation of PLCL coated and noncoated xenograft into bone defect in a ridge preservation rat model showed that the extraction socket was filled with new bone, connective tissue, and residual graft. The ratio between these elements was different between the groups. Previous studies that used BO for ridge preservation demonstrated similar results.[22,23] A comparison between BO and BB revealed higher bone formation with lower percentage of connective tissue and residual scaffold in the BB group. These results can be attributed to the greater biocompatibility of the PLCL reinforced xenograft as well as to faster degradation of the scaffold. A previous study[10] found that the PLCL coating accelerates scaffold degradation and is in positive correlation to the thickness of the coating.

Vascularization and oxygenation are important process in order to create new bone formation.[24] Percentage of new bone formation was higher in the BB group compared to the BO group. This fact may be attributed to the large pores of the BB[10] that favor direct osteogenesis, as they allow vascularization and high oxygenation. Further studies are required to validate this assumption.

Secondary healing was found in six of the 24 animals. This phenomenon can be attributed to the flap design.[25] We did not raise the flap or vertical releasing incisions; thus, the primary closure might have had tension and was not passive. Another possible reason is the heat produced during the use of the bar although constant water irrigation was done. Previous studies[26–28] showed that excessive heat can harm the osteoblast.[28] Heating to 47°C for 1 min caused significantly reduced bone formation,[27] and this temperature or even higher one can be achieved by rotating dental cutting instruments.[26] We cannot exclude other causes as inflammatory reaction[29] due to the higher prevalence of secondary healing in the test and positive control groups compared to the negative control groups (only one in the latter out of all the cases).

TABLE 3 Comparison between groups—*P*-value (ANOVA)

Group	% bone	% connective tissue	% residual graft
BB/BO	0.096	**0.018**	**0.011**
BO/NC	**0.012**	**0.012**	–
BB/NC	0.291	0.88	–

Significant values in bold. $P < 0.05$.

This study has some limitations in terms of the relatively small sample size. Moreover, socket size in rat even though two of them were connected is still small volume that fact together with small amount of xenograft that was placed inside the defect might mask or lower some of the outcomes.

5 | CONLUSIONS

The results of this study demonstrating higher vitality and proliferation of MSCs seeded onto PLCL-coated xenograft (BB) compared with commonly used noncoated xenograft (BO). Furthermore, the greater percentiles of new bone formation with fewer residual scaffolds in the BB group would suggest potentially better regenerative capacity for the PLCL-coated xenografts. Further studies will be necessary to fully understand this mechanism.

ACKNOWLEDGMENT

We would like to thank Prof Samer Srouji, Galilee Medical Center, Nahariya, for kindly providing the bone-marrow-derived human MSCs.

CONFLICT OF INTEREST

The authors declare that they do not have conflict of interest regarding the present study. The study was supported by an educational grant from the Alpha Bio Tec Ltd. The funders had no role in the study design, data collection and analysis; decision to publish; or preparation of the manuscript.

ORCID

Yaniv Mayer DMD http://orcid.org/0000-0001-5500-7961

REFERENCES

[1] Van der Weijden F, Dell'Acqua F, Slot DE. Alveolar bone dimensional changes of post-extraction sockets in humans: a systematic review. *J Clin Periodontol*. 2009;36(12):1048–1058.

[2] 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.

[3] MacBeth N, Trullenque-Eriksson A, Donos N, Mardas N. Hard and soft tissue changes following alveolar ridge preservation: a systematic review. *Clin Oral Implants Res*. 2017;28(8):982–1004.

[4] Iocca O, Farcomeni A, Pardiñas Lopez S, Talib HS. Alveolar ridge preservation after tooth extraction: a Bayesian Network meta-

analysis of grafting materials efficacy on prevention of bone height and width reduction. *J Clin Periodontol*. 2017;44(1):104–114.

[5] Horváth A, Mardas N, Mezzomo LA, Needleman IG, Donos N. Alveolar ridge preservation. A systematic review. *Clin Oral Investig*. 2013;17(2):341–363.

[6] 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(10):950–958.

[7] Scheyer ET, Heard R, Janakievski J, et al. A randomized, controlled, multicentre clinical trial of post-extraction alveolar ridge preservation. *J Clin Periodontol*. 2016;43(12):1188–1199.

[8] Araújo MG, Liljenberg B, Lindhe J. Dynamics of Bio-Oss collagen incorporation in fresh extraction wounds: an experimental study in the dog. *Clin Oral Implants Res*. 2010;21(1):55–64.

[9] Lindhe J, Cecchinato D, Donati M, Tomasi C, Liljenberg B. Ridge preservation with the use of deproteinized bovine bone mineral. *Clin Oral Implants Res*. 2014;25(7):786–790.

[10] Pertici G, Carinci F, Carusi G, Epistatus D, Villa T, Crivelli F, Rossi F, Perale G. Composite polymer-coated mineral scaffolds for bone regeneration: from material characterization to human studies. *J Biol Regul Homeost Agents*. 2015;29(3 suppl 1):136–148.

[11] Fridenshteĭn A. I A, Piatetskiĭ-Shapiro II, Petrakova KV. Osteogenesis in transplants of bone marrow cells. *Arkh Anat Gistol Embriol*. 1969;56(3):3–11.

[12] Petite H, Viateau V, Bensaïd W, et al. Tissue-engineered bone regeneration. *Nat Biotechnol*. 2000;18(9):959–963.

[13] Bruder SP, Kraus KH, Goldberg VM, Kadiyala S. The effect of implants loaded with autologous mesenchymal stem cells on the healing of canine segmental bone defects. *J Bone Joint Surg Am*. 1998;80(7):985–996.

[14] Costa-Pinto AR, Correlo VM, Sol PC, et al. Chitosan-poly(butylene succinate) scaffolds and human bone marrow stromal cells induce bone repair in a mouse calvaria model. *J Tissue Eng Regen Med*. 2012;6(1):21–28.

[15] Mayer Y, Zigdon-Giladi H, Machtei EE. Ridge preservation using composite alloplastic materials: a randomized control clinical and histological study in humans. *Clin Implant Dent Relat Res*. 2016;18(6):1163–1170.

[16] Hämmerle CH, Araújo MG, Simion M, Osteology Consensus Group 2011. Evidence-based knowledge on the biology and treatment of extraction sockets. *Clin Oral Implants Res*. 2012;23(suppl 5):80–82.

[17] Tabatabaei FS, Samadi R, Tatari S. Surface characteristics of three commercially available grafts and adhesion of stem cells to these grafts. *Biomed Mater Eng*. 2017;28(6):621–631.

[18] Meng S, Zhang X, Xu M, et al. Effects of deer age on the physicochemical properties of deproteinized antler cancellous bone: an approach to optimize osteoconductivity of bone graft. *Biomed Mater*. 2015;10(3):035006.

[19] Zigdon-Giladi H, Rudich U, Michaeli Geller G, Evron A. Recent advances in bone regeneration using adult stem cells. *World J Stem Cells*. 2015;7(3):630–640.

[20] Yao W, Guan M, Jia J, et al. Reversing bone loss by directing mesenchymal stem cells to bone. *Stem Cells*. 2013;31(9):2003–2014.

[21] Lin T, Pajarinen J, Nabeshima A, et al. Preconditioning of murine mesenchymal stem cells synergistically enhanced immunomodulation and osteogenesis. *Stem Cell Res Ther*. 2017;8(1):277.

[22] Kuchler U, Keibl C, Fügl A, et al. Dimethyloxalylglycine lyophilized onto bone substitutes increase vessel area in rat calvarial defects. *Clin Oral Implants Res*. 2015;26(5):485–491.

[23] Araújo MG, Lindhe J. Ridge preservation with the use of Bio-Oss collagen: a 6-month study in the dog. *Clin Oral Implants Res*. 2009;20(5):433–440.

[24] Carlier A, Geris L, van Gastel N, Carmeliet G, Van Oosterwyck H. Oxygen as a critical determinant of bone fracture healing—a multiscale model. *J Theor Biol*. 2015;365:247–264.

[25] Plonka AB, Sheridan RA, Wang HL. Flap designs for flap advancement during implant therapy: a systematic review. *Implant Dent*. 2017;26(1):145–152.

[26] Hudson DC, Sweeney WT. Temperatures developed in rotating dental cutting instruments. *J Am Dent Assoc*. 1954;48(2):127–133.

[27] Eriksson RA, Albrektsson T. The effect of heat on bone regeneration: an experimental study in the rabbit using the bone growth chamber. *J Oral Maxillofac Surg*. 1984;42(11):705–711.

[28] Li S, Chien S, Brånemark PI. Heat shock-induced necrosis and apoptosis in osteoblasts. *J Orthop Res*. 1999;17(6):891–899.

[29] Girard B, Baker G, Mock D. Foreign body granuloma following placement of hard tissue replacement material: a case report. *J Periodontol*. 2000;71(3):517–520.

How to cite this article: Mayer Y, Ginesin O, Khutaba A, Machtei EE, Zigdon Giladi H. Biocompatibility and osteoconductivity of PLCL coated and noncoated xenografts: An in vitro and preclinical trial. *Clin Implant Dent Relat Res*. 2018;20:294–299. https://doi.org/10.1111/cid.12596