10.5005/jp-journals-10012-1040

CLINICAL RESEARCH

Histologic and Clinical Evaluation of a Bioactive Calcium Phosphosilicate Bone Graft Material in Postextraction Alveolar Sockets

[1]Aron Gonshor, [2]Stephen A Saroff, [3]Charles R Anderegg, [4]Frederic PC Joachim, [4]Jacques A Charon
[5]Hari Prasad, [6]Srinivas Katta

[1]Lecturer, Faculty of Dentistry, McGill University, Montreal, Quebec, Canada

[2]Periodontal Private Practice, Richmond, Virginia, USA

[3]Assistant Professor, Department of Periodontics, University of Washington School of Dentistry, Washington, USA

[4]Periodontal Private Practice, Lille, France

[5]Senior Research Scientist, Hard Tissue Research Laboratory, University of Minnesota, Minneapolis, USA

[6]Product Manager, NovaBone Products, Alachua, FL, USA

Correspondence: Srinivas Katta, Product Manager, NovaBone Products, LLC, 13631 Progress Blud # 600, Alachua, FL 32615 USA, Phone: 386-462-0418, Fax: 386-412-1636, e-mail: skatta@novabone.com

ABSTRACT

Background: Long-term success of dental implants has been demonstrated when placed simultaneously with or after a socket grafting procedure. Although optimal bone formation can be from 6 to 9 months or longer with grafting materials other than autogenous bone, there is the avoidance of potentially hazardous harvesting autogenous bone.

Methods: This study evaluated bone formation following grafting of 22 postextraction alveolar sockets with a bioactive calcium phosphosilicate putty (CPS putty) graft material.

Results: At 5 to 6 months postgrafting, there was bone regeneration showing both normal clinical attributes and radiographic trabecular appearance. Histomorphometric analysis revealed average vital bone content of 48.2 ± 6.8% to residual graft content of 2.4 ± 1.4% for the 22 sockets in the study, at an average healing period of 5.4 ± 1.5 months.

Conclusions: The high percentage of vital bone content after a relatively short healing phase, suggests that CPS putty can be a reliable choice for osseous regeneration in cases of crest preservation and implant related surgeries.

Keywords: Bone regeneration, Osteoblasts, Alveolar sockets, Bioactive glass, Tissue engineering.

INTRODUCTION

Extractions occur primarily as a result of periodontal disease, caries or trauma. Caries is endemic and a leading cause of tooth loss in the US population. Periodontal disease is responsible for 30 to 35% of extractions in people over 40 years of age.[1] Clinically, it is important to replace missing teeth with the most suitable option for the patient, so that ridge and site preservation at the time of extraction is critical to long-term success, irrespective of the procedure used for tooth replacement.[2]

Autogenous bone has been considered the 'gold standard' for filling bony defects, especially large defects resulting from cysts and tumors, alveolar resorption and periodontal bony defects, all of which leave insufficient bone for the placement of implants. The cancellous portion is usually used and it is rich in mesenchymal cells, which are generally involved in osteogenesis. However, clinical situations, such as the size of the bony defect, absence of enough donor tissue, or the need for a second intervention, may preclude its use.

The ongoing development of biomaterials has improved the characteristics and properties of potential synthetic bony substitutes.[3] The challenge has been to assess the interface between the biomaterial and the host.[4] Alloplastic bioactive graft substitutes are a potential advance in solving this issue. A bioactive material is defined as one that will create a biological response that will prevent a fibrous repair at the interface, but rather lead to a bony union of the material and the host tissue.[5] Bioactive glass ceramics have demonstrated such biocompatibility and direct contact with bone.[6] The first bioactive material was reported in 1971.[7] It was a four-component oxide mixture, consisting of 45% silica dioxide, 24.5% sodium, 24.5% calcium and 6% phosphorous (US Biomaterials Corp., Alachua, Fla). This product has evolved and is now being marketed as a pre-mixed, moldable material called NovaBone Dental Putty® (NovaBone Products, Alachua, Fla), consisting of four components: two bioactive phase components—A 55% standard calcium phosphosilicate (CPS) particulate, and a 14% CPS smaller particulate as well as 12% polyethylene

glycol additive phase and 19% glycerin binder phase (NovaBone Products, Alachua, Fla). In dentistry, this latter putty form of calcium phosphosilicate is designed for osseous regeneration of periodontal bone defects, filling of alveolar sockets, sinuses and augmentation of alveolar ridges.

The purpose of this study was to clinically, radiographically and histotologically evaluate CPS putty when used as a bone graft material in human alveolar postextraction sockets.

MATERIALS AND METHODS

Materials

Bioactivity is initiated immediately upon implantation. The smaller CPS particles release calcium and phosphorous ions into the area, the binder material gets absorbed over a period of a week exposing the larger CPS particulates to blood. In several hours, calcium phosphate is produced in the gel, which then crystallizes into a new surface apatite layer. Bioactivity begins in this surface layer when collagen, glycoproteins and mucopolysaccharides from the surrounding bone are incorporated into the apatite layer. This helps to produce a direct chemical bond with the host bone. *In vivo*, the graft substitute bonds to connective tissues and to bone.[8] The apatite layer helps in the stimulation of osteoprogenitor cells to produce transforming growth factor, by the release of silicon from the surface.[3,9-12]

Methods

There were 22 patients (14 males and 8 females), between the ages of 25 and 79 (mean of 51), requiring tooth extraction. The surgical procedures were performed only in private offices. Patients were screened, and all provided written and oral consent. They were enrolled from October 2008 to August 2010, and the study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000. The case-selection criteria included the absence of acute periodontal or odontogenic disease; women who were neither pregnant nor intended to become pregnant during the study period; no history of cancer or human immunodeficiency virus; no untreated periodontal disease, including periapical disease; and the absence of any medical condition or therapeutic regimen that alters soft and/or hard tissue healing (i.e. osteoporosis, hyperparathyroidism, autoimmune diseases, chemotherapeutic or immunosuppressive agents, steroids, bisphosphonates or similar type drugs).

All cases in this study were of tooth extractions with immediate socket grafting. Care was taken to remove the teeth atraumatically, so as to preserve the surrounding bone. In all cases the sockets had four or five wall defects. After extraction, the sockets were debrided and any inflammatory granulation tissue removed, also ensuring that there was suitable residual bleeding. The sockets were then filled with the CPS putty material (Fig.1), being careful not to touch

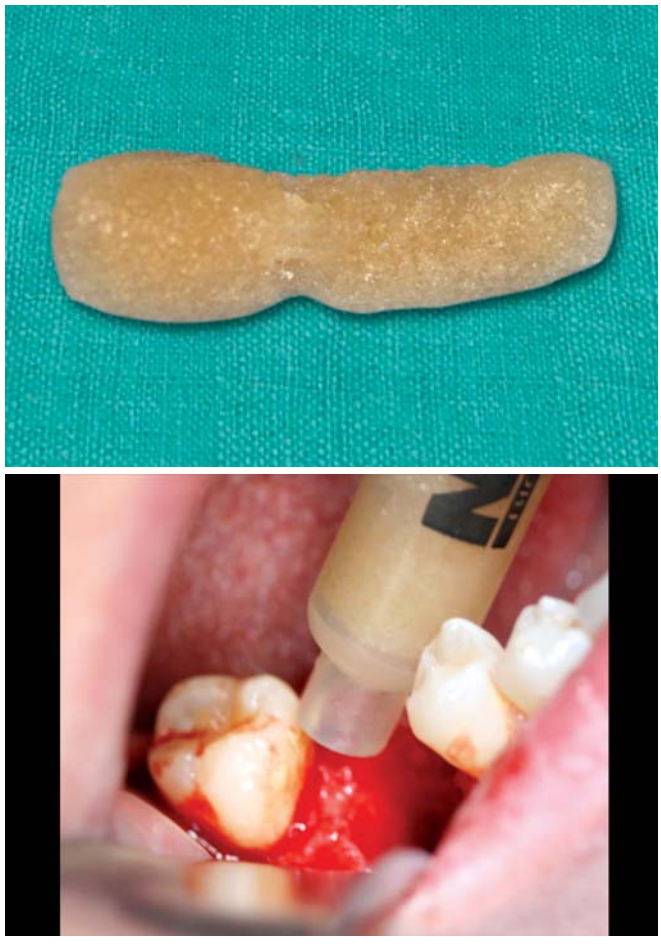


Fig. 1: Calcium phosphosilicate bioactive bone in the two delivery formats used in this study (A) Moldable putty and (B) injectable putty in a syringe

the material with surgical gloves, or to impact the material too tightly. The volume of putty material used varied from 0.5 to 1.0 cc. No membranes were placed. Mucosal and periosteal releasing incisions were created to allow for tension-free primary closure, using 3-0 or 4-0 plain gut or chromic sutures. No pre or postoperative antibiotics were administered, and all patients were placed on chlorhexidine oral rinse postoperatively. Pre and immediate postoperative radiographs were taken. Patients were then followed clinically and radiographically at time intervals of 1 week, 2-3 weeks, 6-8 weeks and 3-4 months. At the latter visit, there was a discussion as to subsequent implant placement, which took place within the 3 to 6 months period. A number of patients decided against implant therapy.

Core Biopsies

On the day of surgery, prior to the implant placement, a trephine bur with a 2.7 mm internal diameter (3.0 mm external diameter) was used to obtain a bone core from the center of the regenerated socket. The cores were left within the trephine and placed in 10% neutral buffered formalin for fixation. Decalcified specimens were prepared in 14 of the 22 cases that opted for subsequent implant placement.

Undecalcified preparations were performed in the remainder (8 cases), with subsequent histomorphometric analysis.

Histologic Preparation

Undecalcified histologies were performed by the Division of Anatomic Pathology, University of Connecticut, Framington, Connecticut. Specimens were fixed in formalin prior to decalcification. A stronger decalcification solution was used for dense bone cores (e.g. mandible). A high speed bone decalcifier—Decal Stat (hydrochloric acid), (Decal Chemical Corp.) was used for decalcification of samples. The slides were stained with modified hemotoxin/eosin and histologic analysis was performed. All histologic preparations for histomorphometrics were performed by the Division of Pathology, University of Minnesota, Minneapolis, Minnesota, USA. Upon receipt, specimens were dehydrated with a graded series of alcohols for 9 days. Following dehydration, the specimens were infiltrated with a light-curing embedding resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). Following 20 days of infiltration with constant shaking at normal atmospheric pressure, the specimens were embedded and polymerized by 450 nm light; the temperature of the specimens never exceeded 40ºC, then cut and ground.[13,14] Specimens were prepared in an apicocoronal direction (parallel to the long axis) and were cut to a thickness of 150 µm on a cutting/grinding system (EXAKT Technologies, Oklahoma City, OK, USA). The cores were polished to a thickness of 45 to 65 µm with a series of polishing sandpaper disks from 800 to 2,400 grit, using a microgrinding system, followed by a final polish with 0.3 µm alumina polishing paste. The slides were stained with Stevenel's blue and Van Gieson's picro fuchsin and coverslipped for histologic analysis using brightfield and polarized microscopy.

Histomorphometry

Following nondecalcified histologic preparation, the cores were evaluated histomorphometrically. The cores were digitized at the same magnification using a microscope (Zeiss Axiolab, Carl Zeiss MicroImaging, Thornwood, NY, USA) and a digital camera (Nikon Coolpix 4500, Nikon, Melville, NY, USA). Histomorphometric measurements were completed using a combination of programs (Adobe Photoshop, Adobe Systems, San Jose, CA, USA; NIH Image, National Institutes of Health, Bethesda, MD, USA). Parameters evaluated were the total area of the core, percentage of new bone formation and percentage of residual graft material. The remainder of the area was considered soft tissue or void. The primary slide evaluated for each specimen was from the most central region of the obtained core. No comparison was made between the apical and coronal sections.

RESULTS

The study consisted of a total of 22 alveolar sockets. Seventeen sockets were in the maxilla, with 11 in the anterior, cuspid-to-cuspid region and 6 in the posterior, premolar-molar region. In the mandible, the remaining five sockets were in the molar region only. At 5 to 6 months (average 5.4) postgraft period, all sockets demonstrated dense bone fill, with no visual evidence of residual graft material. Clinically, there was no significant difference noted in the "tactile feel" when drilling into treated sites as compared to adjacent non-treated sites, with bleeding in the graft site osteotomies showing clear evidence of vascular ingrowth. Radiographs demonstrated very substantial bone fill in the sockets. The trabecular pattern in the regenerated areas appeared very similar to the adjacent (native) bone.

A representative case is that of a 40-year-old female, who presented with mobility and pain in her upper right first molar. After evaluation, the decision was made to extract the tooth, with eventual implant placement. The tooth was extracted atraumatically, at which time it was determined that the quality of bone was insufficient for immediate implant placement, as evidenced by the lack of buccal plate (Fig. 2). CPS putty was placed. No membrane was used since there was sufficient mucosa to obtain primary closure. The patient was recalled 5 months postgraft for

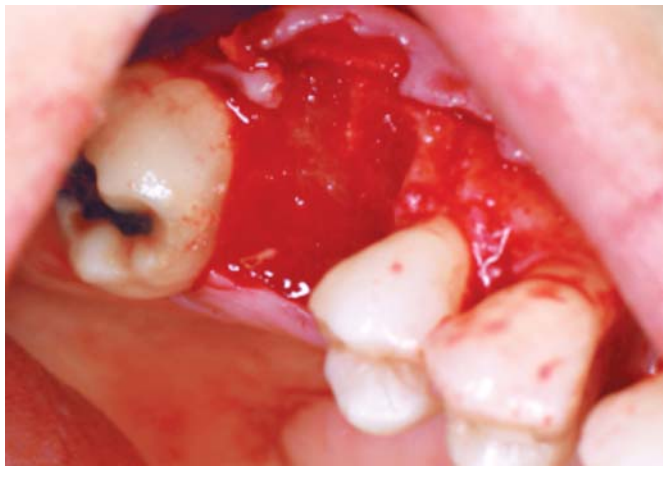


Fig. 2: Immediate postextraction of the upper first molar, showing the buccal dehiscence

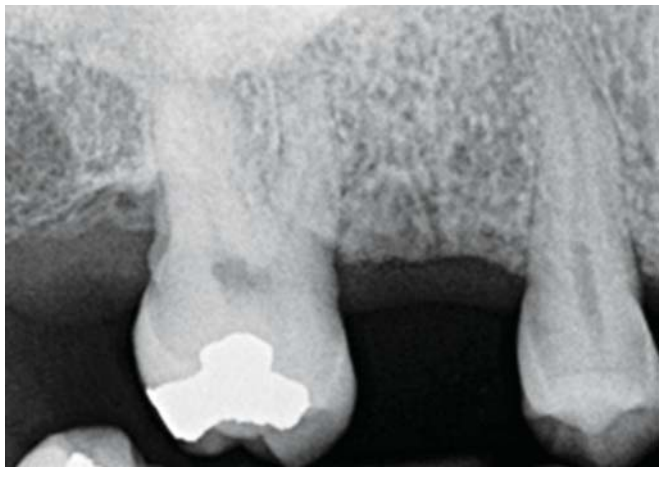


Fig. 3: Periapical of the extraction region prior to implant placement, showing a confluent trabecular pattern of bone

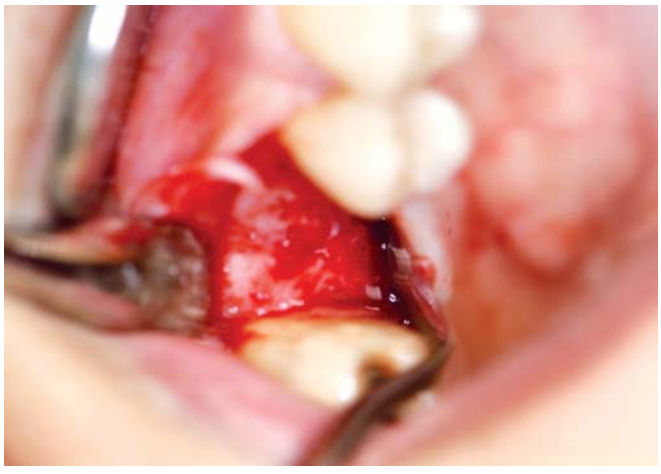
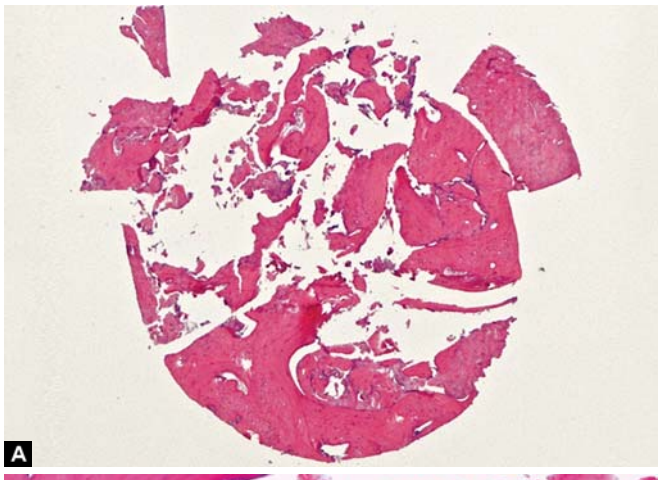
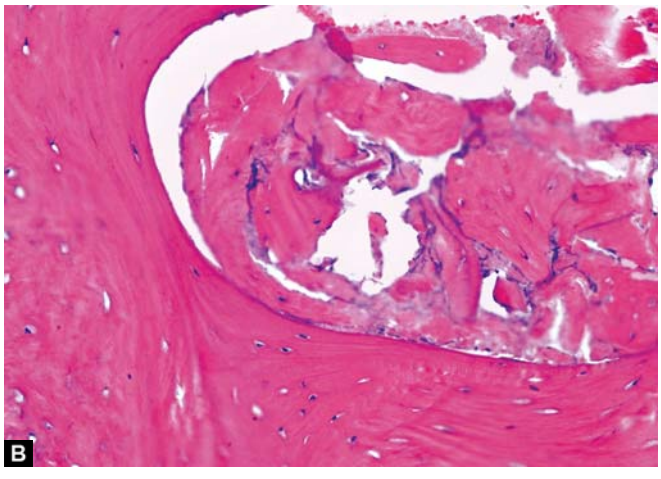
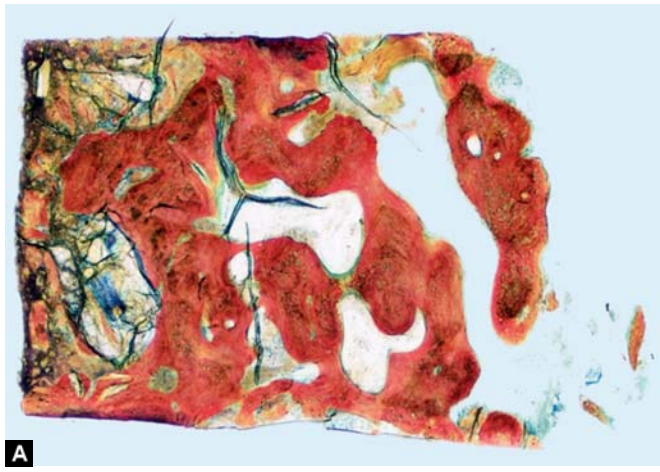


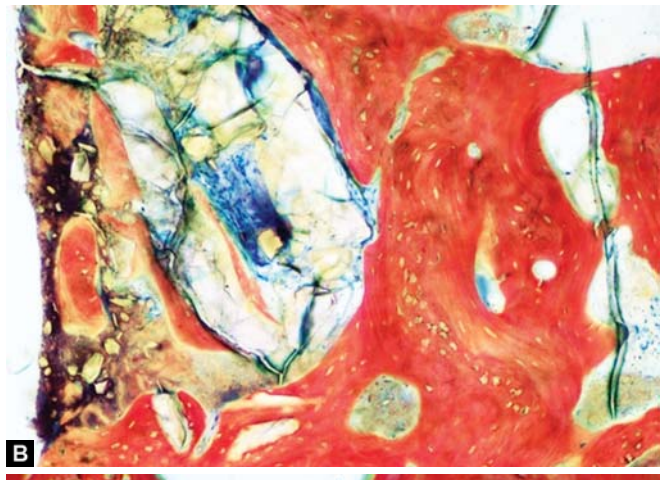
Fig. 4: Regenerated alveolus, including the area of buccal bone loss

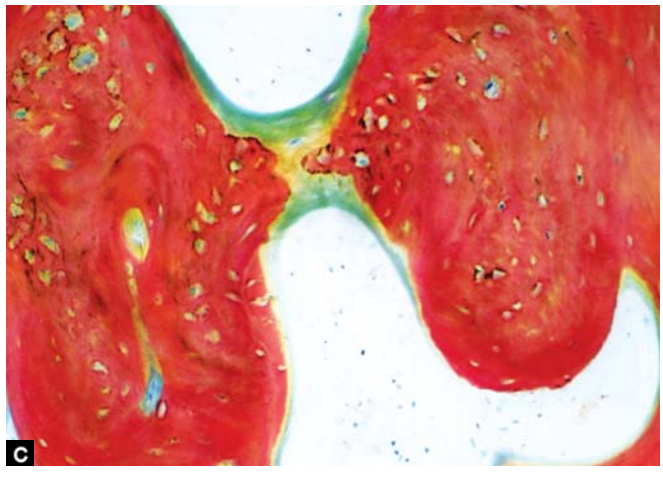




Figs 5A and B: Decalcified core at low (A) and high magnification (B) demonstrating robust vital bone with no evidence of residual graft material







Figs 6A to C: Calcium phosphosilicate bioactive bone undecalcified cores at 5.5 months. Representative images at (A) medium (40x), (B) high (100x) and (C) very high (200x) magnifications. The red-stained tissue is mineralized, newly regenerated bone with visible cell nuclei. Residual graft material can be seen in both A and B. In several cases, the cores between 5 and 6 months showed no evidence of residual graft material

evaluation, prior to implant placement. The radiograph showed complete bone regeneration, with a very confluent trabecular pattern (Fig. 3). The quality of the regenerated bone was excellent with healthy natural bleeding and a completely regenerated buccal plate (Fig. 4), which permitted normal implant placement. A core was taken prior to implant placement. Representative low and high magnification samples of the decalcified sections show substantial portions of dense vital bone, with no evidence of the graft particles (Figs 5A and B).

Figures 6A to C show representative histologic images of an undecalcified core, at increasing magnifications, taken from the bone graft site of a separate case. Histomorphometric evaluation of all the undecalcified cores revealed an average vital bone content of 48.2 ± 6.8% (standard deviation). As a comparison, autogenous trabecular bone

volumes, which can vary widely, have a range from under 20 to 40%.[15] A residual graft content of 2.4 ± 1.4% was found for the calcium-phosphosilicate bone graft, following a healing time of 5.4 ± 1.5 months.

DISCUSSION

The results of the present study show that in postextraction alveolar sockets, CPS putty graft material is able to achieve results that are very similar to those obtained with autogenous bone grafts. In addition, by eliminating the need for second site donor harvesting and the resulting increased risk of morbidity, the use of this synthetic graft substitute becomes very appealing.

In the present study, tissue regeneration after graft placement occurred without complications. Most significantly, there was a very high level of bone formation within the implanted material. This was evidenced by new bone formation, including mature trabecular bone with osteocytes in lacuna as well as marrow formation within the new bone structure. The degree of trabecular bone formation between the implant particles was consistent with the previously reported histologic results in animal models after a similar time frame.[26] In these studies new bone formed around all particles with a progressive thickening of the bone layer as the particles decreased in size. The histologic findings of this study indicate that the graft material followed the same pattern of bone formation as seen in other human and animal studies. A high degree of neovascularization was seen within the grafted area, which is crucial for the support of new bone formation. There was no evidence of reduction of the overall size of the graft material and newly formed bone, although longer study duration would be necessary to demonstrate no long-term resorption. In addition, there was no evidence, either clinically or histologically, of any significant inflammatory reaction surrounding the graft material, suggesting good tissue compatibility.

Historically, the function of biomaterials has been to replace damaged and missing tissues.[7,16] The multi-stage mechanisms and kinetics of surface reactions of CPS and bone have been extensively covered.[17,18] Moreover, Xynos et al were able to show that modulation of the osteoblast cell cycle is achieved by the controlled release of ionic dissolution products from CPS particles.[19-21] Gene array analyses confirmed that after several hours of exposure of human primary osteoblasts to the soluble chemical extracts from CPS particles, several gene families were up-regulated or activated. Genes that encode for nuclear transcription factors and growth factors, especially IGF-II,[20] among the differentially expressed genes were those involve with cell cycle regulation, differentiation and proliferation as well as cell adhesion and bone mineralization.[19,22,23] These studies, all point to a capability possessed by CPS particles to stimulate differentiation toward a cell lineage with therapeutic potential in tissue engineering. In addition there is evidence that these particles possess a transient antimicrobial activity.[24,25]

Human clinical studies and reports of bioactive glasses use have dealt for the most part with repair of periodontal and alveolar ridge defects with more limited studies in orthopedics and other areas of the head and neck. Lovelace et al[29] showed that freeze-dried bone allograft gave similar pocket depth reduction in moderate and deep periodontal osseous defects when compared with CPS particles. Other authors who have treated infra-bony defects with CPS particles have shown similar results with attachment gains of 2.7 to 3.0 mm and 2.8 mm, and reductions in pocket probing depth of 3.7 to 4.4 mm[27,28] with preoperative probing depth at 7.9 to 8.1 mm. In a recent comparative histomorphometric study by Galindo-Moreno et al,[29] bone core biopsies were taken 6 months after sinus grafting with either a bovine hydroxylapatite (HA) or CPS particles. No bone loss was observed radiographically or clinically in both groups. Histologic analysis revealed that both grafts had a high biocompatibility. In the bovine HA-containing group, minimal xenogenic graft absorption was noted. In contrast, the CPS group samples presented a high absorption rate with some remaining particles embedded in new normal bone.

CONCLUSION

The high percentage of vital bone content after a relatively short healing phase suggests that bioactive calcium phosphosilicate putty can be a reliable choice for osseous regeneration in cases of crest preservation and implant related surgeries.

ACKNOWLEDGMENTS

NovaBone Products Ltd (a distributor of the bone graft material used in this study) provided partial research support for this project. The authors gratefully acknowledge the assistance of Dr Michael Rohrer, Director, Hard Tissue Research Laboratory and Oral Pathology Laboratories, University of Minnesota School of Dentistry, for the preparation of the specimens, the histologic data used for histomorphometric analysis, and the assistance of Dr Easwar Natarajan at the University of Connecticut Health Center, Oral Pathology Diagnostic Service, Farmington, CT, for the preparation of the decalcified specimens.

REFERENCES

1. Klinge B, Hultin M, Berglundh T. Peri-implantitis. Dental Clinics of North America 2005;49:661-76.
2. Lekovic V, Kenney E, Weinlaender M, Han T, Klokkeveld P, Nedic M. A bone regeneration approach to alveolar ridge maintenance following tooth extraction: Report of 10 cases. J Perodontol 1997;68:563-70.
3. Anderegg CR, Alexander DC, Freidman M. A bioactive glass particulate in the treatment of molar furcation invasions. Journal of periodontology 1999;70:384-87.

4. Hench LL, West JK. Biological applications of bioactive glasses. Life Chem Rep 1996;13:187-241.
5. Hench LL. Bioceramics: Material characteristics versus in vivo behavior 1988.
6. Gross U, Strunz V. The interface of various glasses and glass ceramics with a bony implantation bed. Journal of Biomedical Materials Research 1985;19:251-71.
7. Hench LL, Splinter RJ, Allen WC, Greenlee TK. Bonding mechanisms at the interface of ceramic prosthetic materials. Journal of Biomedical Materials Research 1971;5:117-41.
8. Zhong JP, LaTorre GP, Hench LL. The kinetics of bioactive ceramics: (Part VII). Binding of collagen to hydroxyapatite and bioactive glass. In: Anderson OH, Yli-Urpo (Eds): Oxford: Butterworth-Heinemann 1994.
9. Price N, Bendall S, Frondoza C, Jinnah R, DS H. Human osteoblast-like cells (MG63) proliferate on a bioactive glass surface. J Biomed Mater Res 1997;37:394-400.
10. Wilson J, Low SB. Bioactive ceramics for periodontal treatment: Comparative studies in the Patus monkey. Journal of Applied Biomaterials 1992;3:123-29.
11. Cancian DC, Hochuli-Vieira E, Marcantonio RA, Garcia JIR. Utilization of autogenous bone, bioactive glasses, and calcium phosphate cement in surgical mandibular bone defects in Cebus apella monkeys. The International Journal of Oral and Maxillofacial Implants 2004;19:73-79.
12. Wilson J, Pigott GH, Schoen FJ, Hench LL. Toxicology and biocompatibility of bioglasses. Journal of Biomedical Materials Research 1981;15:805-17.
13. Donath K, Breuner G. A method for the study of undecalcified bones and teeth with attached soft tissues. Journal of Oral Pathology and Medicine 1982;11:318-26.
14. Rohrer MD, Schubert CC. The cutting-grinding technique for histologic preparation of undecalcified bone and bone-anchored implants: Improvements in instrumentation and procedures. Oral Surgery, Oral Medicine, Oral Pathology 1992;74:73-78.
15. Watzek G, Ulm C, Haas R. Anatomic and physiologic fundamentals of sinus floor augmentation. In: Jensen O (Ed). The sinus bone graft. Chicago: Quintessance 1992:35-37.
16. Hench LL, Polak JM, Xynos ID, Buttery LDK. Bioactive materials to control cell cycle. Materials Research Innovations 2000;3:313-23.
17. Ogino M, Ohuchi F, Hench LL. Compositional dependence of the formation of calcium phosphate films on bioglass. Journal of Biomedical Materials Research 1980;14:55-64.
18. Oonishi H, Hench LL, Wilson J, Sugihara F, Tsuji E, Matsuura M, et al. Quantitative comparison of bone growth behavior in granules of BioglassÆ, AW glass-ceramic, and hydroxyapatite. Journal of Biomedical Materials Research Part A 2000;51: 37-46.
19. Hench LL, Polak JM. A genetic basis for design of biomaterials for in situ tissue regeneration. Key Engineering Materials 2008;377:151-66.
20. Xynos ID, Edgar AJ, Buttery LDK, Hench LL, Polak JM. Gene-expression profiling of human osteoblasts following treatment with the ionic products of BioglassÆ 45S5 dissolution. Journal of Biomedical Materials Research Part A 2001;55:151-57.
21. OrÈfice R, Hench L, Brennan A. Evaluation of the interactions between collagen and the surface of a bioactive glass during in vitro test. Journal of Biomedical Materials Research (Part A) 2009;90:114-20.
22. Bombonato Prado KF, Bellesini LS, Junta CM, Marques MM, Passos GA, Rosa AL. Microarray based gene expression analysis of human osteoblasts in response to different biomaterials. Journal of Biomedical Materials Research (Part A) 2009;88: 401-08.
23. Carinci F, Palmieri A, Martinelli M, Perrotti V, Piattelli A, Brunelli G, et al. Genetic portrait of osteoblast-like cells cultured on PerioGlas 2007.
24. Waltimo T, Brunner TJ, Vollenweider M, Stark WJ, Zehnder M. Antimicrobial effect of nanometric bioactive glass 45S5. Journal of dental research 2007;86:754-57.
25. Gubler M, Brunner TJ, Zehnder M, Waltimo T, Sener B, Stark WJ. Do bioactive glasses convey a disinfecting mechanism beyond a mere increase in pH? International Endodontic Journal 2008;41:670-78.
26. Leatherman BD, Dornhoffer JL. Bioactive glass ceramic particles as an alternative for mastoid obliteration: Results in an animal model. Otology and Neurotology 2002;23:657-60.
27. Froum SJ, Weinberg MA, Tarnow D. Comparison of bioactive glass synthetic bone graft particles and open debridement in the treatment of human periodontal defects: A clinical study. Journal of periodontology 1998;69:698-709.
28. Mengel R, Soffner M, Flores-de-Jacoby L. Bioabsorbable membrane and bioactive glass in the treatment of intrabony defects in patients with generalized aggressive periodontitis: Results of a 12-month clinical and radiological study. Journal of Periodontology 2003;74:899-908.
29. Galindo Moreno P, Avila G, Fern·ndez Barbero JE, Mesa F, O'Valle Ravassa F, Wang HL. Clinical and histologic comparison of two different composite grafts for sinus augmentation: A pilot clinical trial. Clinical Oral Implants Research 2008;19:755-59.