Corticocancellous Porcine Bone in the Healing of Human Extraction Sockets: Combining Histomorphometry with Osteoblast Gene Expression Profiles In Vivo

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Purpose: *Different graft materials have been proposed to minimize the collapse of alveolar bone after tooth extraction. The aim of this study was to examine the use of porcine bone graft in fresh sockets via histomorphometric and in vivo gene expression profiling.* ***Materials and Methods:*** *Thirty fresh extraction sockets with three bone walls in 15 patients were selected. A split-mouth design was employed. On one side of the arch, 15 sockets received corticocancellous porcine bone as a graft, and on the other side, 15 sockets were left unfilled and considered as controls. Four months after surgery, four biopsy specimens were taken from each patient (two from the grafted site and two from the control site). The specimens were analyzed by histomorphometry and ex vivo osteoblast expansion, followed by highly sensitive osteoblast-specific gene expression profiling for Runx2, osteopontin, osteoprotegerin, type I collagen, and alkaline phosphatase by quantitative real-time reverse-transcriptase polymerase chain reaction. Comparisons were made using the Student* t *test.* ***Results:*** *After healing without complications, the grafted sites showed statistically significantly higher mean vital bone and lower mean connective tissue values than the control sites. Statistically significant higher expression of alkaline phosphatase and the matrix formation markers type I collagen and osteopontin were observed in the grafted group compared to the control group, whereas Runx2 and osteoprotegerin expression was comparable.* ***Conclusions:*** *Within the limits of this study, histologic examination and biomolecular evaluation confirmed good biocompatibility and high osteoconductivity of xenogeneic porcine bone in alveolar bone grafting.*
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Several studies have reported significant structural changes and bone resorption in sockets following extraction procedures, with important dimensional changes in the surrounding alveolar bone taking place.[1–3] The healing process of an extraction socket includes the formation of a coagulum that is substituted by connective tissue matrix; after 1 month, the bundle bone is reabsorbed and replaced with woven bone, resulting in reduction of the height of the alveolar crest. This remodeling pattern causes horizontal resorption that may induce additional vertical reduction of the buccal bone.[4,5]

To preserve alveolar bone, several biomaterials have been used immediately following tooth extraction to maintain alveolar bone volume.[6–11] Because of their excellent biocompatibility and bioactivity, hydroxyapatite (HA) ceramics are widely used in bone grafting and dental devices as bone substitutes,[12–14] since HA ceramics have the ability to induce mesenchymal cells to differentiate into osteoblasts, giving HA potential as a scaffolding material for bone tissue engineering.[15,16] Furthermore, a new xenogeneic bone substitute that consists of corticocancellous porcine bone (PB) in the form of particles with a high porosity and a diameter ranging from 600 to 1,000 μm has been used successfully in maxillary sinus augmentation.[17] Histologic and ultrastructural analysis showed that residual graft particles were surrounded by newly formed bone; under transmission electron microscopy, all phases of bone formation were observed in proximity with the corticocancellous PB particles.[18]

In vitro studies have investigated the markers of differentiation of mesenchymal progenitor cells exposed to different biomaterials as bone substitutes.[19–22] The in vitro data have prompted an in vivo investigation of osteoblast differentiation and activity in bone grafting. In particular, analysis of in vivo gene expression could provide valuable information not only about the bone-forming capacity of bone substitutes, but also regarding the osteoblastic potential of regulating osteoclast differentiation and activity, providing a more comprehensive assessment of the functional status of regenerated bone. To date, clinical and histomorphometric parameters have served as the standard parameters to define grafting success, but whether appropriate bone-forming activity can be assessed in osteoblasts from the newly formed bone is unknown.

The aim of this study was to analyze the use of PB grafts in fresh sockets after tooth extraction via histomorphometric analysis and in vivo gene expression profiling.

MATERIALS AND METHODS

Patient Selection

Between September 2009 and February 2010, patients requiring the extraction of two teeth—one on each side of the arch in the molar or premolar regions—were included in this prospective split-mouth study. The inclusion criteria for the sockets were the presence of three bone walls and loss of the buccal plate. All patients needed to be nonsmokers in good health and have no chronic systemic disease. Exclusion criteria were coagulation disorders, signs of acute infection around alveolar bone at the surgical site, and alcohol or drug abuse.

The local ethical committee approved the study, and all patients provided written informed consent. Diagnoses were made clinically and radiographically. The patients were treated by one oral surgeon (RC) at the Department of Dentistry, San Raffaele Hospital, Milan, Italy.

Surgical Protocol

The patients received amoxicillin (1 g) 1 hour prior to surgery and twice a day for a week after the surgical procedure. Surgery was performed under local anesthesia (optocaine, Molteni Dental; 20 mg/mL with adrenaline 1:80,000). The teeth were extracted, and a periodontal probe (Hu-Friedy PGF-GFS, Hu-Friedy) was used to verify the wall assessment of the fresh sockets, which were then debrided before receiving the graft material.

Split-mouth treatment was performed. Half the sockets (randomly selected in one side of the arch) received xenogeneic corticocancellous porcine bone (Tecnoss) (PB group) and the contralateral sockets were left unfilled (control group). The graft material was hydrated with sterile physiologic sodium chloride solution (0.9%, Fresenius-Kabi) for 3 minutes prior to insertion in the sockets and then was packed into the alveolus. A collagen sheet (Condress, Abiogen Pharma) was used to cover the inner denuded biomaterial. The collagen was placed under the detached palatal tissue and secured with silk sutures. A collagen sheet was also placed over the control sites.

Four months after surgery, four cylindric specimens were taken from each patient (two from the PB-grafted site and two from the control site). From each site, one sample was processed for osteoblast expansion and in vivo gene expression analysis, and the other was prepared for histomorphometry. All biopsy specimens were approximately 2 mm in diameter and 7 mm in length and were marked on the occlusal side for orientation during histologic processing. Titanium dental implants (Sweden-Martina), 5.0 mm wide and 10 to 13 mm long, were placed in the biopsy specimen retrieval sites.

Histology and Histomorphometry

The samples were fixed in 4% buffered formaldehyde, dehydrated in a graded series of alcohols (50% to 100%), and embedded in epoxy resin (Epon, Polysciences) according to a previously described procedure.[23] Undecalcified, 30- ± 10-μm-thick sections along the axis of the biopsy were obtained with an Isomet Buehler microtome (Buehler); the sections were stained with toluidine blue and observed with Normasky differential interference contrast microscopy (Fomi III, Carl Zeiss).

The same microscope was connected to a computer and a digital camera (Leica DC 280, Leica Microsystem)

for histomorphometric measurements. Measurements were performed with software (Microcontrol). The percentage of mineralized tissue was calculated in all sections of the same sample, and the measurements were performed at a magnification of ×63. Values reported were percent vital bone (VB), percent connective tissue (CT), and percent graft material (GM). VB was calculated as the amount of mineralized and vascularized bone tissue as a percentage of the total tissue volume (TV). The values of CT and residual GM found in the sample were calculated as percentages of TV. The total amount of VB, CT, and GM corresponded to TV.

Cultures of Primary Osteoblasts

Primary osteoblast cultures were obtained from human bone biopsies by smashing bone chips into smaller pieces in ceramic mortars under sterile conditions. Bone fragments were then digested in Dulbecco minimum essential medium (DMEM) Jocklik medium with 2 mg/mL collagenase IV at 37°C for 2 hours; resuspended in DMEM Iscove with 10% fetal bovine serum supplemented with penicillin and streptomycin (each 100 U/mL), gentamicin (100 U/mL), and amphotericin (50 IU/mL); and incubated at 37°C in a 5% CO_2 atmosphere in tissue flasks. Fresh culture medium was replaced every 3 days.

RNA Preparation and Reverse-Transcriptase Polymerase Chain Reaction

Total RNA was extracted from primary osteoblasts with reagent (TRIZOL, Invitrogen), according to manufacturer's instructions. Two micrograms of RNA were used for reverse transcription (SuperScript First-Strand Synthesis System, Invitrogen) according to the manufacturer's instructions, using oligo(dT) to prime first-strand synthesis in a total volume of 20 µL. The product of each reverse-transcriptase reaction was diluted 1:5 and 1 µL of reactant was used for real-time polymerase chain reactions with 500 nmol/L of each of the following pair of specific primers:

- Type I collagen (col I). FW: 5′-AAC CTG GTG CTA AAG GCG AAC C-3′; RW: 5′-CAG CAC CAG GGA AAC CAG TAG C-3′; annealing temperature (AT) = 61°C.
- Runx2. FW: 5′-GAG GTA CCA GAT GGG ACT GTG G-3′; RW: 5′-GGA ATG CGC CCT AAA TCA CTG A-3′; AT = 61°C.
- Osteoprotegerin (OPG). FW: 5′-TGG CAC CAA AGT AAA CGC AGA GAG-3′; RW: 5′-GGT CAC TGG GTT TGC ATG CCT TTA-3′; AT = 67°C.
- Osteopontin (OPN). FW: 5′-TGG CCG AGG TGA TAG TGT GGT TTA-3′; RW: 5′-TGTG GAA TTC ACG GCT GAC TTT GG-3′; AT = 67°C.
- Alkaline phosphatase (ALP). FW: 5′-TTA ATG CCA CCG ACA TCC CAT CTG-3′; RW: 5′-ACC TCG ATG CTG ATT TCC TCT CCA-3′; AT = 67°C.
- Glyceraldehyde phosphate dehydrogenase (GAPDH). FW: 5′-CGG AGT CAA CGG ATT TGG TCG-3′; RW: 5′-TGG TGA TGG GAT TTC CAT TGA TG-3′; AT = 61°C.

The final 10 µL of reaction mix contained 1× SYBR/green I mix (QuantiTect SYBR Green PCR Kits, QIAGEN), deoxynucleotide triphosphate mix [with deoxyuridine triphosphate instead of deoxythymidine triphosphate]), reaction buffer, and a Taq DNA polymerase (FastStart Taq DNA polymerase, Roche); SYBR green I dye; and magnesium chloride. Amplifications were run in a cycler apparatus (Light Cycler LC480, Roche) in triplicate, using a standard curve for each gene, with the following protocol:

1. Hot start (denaturation): 95°C for 5 minutes;
2. Amplification (three steps): denaturation at 95°C for 10 seconds; annealing at the indicated temperature for 5 seconds; and extension at 72°C for 20 seconds;
3. Melting curve: 95°C for 10 minutes; and
4. Cooling: to 30°C.

Mean cycle threshold (Ct) values for Runx2, OPG, OPN, ALP, and col I transcripts were calculated from all biopsy specimens. For each gene product, the mean crossing-point value (number of cycles at which a preset fluorescence threshold is reached in each sample) was calculated in triplicate (Ct). The exact mRNA concentration was calculated by crossing the Ct value on the standard curve generated from purified amplified cDNA. Average transcript levels of each gene were then normalized by GADPH (housekeeping).

Statistical Analysis

A dedicated software program was used for all statistical analyses (SPSS 11.5.0, SPSS Inc). All values were calculated as means ± standard deviations. For histomorphometry, the values of VB, CT, and GM for each group were represented by the mean percentage of all sections. The significance of differences between PB and control groups in relation to VB, CT, and GM values was determined by a two-tailed Student *t* test ($P < .05$). Statistical power calculations showed that the minimal sample size was 13, with an alpha value of 0.05% and 80% power. For gene expression analysis, transcript levels from osteoblasts derived from PB and control group biopsy specimens were compared. A two-tailed Student *t* test was again adopted, with a P value < .05 considered significant. Statistical power calculations showed that the minimal sample size was 13, with an alpha value of 0.05% and 80% power.

Table 1 No. and Positions of Extracted Teeth (n = 30)

	Right				Left				
Arch	Second molar	First molar	Second premolar	First premolar	First premolar	Second premolar	First molar	Second molar	Total
Maxilla	1	2	2	1	2	3	1	3	15
Mandible	1	3	3	2	2	2	1	1	15

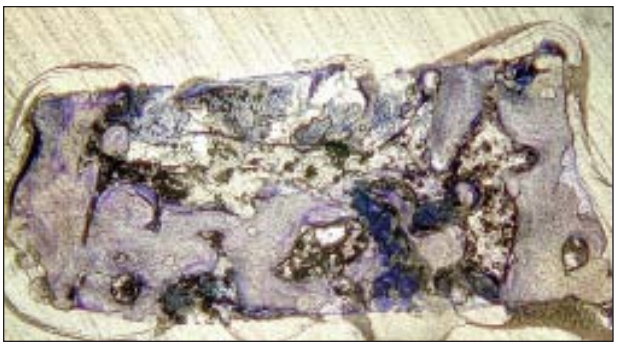

Fig 1a Histologic section from a control site. Trabecular deposition of vital lamellar bone is apparent. Large medullary spaces are present and inflammatory cells are absent (toluidine blue; original magnification ×10).

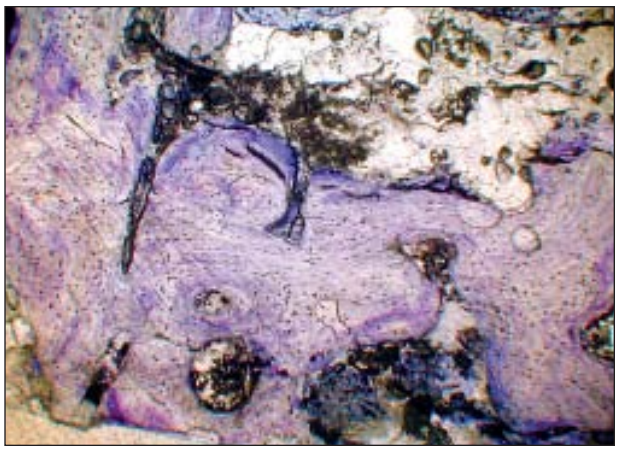

Fig 1b Histologic section from a control site. Lines of osteoblasts are present, with interposed incremental and reversal lines (toluidine blue; original magnification ×63).

RESULTS

Fifteen patients (nine women and six men) with a mean age of 53.7 years (range, 32 to 70 years) were included in this prospective split-mouth study. Thirty teeth were extracted (Table 1). All patients completed the healing period following extraction and grafting procedures without complications and without clinical symptoms of infection.

Qualitative Histologic Analysis

In total, 60 biopsy specimens were obtained (30 from the PB group and 30 from the control group). Control sites showed more shrinkage in the buccolingual dimension and the histologic sections showed less newly formed bone than did the grafted sites (Fig 1). Histologic examination revealed an absence of inflammatory cells, along with bone formation in all grafted sites and the presence of biomaterial particles and connective tissue.

In the PB group the histologic analysis showed that some demineralized bone particles were present; the grafted bone material was easily distinguished from the natural bone by staining (Fig 2). New bone and osteoid were observed surrounding the remaining graft particles, which were incompletely resorbed but well integrated and in complete continuity with the new bone tissue formation. In the medullary space, density of the vascular and connective tissue was present, and no evidence of inflammatory infiltrate or necrosis or foreign-body reaction was observed in any of the specimens.

Histomorphometric Findings

Data from histomorphometric analysis were reported in Table 2. The PB group showed statistically significantly ($P < .05$) higher mean VB values than the control group. The PB group showed statistically significantly ($P < .05$) lower mean CT values than the control group. Residual GM composed a mean of 34.4% ± 5.1% of the PB-grafted sites.

Osteoblast Gene Expression Findings

Osteoblasts derived from PB grafts and control group sites grew comparably and maintained an osteoblast-like morphology. The mRNA expression of the different genes examined (Runx2, OPG, OPN, col I, and ALP) relative to controls, as well as the comparisons between the grafted and control groups, are documented in Fig 3. The PB group showed an increase in OPN, ALP, and col I gene expression that was statistically significantly different ($P < .05$) versus the control group.

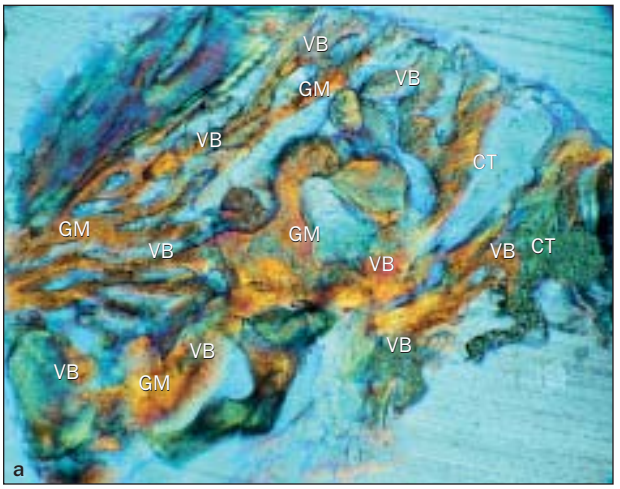

Fig 2a Histologic section from an experimental site. Trabeculae of vital bone (VB) surround the graft material particles (GM). Some connective tissue (CT) is present (differential interference contrast microscopy; original magnification ×10).

Fig 2b Bundles of vital bone (VB) surround the graft material particles (GM) (differential interference contrast microscopy; original magnification ×160).

Fig 2c Osteoblasts (O) can be seen in the secreting phase in contact with PB particles (GM) (differential interference contrast microscopy; original magnification ×400).

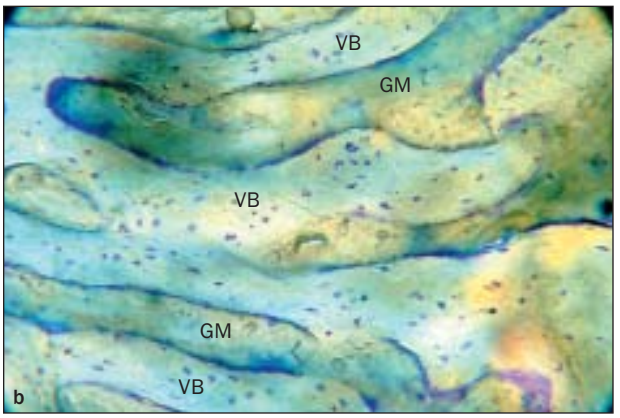

Table 2 Histomorphometric Data (Mean Values ± Standard Deviations) of 30 Sockets Treated in the Study

Group	No. of sockets	VB (%)	CT (%)	RGM (%)
PB graft	15	39.6 ± 9.4	26.0 ± 9.9	34.4 ± 5.1
Control	15	29.5 ± 5.0	57.7 ± 6.9	0

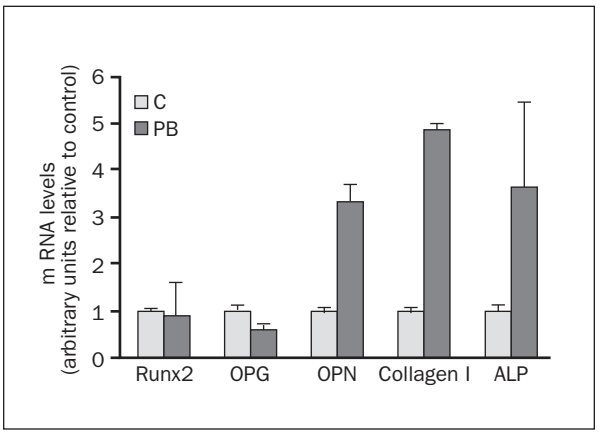

Fig 3 *(Right)* Real-time gene expression profiles. Mean ± SD of mRNA expression of the different genes examined.

DISCUSSION

Graft materials may minimize bone resorption and simultaneously improve the healing process by acting as a scaffold for lamellar bone formation, reducing the time required for healing. Many biomaterials have been used for this purpose, and each has different features and biologic behavior.[21,24,25]

The histologic findings from the present study showed that corticocancellous PB could be used successfully for ridge preservation. The absence of inflammatory signs around the graft particles, the close contact between graft particles, and the newly formed lamellar bone present in the specimens suggested that this biomaterial may be safe and biocompatible.

The efficacy of PB as a bone graft material may be the result of a combination of factors, the foremost of which is its osteoconductive capacity, a property resulting from its chemical and structural features.[26] In particular, the histomorphometric analysis revealed significantly higher percentages of VB in the PB group (39.6% ± 9.4%) compared to the control group (29.5% ± 5.0%). The inclusion of graft particles in bone tissue provided a thick tissue network in which the graft particles were completely surrounded by vital bone. This might indicate that the biomaterials were osteoconductive and acted as a natural scaffold for new bone formation, preventing alveolar resorption. These data were in agreement with those of a previous study[26] in which PB was used in combination with collagen membrane for ridge preservation after tooth extraction. Histologic analysis showed a significantly higher percentage of trabecular bone and total mineralized tissue in ridge preservation sites (35.5% ± 10.4%) 7 months after tooth removal compared to an unfilled control socket (25.7% ± 9.5%).

The percentage of VB obtained in the present study could be explained by the greater bone-forming activity of osteoblasts around grafted PB and may possibly be associated with a lower osteoclastogenic capacity. To examine this possibility, the gene expression profiles of primary osteoblasts expanded from the grafted site were determined. Indeed, a recent study of osteoblast gene expression profiles from magnesium-enriched HA grafts revealed higher expression of certain specific markers of osteoblast differentiation and bone formation. This was associated with a lower osteoclastogenic potential; the graft material attracted circulating biocomponents (bone sialoprotein and OPN) at sites of tissue repair, thus promoting bone regeneration.[27]

In the present study, the expression of a number of genes involved in osteoblast differentiation, bone matrix formation (OPN, OC, col I, and Runx2), and osteoclast differentiation (OPG) was examined. Bone samples from the PB-grafted sites showed an increase in ALP, col I, and OPN expression, ie, they demonstrated a better potential in mRNA encoding for col I, the main component of the mineralized matrix; better metabolic activity (ALP enzymatic activity) to produce mineralized matrix[28]; and a less-differentiated osteoblast phenotype (as shown by the increased OPN expression). OPN is an arginine-glycine-aspartate–containing adhesive glycoprotein[28] that can bind to various extracellular molecules, including col I, fibronectin, and osteocalcin, likely conferring increased biomechanical competence to extracellular matrices.[28] OPG is an osteoblast-secreted factor that acts as a decoy receptor for receptor activator of nuclear factor kappa B ligand (RANKL) and prevents its interaction with its receptor (RANK), thereby decreasing osteoclastogenesis and bone resoption.[29] Runx2 is a crucial integrator of the cell-signaling pathways required for osteoblast differentiation.[30] In the present study, no statistically significant differences were found in OPG and Runx2 levels between the PB and control groups.

Therefore, because the development of the osteoblast phenotype requires transcriptional mechanisms that regulate induction of a concerted and orderly program of gene expression, in the present study PB seems to be associated with a differentiated osteoblast phenotype, promoting mineralized matrix production and maturation as well as cell-matrix interaction. Together, the data are compatible with greater bone-forming activity for the PB group and may explain why similar higher levels of VB were generated in the biomaterial grafts than in control sites. Moreover, the present study strongly suggests that osteoblasts from the PB group produced osteoid matrix around the biomaterial, emphasizing the remarkable role of residual graft material in bone production.

CONCLUSION

Within the limits of this study, histologic examination and biomolecular evaluation confirmed good biocompatibility and high osteoconductivity of xenogeneic porcine bone in grafting fresh extraction sockets. However, further studies are needed to better understand the long-term clinical and biologic outcomes of this biomaterial.

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REFERENCES

1. Araujo MG, Lindhe J. Dimensional ridge alteration following tooth extraction. An experimental study in the dog. J Clin Periodontol 2005;32:212–218.
2. 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.
3. Zubillaga G, Von Hagen S, Simon BI, Deasy MJ. Changes in alveolar bone height and width following post-extraction ridge augmentation using a fixed bioabsorbable membrane and demineralized freeze-dried bone osteoinductive graft. J Periodontol 2003;74:965–975.
4. Amler MH. The time sequence of tissue regeneration in human extraction wounds. Oral Surg Oral Med Oral Pathol 1969;27: 309–318.

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. Sclar AG. Strategies for management of single-tooth extraction sites in aesthetic implant therapy. J Oral Maxillofac Surg 2004;62(suppl 2):90–105.
7. Becker W, Dahlin C, Becker BE, et al. The use of e-PTFE barrier membranes for bone promotion around titanium implants placed into extraction sockets: A prospective multicenter study. Int J Oral Maxillofac Implants 1994;9:31–40.
8. Nevins M, Mellonig J. Enhancement of damaged edentulous ridge to receive dental implants: A combination of allografts and the Gore-Tex membrane. Int J Periodontics Restorative Dent 1992;12:97–111.
9. Cammack GV II, Nevins M, Clem DS III, et al. Histologic evaluation of mineralized and demineralized freeze-dried bone allograft for ridge and sinus augmentations. Int J Periodontics Restorative Dent 2005;25:231–237.
10. Artzi Z, Tal H, Dayan D. Porous bovine bone mineral in healing of human extraction sockets. Part I. Histomorphometric evaluation at 9 months. J Periodontol 2000;71:1015–1023.
11. Benque EP, Gineste M, Heughebaert M. Histological study of the biocompatibility of hydroxyapatite crystals in periodontal surgery. J Biol Buccale 1985;13:271–282.
12. Ripamonti U. The morphogenesis of bone in replicas of porous HA obtained from conversion of calcium carbonate exoskeletons of coral. J Bone Joint Surg 1991;73A:692–703.
13. Zhang XD. A study of porous block HA ceramics and its osteogenesis. In: Ravaglioli AA, Krajewski A (eds). Bioceramics and the Human Body. Amsterdam: Elsevier, 1991:408–415.
14. Yuan H, Li Y, Yang Z, Feng J, Zhang XD. An investigation on the osteoinduction of synthetic porous phase-pure HA ceramics. Biomed Eng Appl Basis Com 1997;9:274–278.
15. Okumura M, Ohgushi H, Dohi Y, et al. Osteoblastic phenotype expression on the surfaceof HA ceramics. J Biomed Mater Res 1997;37:122–129.
16. Norman ME, Elgendy HM, Shors EC, el-Amin SF, Laurencin CT. An in-vitro evaluation of coralline porous HA as a scaffold for osteoblast growth. Clin Mater 1994;17:85–91.
17. Barone A, Crespi R, Aldini NN, et al. Maxillary sinus augmentation: Histologic and histomorphometric analysis. Int J Oral Maxillofac Implants 2005;20:519–525.
18. Orsini G, Scarano A, Piattelli M, et al. Histologic and ultrastructural analysis of regenerated bone in maxillary sinus augmentation using a porcine bone-derived biomaterial. J Periodontol 2006;77:1984–1990.
19. Zreiqat H, Markovic B, Walsh WR, Howlett CR. A novel technique for quantitative detection of mRNA expression in human bone-derived cells cultured on biomaterials. J Biomed Mater Res (Appl Biomater) 1996;3:217–223.
20. Frank O, Heim M, Jakob M, et al. Real-time quantitative RT-PCR analysis of human bone marrow stromal cells during osteogenic differentiation in vitro. J Cell Biochem 2002;85: 737–746.
21. Kubler A, Neugebauer J, Oh JH, Scheer M, Zoller JE. Growth and proliferation of human osteoblasts on different bone graft substitutes: An in vitro study. Implant Dent 2004;13:171–179.
22. Carinci F, Piattelli A, Stabellini G, et al. Calcium sulfate: Analysis of MG63 osteoblast-like cell response by means of a microarray technology. J Biomed Mater Res Part B Appl Biomater 2004;71:260–267.
23. Crespi R, Grossi SG. A method for histological examination of undecalcified teeth. Biotechnic Histochem 1992;67:202–206.
24. Vance GS, Greenwell H, Miller RL, Hill M, Johnston H, Scheetz JP. Comparison of an allograft in an experimental putty carrier and a bovine-derived xenograft used in ridge preservation: A clinical and histologic study in humans. Int J Oral Maxillofac Implants 2004;19:491–497.
25. Froum S, Cho S-C, Rosemberg E, Rohrer M, Tarnow D. Histological comparison of healing extraction sockets implanted with bioactive glass or demineralized freeze-dried bone allograft: A pilot study. J Periodontol 2002;73:94–102.
26. Barone A, Aldini NN, Fini M, Giardino R, Guirado J, Covani U. Xenograft versus extraction alone for ridge preservation after tooth removal: A clinical and histomorphometric study. J Periodontol 2008;79:1370–1377.
27. Crespi R, Mariani E, Benasciutti E, Capparè P, Cenci S, Gherlone E. Magnesium-enriched hydroxyapatite versus autologous bone in maxillary sinus grafting: Combining histomorphometry with osteoblast gene expression profiles in vivo. J Periodontol 2009;80:586–598.
28. Denhardt DT, Noda M. Osteopontin expression and function: Role in bone remodeling. J Cell Biochem Suppl 1998;30–31: 92–102.
29. Roodman GD. Regulation of osteoclast differentiation. Ann NY Acad Sci 2006;1068:100–109.
30. Lian JB, Javed A, Zaidi SK, et al. Regulatory controls for osteoblast growth and differentiation: Role of Runx/Cbfa/AML factors. Crit Rev Eukaryot Gene Expr 2004;14:1–41.