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Original Article

Effectiveness of buccal pouch grafting in minimizing loss of alveolar dimension: A canine investigation

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ABSTRACT

Background: The study's purpose was to study buccal pouch grafting (BPG) with xenograft, freeze-dried bone allograft (FDBA), or FDBA + decalcified FDBA (DFDBA) on alveolar ridge width preservation and overlying soft tissue thickness at dog premolar extraction sites.

Materials and Methods: In this animal study, 4 dogs had their mandibular first premolar (P1) and distal roots of P2, P3, and P4 extracted (after endodontic treatment of the mesial roots) bilaterally. A small buccal pouch was created at each extraction socket and four treatments tested: nothing, xenograft, FDBA, or FDBA + DFDBA. Casts made pretreatment and at 1 and 3 months after treatment allowed measurements of buccolingual alveolar ridge width (BLRW), while overlying buccal soft tissue thicknesses were measured clinically. Data were assessed using analysis of variance to compare changes in soft tissue thickness and BLRW between times and treatments. Tukey–Kramer adjustment for multiple comparisons was applied for doing *post hoc*, pairwise comparisons. Results were considered significant if $P < 0.05$.

Results: Control sites showed significant ($P = 0.0067$) decreases in soft tissue thickness over time while there was a trend for increased soft tissue thickness at all grafted sites. There were significant losses in BLRW over time for control ($P = 0.0032$) and FDBA groups ($P = 0.015$) with a trend for loss with FDBA + DFDBA. Pairwise comparison using Tukey–Kramer adjustment revealed significant increases in BLRW from T1 to T3 for the xenograft group relative to all the others.

Conclusion: BPG using xenograft is effective in maintaining hard and soft tissue stability following tooth extraction.

Key Words: Allografts, osteogenesis, soft tissue

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INTRODUCTION

It is well documented that significant alveolar ridge shrinkage occurs shortly after tooth extraction.[1] As much as 50% of ridge width and a variable amount of ridge height can disappear within the first 12 months[2] which is most prominent midbuccally.[3] These changes in alveolar ridge anatomy can make restoring the affected sites challenging, especially if the patient wishes to have a dental implant-supported prosthesis. Various approaches have been developed

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to allow implant placement in edentulous sites with suboptimal ridge anatomy including guided bone regeneration grafting,[4] block grafting,[5] and ridge splinting.[6] However, the simplest approach is to prevent the ridge shrinkage from happening by employing socket preservation grafting (SPG) at the time of tooth extraction.[7,8] Many experimental and clinical investigations have been done to better understand the ideal conditions and materials for SPG, and the current consensus is that a mineralized, slowly resorbing particulate graft such as Bio-Oss®(Geistlich Biomaterials, Princeton, NJ, USA) or mineralized allograft covered with a barrier works best.[9] Using this approach definitely reduces the degree of alveolar ridge shrinkage. However, particulate graft materials placed into extraction sockets may impair normal bone healing as the mineralized graft particles will remain indefinitely.[10,11]

One promising way to avoid using particulate grafting may be to prepare autogenous, platelet-rich fibrin clots from the patient's peripheral blood and use these as the socket graft materials.[12] This makes good biologic sense but introduces the technical challenges of drawing and handling the patient's blood, and the added costs of purchasing expensive equipment were needed to prepare the clots. A simpler and innovative alternative to SPG for sites with intact buccal plate was proposed by Caiazzo *et al*. and Brugnami and Caiazzo termed it as "buccal plate preservation" (BPP).[13,14] Instead of placing particulate graft in the actual socket, these clinicians suggested placing it buccally in a small subperiosteal pouch. We found this idea intriguing and therefore undertook the present animal investigation.

MATERIALS AND METHODS

In this animal study (committee number 23810201922031), four (6–12 months of age) mongrel dogs (10–11.5 kg body weight) in good general health were enrolled in this study. All provisions of the Declaration of Helsinki on care and treatment of laboratory animals in research were respected. The dogs were kept in individual cages at the Torabinejad Research Center, Isfahan Medical School. Following a 10-day acclimatization quarantine for veterinary care and necessary vaccinations, thorough supragingival scaling was performed and preliminary dental arch alginate impressions were taken with the animals anesthetized using acepromazine 1% and ketamine 10 mg/kg (Alcomed B. V., Netherlands). The impressions were then used to make casts and custom trays for polyvinyl silicone (Happi-Den, South Korea) impressions and pouring of stone casts.

All mandibular premolar tooth sites were used in the experiment. Premolar 1 (P1) and the distal roots of premolars 2, 3, and 4 provided the necessary treatment sites. Transparent stents were made on the casts to assist with data collection preoperatively and again at 1 and 3 months. Small openings in the stents were made on the buccal aspect of each intended treatment site and 5 mm from the gingival crest. This allowed access for a standard Williams' periodontal probe to record soft tissue thickness with the aid of rubber markers [Figure 1]. Afterward, a digital slide caliper with precision 0.01 mm was used to determine the depth of probe penetration (i.e., probe tip to yellow rubber marker). The buccolingual alveolar ridge widths (BLRWs) at each treatment site were measured on casts using the same digital slide caliper.

All surgical procedures were performed under general anesthesia (0.04 mg/kg atropine subcutaneously; 0.02 cc/kg of acepromazine 1% and 15 mg/kg of ketamine; halothane intubation). Small localized injections of lidocaine with epinephrine (1:80,000) were used to reduce bleeding during the procedures. Following intrasulcular incisions, P1 was atraumatically extracted. Then, the crowns of P2, P3, and P4 bilaterally were sectioned [Figure 2] and root canal treatments were done for their mesial roots. Afterward, the distal roots were removed atraumatically [Figure 3].

Small pouches were then created on the buccal aspect of each treatment site (P1 and distal sockets

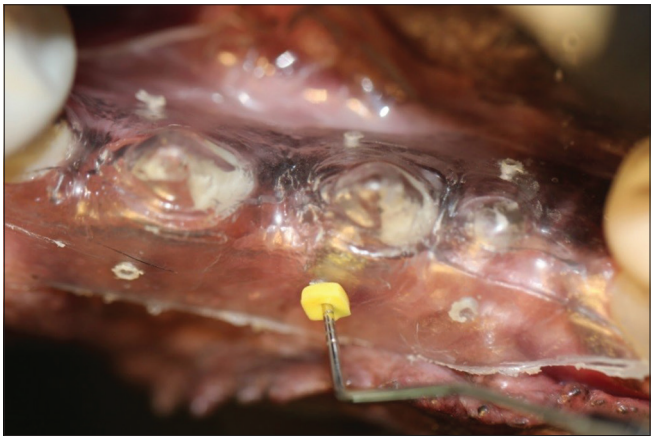

Figure 1: Gingival tissue thickness at all treatment sites was measured with a periodontal probe and rubber endodontic file markers.

of P2, P3, and P4) using a blunt periosteal elevator to raise the full-thickness mucoperiosteum taking care to leave the periosteum as intact as possible [Figure 4]. Each of the 8 pouches in each dog mandible was randomly selected for a particular intervention. The control pouch received no further treatment, while the 3 remaining pouches in each quadrant randomly received one of the following materials as a graft: xenograft (Bio-Oss®, Geistlich Biomaterials, Princeton, NJ, USA), freeze-dried human allograft (FDBA) (Tissue Regeneration Corp., Kish Island, Iran), or FDBA + demineralized human bone allograft (DFDBA) (Tissue Regeneration Corp., Kish Island, Iran). No graft material was placed in the actual root sockets. Finally, interrupted sutures (4/0 Vicryl, Ethicon Corp.) were placed both mesially and distally at each site to close the pouches. Immediately following surgery, the animals received ampicillin 20 mg/kg for 7 days every 8 h and metronidazole 20 mg/kg every 12 h, both drugs being given orally. A soft diet was provided, and after each meal, for the first 2 weeks, all sites were gently wiped using 0.12% chlorhexidine-soaked gauze. Remaining sutures were removed at 2 weeks.

The soft tissue thickness measurements were repeated at 1 and 3 months postsurgery, again under general anesthesia. Mandibular impressions and stone casts also were made at these times to detect any changes in buccolingual ridge width. After the 3-month record collection session, the animals were terminated using the American Veterinary Medical Association protocol (AVMA Guidelines for the Euthanasia of Animals: 2013 Edition; ISBN 978-1-882691-21-0).

Retrieved tissue blocks were fixed for 10 days in 10% formalin and then demineralized for 20 days in 10% nitric acid. The demineralized tissue blocks were then coded for later blind assessment and embedded in paraffin to prepare 5 μm thick histological sections from the central area of each treatment site. Staining was with hematoxylin and eosin. Three sections from each block were examined blindly under light microscopy (Nikon #400, Japan) at 40 times magnification by a pathologist. Digital micrographs of each section were assessed for the percentages of new bone versus residual graft particles and for soft tissue thickness using computer software IHMM (Ver, Sbmus, Iran). The notice was also taken of the presence of inflammation and of any foreign body reaction.

Figure 2: Teeth P2, P3 and P4 were sectioned through their furcations.

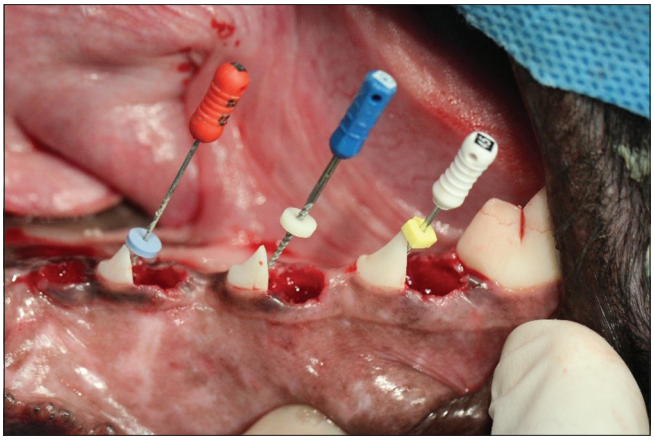

Figure 3: Endodontic treatments for the mesial roots of teeth P2, P3, and P4 were performed followed by removal of their distal roots.

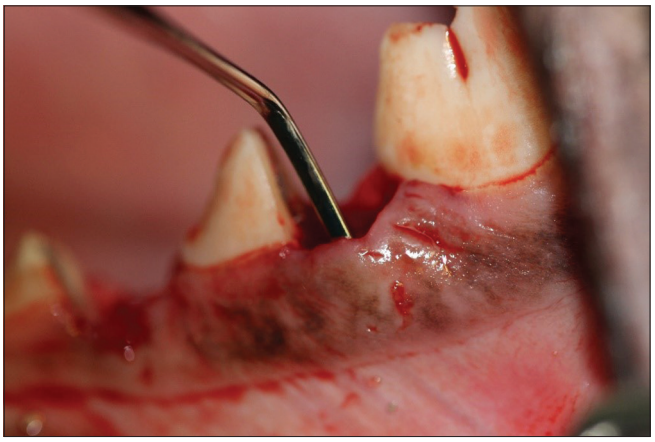

Figure 4: A small periosteal elevator was used to create a pouch under the mucoperiosteum on the buccal aspect of each root extraction socket.

The categorical variables group and position were summarized with counts and percentages, while the continuous variables soft tissue thickness and

buccolingual ridge width were summarized with means, standard deviations, medians, and/or ranges as appropriate. Kolmogorov–Smirnov normality tests were evaluated per group to check for normality assumption. As there were repeated measures over time and different sites in each dog, mixed model analysis was used to accommodate the collinearity within measurements of dogs. Analysis of variance (ANOVA) was used to compare changes in soft tissue thickness and buccolingual width between times and between treatment modalities. Tukey–Kramer adjustment for multiple comparisons was applied for doing *post hoc*, pairwise comparisons for variables that were found to be significant to determine the pairs that contributed to the differences. Results were considered significant if $P < 0.05$. Statistical analyses were performed using version 9.4 of the SAS System for Windows (Copyright© 2002–2010 SAS Institute, Inc., Cary, NC, USA).

RESULTS

Kolmogorov–Smirnov testing confirmed normal distributions for both the clinical soft tissue thickness and buccolingual ridge width measurements in all groups and at the three-time intervals. The measured changes in soft tissue thickness are shown in Table 1. For control sites, there were statistically significant ($P = 0.0067$) decreases in soft tissue thickness over time. It was confirmed with further ad-hoc test that there was a significant difference (soft tissue shrinkage) between time T1 (just before surgery) and T3 (3 months after surgery) (Adj $P = 0.0051$). In contrast, there was a trend for increased soft tissue thickness in all grafted sites. ANOVA testing showed statistically significant changes in soft tissue thickness between different groups from T1 to T3 ($P = 0.0106$). Further ad-hoc testing revealed significant differences between T1 and T3 between the control group and each of the others: xenograft (adj $P = 0.0260$); FDBA (adj $P = 0.0266$); and FDBA + DFDA (adj $P = 0.0284$).

Table 2 displays the data for changes in BLRW. Mixed model analysis showed a statistically significant reduction in buccolingual ridge width over time for the control ($P = 0.0032$) and FDBA groups ($P = 0.015$). There was also a trend for reduction of ridge width with the FDBA + DFDBA group, but this did not reach statistical significance. The xenograft group showed a trend for increased buccolingual width, but again the change was not statistically significant ($P = 0.2867$).

Further ad-hoc testing indicated significant losses in BLRW between times T1 (just before surgery) and T2 (1 month after surgery) for both the control (adj $P = 0.0028$) and FDBA (adjp = 0.0133). Significant changes were also seen between times T1 and T3 (3 months after surgery) for control (adj $P = 0.0094$) and between times T2 and T3for FDBA (adj $P = 0.0404$). ANOVA testing showed statistically significant changes in BLRW between the 4 different groups from T1 to T3 ($P = 0.003$). Pairwise comparison with Tukey–Kramer adjustment further showed that there was a significantly less reduction in BLRW from T1 to T3 for the xenograft group relative to all the others: (i) FDBA, (adj $P = 0.0058$); (ii) FDBA + DFDA, (adj $P = 0.032$); and (iii) control, (adj $P = 0.0072$).

The mean measurements following blind assessment of the histological sections are displayed in Table 3. All three graft materials resulted in recognizable new

Table 1: Analysis of soft tissue thickness (mm)

Group	Time	*n*	Mean	SD	Median	Minimum	Maximum	*P*
Control	T1	8	1.65	0.65	1.53	0.77	2.57	0.0067
	T2	8	1.74	0.86	1.40	1.00	3.42	
	T3	8	1.27	0.44	1.29	0.69	1.80	
FDBA	T1	8	1.80	0.38	1.89	1.27	2.28	0.0784
	T2	8	2.41	0.72	2.26	1.23	3.30	
	T3	8	2.21	0.28	2.16	1.88	2.72	
FDBA + DFDA	T1	8	1.45	0.33	1.38	0.99	1.93	0.0734
	T2	8	2.26	0.85	2.12	1.27	3.39	
	T3	8	1.86	0.51	1.81	1.31	2.73	
Xenograft	T1	8	1.34	0.33	1.29	0.96	1.93	0.1649
	T2	8	2.22	1.02	2.21	0.95	3.65	
	T3	8	1.76	0.43	1.78	0.91	2.32	

T1: Pretreatment; T2: 1-month posttreatment; T3: 3-month posttreatment. SD: Standard deviation; FDBA: Freeze-dried bone allograft; DFDBA: Decalcified FDBA

Table 2: Changes in buccolingual alveolar ridge width (mm) with time for the 4 experimental groups

Group	Time	*n*	Mean	SD	Median	Minimum	Maximum	*P*
Control	T1	8	8.16	1.16	8.10	6.85	9.80	0.0032
	T2	8	7.15	1.19	7.05	5.60	8.85	
	T3	8	6.99	1.35	7.58	4.50	8.30	
FDBA	T1	8	8.41	0.93	8.45	6.95	9.70	0.0150
	T2	8	7.96	1.31	7.88	5.90	10.00	
	T3	8	7.20	1.05	6.78	6.05	8.95	
FDBA + DFDA	T1	8	8.08	1.07	8.18	6.15	9.50	0.0956
	T2	8	7.69	1.27	7.50	5.90	9.60	
	T3	8	7.18	0.98	7.33	5.25	8.45	
Xenograft	T1	8	8.27	1.09	8.28	6.70	10.00	0.2867
	T2	8	8.61	0.89	8.53	7.00	10.00	
	T3	8	8.68	0.96	8.80	6.95	10.00	

T1: Pretreatment; T2: 1-month posttreatment; T3: 3-month posttreatment. SD: Standard deviation; FDBA: Freeze-dried bone allograft; DFDBA: Decalcified FDBA

Table 3: Measurements obtained from assessment of the micrographs using computer software

Graft material	Mean percentage new bone (%)	Mean percentage remaining graft particles (%)	Mean soft tissue thickness (mm)
None			0.16
FDBA	26.5	22.0	0.82
FDBA + DFDBA	17.4	20.8	0.61
Xenograft	30.0	24.9	1.20

FDBA: Freeze-dried bone allograft; DFDBA: Decalcified FDBA

bone formation in the implanted buccal pouches. While there was insufficient data available to conduct a statistical analysis of the results, the xenograft appeared to promote the greatest amount of new bone.

DISCUSSION

SPG at the time of tooth extraction has been shown repeatedly to be effective in reducing but not eliminating shrinkage in alveolar ridge width during tooth extraction site healing.[7,8] There are some limitations; however, the principal one being that the added graft particles, which need to be slowly or nonresorbing for best results,[10] may interfere with normal bone healing and result in a healed socket with reduced vital bone and substantial retained graft material.[15] Demineralized bone allograft with a collagen barrier was earlier shown to be ineffective with SPG.[16] The subperiosteal grafting procedure (BPG) investigated here may be a preferred alternative for SPG at least in situations where the buccal bone wall remains intact following tooth removal. Brugnami and Caiazzo[14] reported a human case series using this approach with particulate Bio-Oss® as the graft in comparison to unfettered socket healing. No barrier materials were used to isolate the graft particles from the overlying mucoperiosteal flap, as is customary with traditional guided bone regeneration for ridge augmentation.[17] Measurements of ridge width changes for test and control sites at their mesiodistal midpoints were made on study casts by a blinded lab technician. The measurements were made pretreatment, and after 6 weeks, site healing, at which time dental implants were inserted. At test sites, the changes in buccolingual ridge width ranged from a loss of 0.5 mm to a gain of 2 mm. At the control sites, the changes ranged from zero to a loss of 2.5 mm. Just how the xenograft functioned in this application could not be determined. However, it is known from histology obtained from SPG sites treated with xenograft in humans that many of the graft particles become engulfed in fibrous tissue.[18] It could be assumed therefore that this same particle encapsulation contributed to the findings with BPG. Whether this early benefit of the BPG procedure can be maintained for much longer extraction site healing intervals was not reported. However, since socket healing was unfettered, treatment time was shortened with dental implant placement being possible at 6 weeks healing ("earlyplacement")[19] as compared to the usual 6 months healing required before implant placement following SPG. While significant remodeling with crestal bone loss would be expected with unfettered socket healing, BPG with xenograft may have reduced the degree of this resorption. It is known for example that applying a layer of xenograft over autogenous block bone grafts does lessen resorption of the latter.[20] It is interesting

to note, however, that using xenograft to fill buccal gaps following immediate implant placement without also using it as a buccal overgraft (BPG) does not prevent resorption and thinning of buccal bone.[21,22] Thus, for example, Benic *et al*.[22] followed the status of single immediate implants placed in 14 patients using xenograft covered by a collagen membrane to fill residual buccal gaps. No graft material was placed over intact buccal bone. Cross-sectional cone beam computed tomography (CBCT) assessment after 7 years in function showed that 5 of the 14 implants showed virtually no buccal bone remaining. These same implants showed a mean of 1 mm greater soft tissue recession.

Fickl *et al*.[23] studied alveolar ridge dimensional changes in canine premolar extraction sites following four different socket preservation protocols. After endodontic treatment of their mesial roots, the distal roots of the mandibular third and fourth premolar teeth in five dogs were removed bilaterally. The four extraction sites in each animal were then used to assess the effectiveness of four treatments as follows: (i) no treatment other than sutures to reposition the soft tissues; (ii) Bio-Oss Collagen® alone; (iii) Bio-Oss Collagen® placed in the socket and covered with a 3 mm thick free gingival autograft (SP) from the palate; and (iv) Bio-Oss Collagen® covered with a resorbable porcine, cross-linked collagen membrane. Many of the sites treated with this last protocol suffered early infection with pus formation, and therefore, only three of the four treatment protocols could be investigated as originally planned. Impressions and models were obtained pretreatment and at 2 and 4 months after treatment to assess volumetric changes in alveolar ridge dimension using computer-aided software and an optical scanner. Both the Bio-Oss Collagen® alone and when covered with a soft tissue autograft showed less loss in alveolar ridge buccal volume than seen at the control sites. Neither of the graft protocols led to complete preservation or an increase of the pretreatment ridge contours leading the investigators to suggest that buccal overgrafting with xenograft might be added to either procedure. Such buccal overgrafting was subsequently shown by others to be a benefit with human alveolar ridge width preservation using SPG.[24] However, adding a dense polytetrafluorethylene barrier over the BPG site isolating it from the overlying soft tissue and periosteum appeared to have a negative impact.[25] Fickl *et al*.[26] later conducted another canine project in which one of their SPG protocols was to place Bio-Oss Collagen® in the socket in combination with a combined free gingival/connective tissue autograft with the computed tomography portion placed under the buccal flap as tissue volume augmentation. This treatment also failed to preserve the pretreatment alveolar ridge buccal volume after 4 months site healing.

In the present animal investigation, BPG with particulate Bio-Oss® alone was the most effective of the four protocols tested for their ability to minimize loss of buccal alveolar ridge volume. Measurements of alveolar ridge width and the thickness of overlying keratinized soft tissues were obtained before and then 1 and 3 months after BPG. Control sites were sham operated, while the three test site groups received Bio-Oss® alone, human FDBA alone, or a combination of FDBA + DFDBA (both human tissue). Both the control and FDBA sites showed significant losses in BLRW, while the combination FDBA + DFDBA sites showed a nonsignificant trend to lose width. In contrast, the xenograft (Bio-Oss®)-treated sites showed a trend for increased ridge width. Further testing using Tukey–Kramer adjustment showed that using xenograft alone led to significant increases from baseline to 3 months compared to the other three treatment groups. In the control and two treatment groups that lost ridge width, at least part of the loss would have been due to bone resorption precipitated by raising the periosteum[27] to create the buccal pouches. Flap elevation in the xenograft group, however, clearly had less impact, perhaps as already mentioned, because of a protective effect by the xenograft particles.[20]

Measurable clinical changes in keratinized soft tissue thickness also were detected in the present experiment. The sham-operated sites showed a significant decrease in soft tissue thickness from baseline to 3-month postextraction. In contrast, there were significant increases in soft tissue thickness with time in all BPG-grafted sites compared to the controls, likely at least partially due to a localized fibrotic reaction to the graft materials. The greatest increase in soft tissue thickness was seen in the xenograft group. This thickening clearly could be an advantage in obtaining and maintaining peri-implant soft tissue profiles and reducing the risk of unesthetic soft tissue recession resulting in unwanted exposure of implant surfaces.[28]

The xenograft group appeared to have the greatest amount of new bone formation in the examined

histological sections although sufficient data were not available for statistical assessment. Thus, the positive impact of xenograft on ridge width changes appears to have been due to a combination of new bone formation and increased soft tissue thickness. New bone was readily seen around many of the xenograft particles despite the fact that the graft particles had not been isolated from the overlying soft tissues with a barrier material. This bone most likely was formed by osteoprogenitor cells in the existing buccal bone wall migrating into the xenograft deposits, based on the findings of others. For example, Abbas[29] studied the osteoconductive properties of Bio-Oss® particulate onlay grafts applied to rabbit mandible and isolated by resorbable domes. He reported that new bone was formed within the grafted area as an outgrowth from the underlying mandibular bone. New bone formation also was seen in the other two BPG treatment groups in the present experiment but was not sufficient to prevent an overall loss in alveolar ridge width. Like Bio-Oss®, FDBA has primarily osteoconductive properties, while DFDBA depending on how it is processed often has osteoinductive properties as well. However, some resorption of DFDBA is needed to release bone-stimulating proteins from the graft particles, and this may somehow have complicated the healing process when used along with the FDBA for BPG. In any event, the present findings support the human clinical findings of Caiazzo *et al*.[13] that BPG with xenograft helps to minimize loss of buccal alveolar ridge volume following tooth extraction. Since normal internal postextraction socket healing will not be inhibited by the procedure, placement of dental implants can be done earlier than if the sockets had been preserved internally as is the more common approach. BPG with xenograft also has been shown to help to retain or enhance buccal alveolar ridge contours when used at the time of immediate implant installation[30] or as an esthetic graft on the buccal aspect of implants to compensate for irregular ridge anatomy.[31] Since xenograft is basically nonresorbable, the impact on alveolar anatomy is likely to be maintained over time offering the benefits of a stable buccal bone and soft tissue profile. As well, the apparent lack of need for a barrier membrane with the xenograft helps to decrease treatment expense and difficulty. Others[32] have recently reported that the placement of xenograft onto the surface of alveolar bone through a minimally invasive buccal tunneling procedure led to substantial new bone formation. Their technique did not include the use of barriers, space-maintaining devices, or bone decortication but did include the addition of the synthetic growth factor rhPDGF-BB. This finding suggests that "sticky bone,"[33] i.e., the mixing of xenograft with platelet-rich plasma, rather that xenograft alone may produce superior results with BPP than achieved in the present study. We look forward to further investigations of the BPP/xenograft procedure using advanced imaging techniques such as CBCT[34,35] to document in greater detail this simple and effective treatment.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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