Socket Grafting with Calcium Phosphosilicate Alloplast Putty: A Histomorphometric Evaluation

Lanka Mahesh BDS, MBA (1)/ Maurice A. Salama, DMD (2)/ Gregori M. Kurtzman, DDS (3)/ Frederic P.C. Joachim, DDS, MSc (4)

ABSTRACT
Background: Socket grafting immediately after extraction with a bone graft substitute is essential to preserve the ridge architecture for implant placement. There are several bone graft substitutes that have been tested to effectively regenerate osseous tissue in the sockets. Evidence suggests that socket bone typically regenerates between 6-8 months or longer dependent on several factors including the original ridge dimensions, type of graft and the overall systemic health of the individual. The purpose of this study is to histologically evaluate the bone regeneration potential of a novel synthetic calcium phosphosilicate putty (CPS) graft substitute. Methods: After extraction of the involved teeth, CPS putty graft was placed and the sockets covered with a collagen plug (Collaplug, Zimmer Dental, Carlsbad, CA). Cores were taken from 20 patients for histological evaluation prior to implant placement. Ten cores were processed decalcified with H&E stain and the remaining ten were processed undecalcified. Histomorphometric data obtained from both sets is presented.

Results: Histomorphometric analysis revealed an average vital bone content of 49.5 (±21.7). A residual graft content of 4.3% (± 7.8) was observed following a healing time of 4.9 (± 0.8) months.

Conclusions: Clinical and histomorphometric data suggests that CPS Putty is a good choice for socket bone regeneration in implant-related surgeries.

Key words: bone regeneration; alveolar sockets; Alloplast putty; Calcium Phosphosilicate, Bioglass
1. Private Practice, New Delhi, India
2. Private Practice, Atlanta, GA
3. Private Practice, Silver Spring, MD
4. Periodontal Private Practice, Lille, France.

Correspondence: Dr Lanka Mahesh, S – 382, Panshila Park, New Delhi 110019, India
e-mail: drlanka.mahesh@gmail.com

Disclosure: NovaBone Products provided graft material for support of this project.
INTRODUCTION

Periodontal disease, caries or trauma may necessitate the need to extract teeth. It is important to replace missing teeth so that ridge and site preservation at the time of extraction aids in long-term success and preservation of the osseous structure, irrespective of the procedure selected for tooth replacement\(^2\).

Several bone graft substitutes are available currently for replacing and regenerating the osseous structures. Autogenous bone has been the ‘gold standard’ for its ability to supply the scaffold for osteoconduction, the growth factors for osteoinduction and bone cells for osteogenesis\(^2\).a.

Bone graft substitutes can be classified based on the biological function they impart at the defect site: osteoinductive and/or osteoconductive. Osteoinduction is a biochemical process resulting in the recruitment and differentiation of the surrounding viable cells into bone forming cells by the molecules contained within the graft while osteoconduction is a physical phenomenon by which the matrix of the graft forms a scaffold on which cells in the recipient site are able to form new bone. It is a three-dimensional process of ingrowth of capillaries, perivascular tissue and osteoprogenitor cells from the surrounding tissues into the graft. Most commercially available bone substitutes are osteoconductive while some exhibit osteoinductive properties. Osteoinductive properties of a graft material vary dependent on the methods of processing of donor tissue. All xenografts and alloplasts are osteoconductive and provide an excellent scaffold for bone regeneration.

Biomaterial development has improved the characteristics and properties of potential synthetic bony substitutes\(^3\). A new class of graft substitutes that are bioactive, indicating they interact with the surrounding tissues both physically and chemically, have evolved unlike most osteoconductive graft substitutes that are bioinert only providing a physical scaffold for bone to grow through. These graft substitutes are helping to understand the graft-host interface which has not been assessed completely to evaluate the bone fill potential\(^4\).
Bioactive glass-ceramics have demonstrated biocompatibility resulting in direct contact with bone in the healed sites\(^{(5,6)}\). The first bioactive material was reported in 1971, a four-component oxide mixture, consisting of 45% silicon, 24.5% sodium, 24.5% calcium and 6% phosphorous \(^{(7)}\). This product has evolved and is now being marketed as a pre-mixed, moldable material called NovaBone Dental Putty\(^{®}\) (NovaBone Products, Alachua, Fla.) (CPS Putty). It consists of a bimodal bioactive phase with an additive and a glycerin binder. The CPS putty has been approved for bone regeneration in osseous defects throughout the body including spine, orthopedic, craniofacial and dental defects.

This article’s purpose is to histologically evaluate CPS putty as a bone graft substitute when used in forty-four human alveolar post-extraction sockets.

**MATERIALS AND METHODS**

There were 42 patients (27 Male, 15 Female) between the ages of 25 and 79 (mean: 46). The study consisted of 49 alveolar sockets: 30 sockets were in the maxilla with 16 in the anterior area (cuspido-to-cuspid) and 14 in the posterior area (premolar-molar). The remaining 19 sockets were in the mandible with 6 in the anterior and 13 in the posterior area.

Usual case selection criterion was followed which excluded acute periodontal disease; pregnant women, human immunodeficiency virus patients; and any systemic medical condition that may interfere with healing (i.e., osteoporosis, steroid therapy, autoimmune diseases, etc).

The involved teeth were extractedatraumatically under local anesthesia to preserve as much socket architecture as possible. After extraction, the sockets were debrided and an effort was made to completely remove all the inflammatory granulation tissue. CPS putty was injected into the sockets (Figure 1) and a
spatula used to gently adapt the material to the socket walls and care was taken not to compact the material too tightly. About 0.5cc-1.0cc CPS Putty was used in each socket. The putty consistency helped contain the graft substitute in the defect and the unique cartridge delivery system enhanced the experience and minimized graft wastage. No membranes were used but the socket was covered with Collaplug (Zimmer Dental, Carlsbad, CA) to help retain the material and the mucosa was sutured with resorbable sutures.

Standard post-operative instructions were given and 27 (done in India) of the 49 patients were given postoperative antibiotics (amoxicillin, 250mg TID). All patients were placed on Chlorhexidine oral rinse post-operatively. Pre and immediate post-operative radiographs were taken. Patients were then recalled 2-3 weeks post operatively to evaluate the clinical healing. Post-operative radiographs were taken between 4-6 months (average 4.9 months) to evaluate the bone regeneration prior to implant placement.

**HISTOLOGY**

Prior to implant placement, a 2.7mm inner diameter (3.5mm outer diameter) trephine bur was used to obtain a bone core. All efforts were made to obtain the core from the center of the regenerated socket where the implant was planned to be placed. The trephines with the biopsy cores were placed in 10% formalin for fixation and sent for histological study. All twenty cores were processed non-decalcified in two different locations. Of the twenty cores that were obtained 10 cores were processed decalcified by the department of Oral Pathology, Rajiv Gandhi University in India and the remainder processed non-decalcified by the hard tissue laboratory at University of Minnesota.
DECALCIFICATION

Decalcified histologies were performed by the Department of Oral Pathology, Rajiv Gandhi University, Bangalore, India. All samples were subjected to microwave decalcification with 5% Nitric acid solution (95ml de-ionized water with 5ml Nitric acid). The tissue specimen was immersed in the above solution and placed in the microwave and heated up to 800w for 20 seconds and this cycle was repeated thrice with one hour interval between each cycle. This was followed by routine automatic tissue processing, embedding, sectioning and finally the sections were stained with H & E stains.

HARD TISSUE HISTOLOGY

Division of Pathology, University of Minnesota, Minneapolis, Minnesota, USA performed non-decalcified histology and provided histomorphometric data on the remainder of the cases. 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). This was then followed by 20 days of infiltration with constant shaking at normal atmospheric pressure, the specimens were then embedded and polymerized by 450 nm light. Care was taken during specimen preparation that the temperature of the specimens never exceeded 40ºC, which were then cut and ground. Specimens were prepared in an apico-coronal 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). The cores were polished to a thickness of 45-65 µm with a series of polishing sandpaper disks from 800 to 2,400 grit, using a microgrinding system, which was then 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 a cover slip placed for histologic analysis using bright field and polarized microscopy.
RESULTS

Clinically and radiographically all the sockets healed without any complications or adverse reactions. No signs of inflammation or infection were observed during the healing period. No particles of CPS Putty were observed upon re-entry and all the sockets appeared to be filled with a dense hard tissue. Clinically, there was no significant difference noted in the "tactile feel" when drilling into treated sites compared to adjacent non-treated sites, with bleeding in the graft site osteotomies showing clear evidence of vascular ingrowth of the grafted sites. Radiographs taken at 5-6 months (average 4.9) post grafting demonstrated dense bone fill in all study participants. The trabecular pattern within the grafted area presented with very similar trabecular patterns to the adjacent native bone indicative of graft resorption and remodeling.

Representative case 1:

A 50 year old female, in stable health, presented with a failed root canal restoration and a tooth fracture associated with a bridge (#45) (Figure 2A). Following evaluation, the decision was made to extract the tooth with planned 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, due to the loss of the buccal plate (Figure 2B).

The socket was debrided and CPS Putty was placed filling the socket to the level of the lingual crest and shaping it on the buccal side to simulate the natural anatomy in the region (Figure 3A). A two week follow up visit reveals the tissue healing in the area and the contour of the ridge (Figure 3B). The patient was recalled at 6 months post graft for evaluation. Clinically the ridge looked healthy and well formed (Figure 4A). Radiographically at 6 months the site demonstrated good bone fill (Figure 4B) which was confirmed clinically upon reentry with a full thickness flap (Figure 4C). The trabecular pattern in the regenerated area demonstrated similar characteristics to the trabecular pattern in the ungrafted area. It
was noted that the defect completely regenerated including the buccal wall, with no visual evidence of residual CPS putty graft particles.

A core was taken prior to implant placement. The defect demonstrated excellent bleeding indicative of healthy osseous tissue with no evidence of CPS Putty particles (Figure 5).

Histologic images at 40x and 100x (Figure 6a & 6b) magnifications reveal dense interconnected vital bone with remnants of graft material. At 100x the osteoblasts in the lacunae can be clearly seen indicative of a healthy cancellous osseous tissue.

**Representative case 2:**

A 22 year old female presented with pain in the lower left molar. Upon examination tooth #36 was seen to be grossly decayed with no clinical crown (Figure 7). Radiographic examination of the area did not reveal any periapical pathology (Figure 8). For best prognosis, it was decided that the tooth be extracted, the area grafted with CPS Putty graft and re-entered after 4 months for implant placement. On the day of the surgery, the attached gingiva was detached using a ‘Buser’ elevator under local anesthesia. The tooth was then extracted with luxators and care was taken to preserve the buccal/lingual plates.

After extraction, the socket was debrided and irrigated with saline. CPS Putty was placed into the socket using the unique cartridge delivery system (Figure 9). About 0.5cc of the graft material was used to fill the socket which was then covered with a collagen plug (CollaPlug, Zimmer Dental, Carlsbad, CA) (Figure 10) and stabilized with 3-0 Cytoplast sutures (Osteogenics, Texas). A radiograph was taken at 4 months to evaluate the bone fill. A core was taken prior to implant placement and was processed by the Oral Pathology Department at Oxford Dental College, Bangalore, India.
The histology slide (Figure 11) demonstrates good bone fill surrounded by vascular marrow tissue. Also noticeable are the osteocytes in mature bone. Some remnants of the bone graft substitute can also be seen.

**HISTOMORPHOMETRY**

Following non-decalcified histologic preparation, the cores were evaluated histomorphometrically. Cores were digitized at the same magnification using a microscope (Zeiss AxioLab, Carl Zeiss MicroImaging, Thornwood, NY) and a digital camera (Nikon Coolpix 4500, Nikon, Melville, NY). Histomorphometric measurements were completed using a combination of programs (Adobe Photoshop, Adobe Systems, San Jose, CA) (NIH Image, National Institutes of Health, Bethesda, MD). Parameters evaluated within the study were 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 section evaluated for each specimen was taken from the most central region of the obtained core. No comparison was made between the apical and coronal sections.

Histomorphometric evaluation of the non-decalcified cores revealed an average vital bone content of 49.5 (±21.7). In comparison, autogenous trabecular bone volumes, which can vary widely, have a range from under 20% to 40%\(^8\). A residual graft content of 4.3% (± 7.8) was found for the CPS bone graft, following a healing time of 4.9 (± 0.8) months.

**DISCUSSION**

The results from this study show a very high level of bone regeneration as evidenced radiographically by a trabecular pattern that resembles closely the unrestored area and histologically with osteocytes in lacuna. A high degree of revascularization was observed within the grafted area, essential for the support of new bone formation.
CPS Putty is a third generation bioactive graft material that not only provides physical scaffold for the bone tissue to grow but also interacts chemically with the surrounding tissue to impart an increased level of osteoblastic activity at the defect site. Upon implantation of the CPS Putty, the smaller CPS particles release calcium and phosphorous ions into the area; with the binder material getting absorbed over a period of a week, exposing the larger CPS particles to blood. Once the clot is organized the dissolution of binder and the smaller CPS particles create a porous network for fluid flow through the clot thus exposing the larger bioactive particles to blood. Within hours a calcium phosphate is produced, which crystallizes into a new surface apatite layer (hydroxycarbonate apatite). The graft substitute has been shown to bond to the connective tissue and also adjacent bone\(^9\). This apatite layer assists in the stimulation of osteoprogenitor cells to produce transforming growth factor, by the release of silicon from the surface\(^3\)(10-13). The surface reactions take place within a short, 2-4 day time frame\(^{14}\), with attachment of stem cells and the subsequent proliferation and differentiation of osteoblasts rapidly occurring on the surface of the bioactive material\(^{15, 16}\).

Several publications have tried to explain the various mechanisms and surface reactions of CPS particulate\(^{17, 18}\). The mechanism is complex and involves ionic dissolution and calcium release that eventually stimulates genes that are involved in regulation of osteoblast differentiation and proliferation\(^{14, 19, 20}\). All studies indicate a capability possessed by CPS particles to stimulate differentiation toward a cell lineage with therapeutic potential in tissue engineering. This phenomenon has been termed ‘osteostimulation’. Osteostimulation is officially defined as “an active stimulation of osteoblast proliferation and differentiation as evidenced during in vitro studies by increased levels of DNA synthesis and of the osteoblast markers osteocalcin and alkaline phosphatase” (FDA 510k 2005).

Osteostimulative materials support a higher level of osteoblast expression and activity than seen with materials that are merely osteoconductive. A recent comparative histomorphometric study by Galindo-
Moreno et al, found that bone core biopsies taken 6 months after sinus grafting with either a bovine hydroxyapatite (HA) or CPS particles that no bone loss was observed radiographically or clinically in both groups\textsuperscript{21}. Histologic analysis demonstrated that both grafts had high biocompatibility. But in the bovine HA containing group, minimal xenogeneic graft absorption was noted. In contrast, the CPS group presented a high absorption rate with occasional remaining particles embedded in new normal bone.

Another recent publication by Kotsakis et.al., also evaluated the performance of CPS Putty graft in\textsuperscript{12} extraction sockets. The average ridge width decreased by 1.1mm (±0.44) and the ridge height decreased by 0.83mm (±0.22) ) (P>0.05). Histomorphometric analysis revealed good bone regeneration with an average of 39.3% (±9.3) in the socket accompanied by rapid graft absorption (>90%) \textsuperscript{22}.

This study corroborates the findings from the earlier studies with CPS graft substitute. However, longer duration studies are essential to demonstrate absence of shrinkage associated with grafting in sites that will not receive implants or in large defect in which implant were placed. Additionally, no evidence was noted either clinically or histologically of any significant inflammatory reaction surrounding the graft material, suggesting good tissue compatibility.

**CONCLUSION**

Results suggest that CPS Putty is a reliable choice for osseous regeneration in cases of socket grafting and implant related surgeries. The putty consistency of the material and its unique delivery system also increases its clinical appeal.
ACKNOWLEDGMENTS

NovaBone Products (Manufacturer of the bone graft material used in this study) provided partial research support for this project. The authors acknowledge Srinivas Katta, Director at NovaBone Products for his assistance in authoring the discussion section of this manuscript. The authors also 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. Narayan Venkataraman at The Oxford Dental College, Oral Pathology Department, Bangalore, India for the preparation of the non-decalcified specimens in India.
REFERENCES


FIGURE LEGENDS:

Figure 1: Placement of CPS Putty into a fresh extraction socket.

Figure 2A: Radiograph of tooth #5 demonstrating fractured crown.

Figure 2B: Tooth #5 extracted; note loss of buccal wall.

Figure 3A: CPS Putty placed into #5 extraction socket; note the absence of the buccal plate.

Figure 3B: Two week follow up clinical picture of the defect site; note the ridge contour.

Figure 4A: Clinical picture of the ridge demonstrating good ridge dimensions and contour. The bone in the area appears remodeled when compared to Figure 3B.

Figure 4B: Radiograph of the regenerated socket demonstrating a trabecular pattern blending with the adjacent native bone.

Figure 4C: Re-entry into the site showing regeneration of the buccal plate that had been previously lost and an absence of any visible graft particles.

Figure 5: Occlusal view of a fully submerged implant surrounded by healthy bone.

Figure 6A: Specimen section at 40x magnification, demonstrating dense vital bone.

Figure 6B: Specimen section at 100x magnification demonstrating mature bone with osteoblasts and minimal remnants of graft particulate.

Figure 7: Grossly decayed tooth #36; note absence of a clinical crown.

Figure 8: Radiograph of #36; note fractured crown showing no periapical pathology.

Figure 9: CPS Putty delivered into the socket using the new cartridge delivery system.

Figure 10: Socket occluded with CollaPlug prior to suturing.

Figure 11: Decalcified specimen demonstrating good bone fill along with richly vascular marrow tissue. Also noticeable are the osteocytes embedded in mature bone and some remnants of the graft substitute.