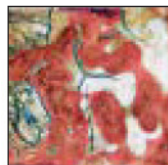# Histomorphometric Evaluation of a Calcium-Phosphosilicate Putty Bone Substitute in Extraction Sockets

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The objective of this study was to evaluate bone regeneration in 24 sockets grafted with a calcium phosphosilicate putty alloplastic bone substitute. A core was obtained from 17 sockets prior to implant placement for histomorphometry at 5 to 6 months postextraction. Radiographic analysis during the same postextraction healing period showed radiopaque tissue in all sockets. Histomorphometric analysis revealed a mean vital bone content of 31.76% (± 14.20%) and residual graft content of 11.47% (± 8.99%) after a mean healing period of 5.7 months. The high percentage of vital bone in the healed sites in combination with its timely absorption rate suggest that calcium phosphosilicate putty can be a reliable choice for osseous regeneration in extraction sockets. (Int J Periodontics Restorative Dent 2014;34:233–239. doi: 10.11607/prd.1855)

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Ridge preservation at the time of extraction is critical for the long-term esthetic and functional success of the future restoration, irrespective of the procedure used for the rehabilitation of the edentulous site.[1–3] A variety of bone graft substitutes have been introduced for the purpose of ridge preservation, including autografts, allografts, xenografts, and alloplasts.[4–6] The challenge has been to assess the interface between the biomaterial and the host tissues.[7] Alloplastic bioactive bone substitutes are a potential advancement in enhancing this interface.[8] A bioactive material is defined as one that will create a biologic response, prevent a fibrous repair at the surgical site, and lead to a bony union of the material and the host tissue.[9] Glass-ceramics have demonstrated such biocompatibility and direct contact with bone in vitro.[10]

Calcium phosphosilicate (CPS) materials have been classified as bioactive since they have been shown to undergo both physical and chemical dissolution when implanted.[11] This is a result of an ion release mechanism that occurs between the biomaterial and

the body tissues, resulting in in situ formation of hydroxycarbonite apatite (HCA), which remodels into bone. This phenomenon has been termed osteostimulation as it has demonstrated a significant bone turnover potential compared with osteoconductive graft substitutes. This product has evolved and is available as a putty, moldable material (Dental Putty, NovaBone) consisting of four components: two bioactive phase components, an additive phase consisting of polyethylene glycol, and a binder phase composed of glycerin. CPS putty has been used in a variety of defects, including postextraction sockets and bone augmentation procedures, with very promising results.[12,13]

The aim of this multicenter study was to clinically and histologically evaluate the efficacy of CPS putty for ridge preservation in alveolar postextraction sockets. A secondary aim was to report any adverse events associated with the use of CPS putty.

Method and materials

Twenty-four patients (11 men and 13 women) between the ages of 25 and 79 years with a median age of 51 years who were scheduled for tooth extraction and ridge preservation were included. The surgical procedures were performed in four different private practices. All participants were screened and enrolled from October 2009 to August 2011. Willing participants signed the consent form and were

enrolled in the study. 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 not pregnant nor intended to become pregnant during the study period, no history of cancer or human immunodeficiency virus, and the absence of any medical condition or therapeutic regimen that alters soft and/or hard tissue healing. Smokers were included if they smoked fewer than 10 cigarettes/day and were encouraged to abstain from smoking 1 week before as well as 4 weeks after the surgery. To be included in the study, patients had to present with type I or II extraction sockets following extraction, according to the Salama and Salama classification.[14] If a socket after extraction was composed of a mostly defect environment (type III extraction socket), it was excluded from the evaluation.

All participating clinicians attended a calibration course prior to the start of patient recruitment to ensure reproducibility of the clinical technique used for ridge preservation. Consenting participants were treated either by attempting primary coverage over the extraction sockets with a coronally advanced flap or by using the socket plug technique, as previously described by Kotsakis et al,[15] depending on the operator's preference. Cell occlusive membranes were not used in any of the treated cases.

Briefly, care was taken to extract the teeth atraumatically in an

attempt to preserve the remaining bone structure. As previously mentioned, any type III sockets with a significant defect environment were excluded from the study. According to the classification used in this study, a defect environment was defined as a socket with three walls or fewer that was associated with less predictable bone regeneration.[15] Following extraction, the sockets were meticulously debrided and CPS putty material was placed into the sockets using either the syringe or a cartridge delivery system. According to the operating surgeon's preference, either a collagen plug (Collaplug, Zimmer Dental) was used to prevent migration of the putty and to occlude the socket according to the socket plug technique, or a full-thickness mucoperiosteal flap was raised to the level of the mucogingival junction on the buccal aspect followed by a periosteal releasing incision to allow coronal advancement of the flap and tension-free primary closure. In the first case, the collagen plug was stabilized using a horizontal or a figure-eight mattress suturing technique with 4-0 vicryl sutures. In the second technique, primary closure was achieved using an interrupted suturing technique with the same suture material. No pre- or postoperative antibiotics were administered, and all patients were placed on 0.12% chlorhexidine oral rinse postoperatively. Pre- and immediate postoperative radiographs were obtained. Postoperative evaluations were performed at 7 and 14 days and at 4 weeks to assess wound healing

and record any adverse events. Following 5 to 6 months of healing, surgical reentry was performed on patients that decided to proceed with implant placement at the healed sites to clinically evaluate the regenerated bone in the original defect and to retrieve a bone biopsy specimen. Prior to implant placement, a trephine bur with a 2.7- to 3.0-mm internal diameter was used to obtain a bone core from the healed sites. The cores were placed in 10% neutral buffered formalin for subsequent histomorphometric analysis.

Histomorphometric analysis

Histomorphometric analysis was 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). 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 40°C) and then cut and ground.[16] Specimens were cut to a thickness of 150 μm on a grinding system (EXAKT Technologies). 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. The cores were digitized at the same magnification using a microscope (Zeiss Axiolab, Carl Zeiss MicroImaging) and a digital camera (Coolpix 4500, Nikon). Histomorphometric measurements were completed using a combination of programs (Adobe Photoshop, Adobe Systems; NIH Image, National Institutes of Health). 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.

Results

Each patient contributed a single extraction site, for a total of 24 sockets treated with 12 sockets located in the maxilla, 7 in the anterior canine-to-canine region, and 5 in the posterior, premolar-molar region. The remaining sockets were located in the mandibular molar region. The volume of putty material used in each socket varied from 0.5 to 1.0 mL with no single rooted tooth requiring more than 0.5 mL of CPS putty. Healing was uneventful at all sites, and there were no reports of the adverse events frequently associated with extractions, such as bleeding, wound dehiscence, postoperative pain, and/or alveolar osteitis (dry socket).

At the 5- to 6-month postextraction period (with a mean observation period of 5.7 months), 17 patients decided to proceed with implant placement. Four were light smokers. Clinically, all sockets demonstrated dense bone fill with no signs of visible residual biomaterial. Most sites had a slight indentation at the coronal level of the facial wall of the ridge, but overall exhibited favorable dimensions for implant placement. The clinical appearance of the grafted sites was very similar to that of the neighboring bone. All sites had a deep red color, and there was bleeding in the graft site osteotomies showing clear evidence of vascular ingrowth (Figs 1 to 3). During implant site preparation, the quality of the regenerated bone gave the tactile impression of D2 to D3 type depending on the site. Radiographic examination revealed complete fill of the sockets, with radiopaque tissue visible in the sockets. The trabecular pattern in the regenerated areas appeared very similar to that of the adjacent native bone.

Light microscopy of the biopsy specimens revealed that all bone cores had some residual graft particles. The particles were most commonly surrounded by woven bone and osteoid, while in some cases particles were encapsulated by dense, cell-rich connective tissue. A thick seam of green-staining osteoid was frequently observed on the periphery of the newly formed bone. This finding is

Fig 1 *Initial presentation of a 46-year-old man with a hopeless mandibular second premolar. The treatment plan included extraction due to root fracture followed by ridge preservation.*
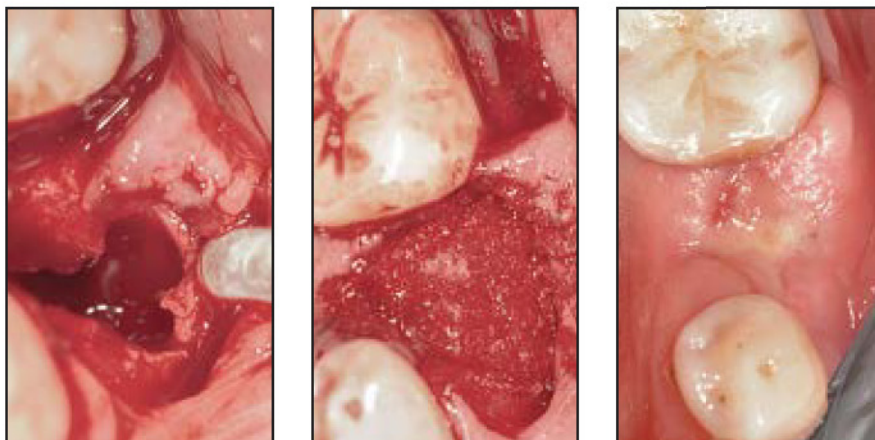

Fig 2 *Occlusal view of (a) postextraction socket, (b) socket grafting with the CPS putty, and (c) healing at 2 weeks postsurgery.*

Fig 3 *Occlusal view of the ridge (a) at 1 month postsurgery, (b) 4 months postsurgery, and (c) during implant placement. Note that the preservation of the ridge dimensions allowed for the placement of an implant surrounded by at least 1 mm of bone.*

osteons. These structures are indicative of bone maturity in these sites. No empty bone lacunae were found, indicative of vital bone formation. Histomorphometric evaluation revealed a mean vital bone content of 31.76% ± 14.2% with a residual graft content of 11.47% ± 8.99% (Table 1). There was no significant difference in the percentage of new bone growth between patients treated with the socket plug technique or where primary flap closure was achieved (Figs 4 and 5). No inflammatory cells were seen in any of the cores processed, and all cores displayed a healthy bone volume.

Discussion

The multistage mechanisms and kinetics of surface reactions of CPS and bone have been extensively investigated in vitro.[17,18] The surface reactions take place within a short, 2- to 4-day timeframe,[19] with attachment of undifferentiated cells and the subsequent proliferation and differentiation of osteoblasts rapidly occurring on the surface of the bioactive material.[20] Upon implantation, the smaller CPS particles release calcium and phosphorous ions in the area. The binder material gets absorbed over a period of a week, exposing the larger CPS particulates to blood. Breaking the silicon-oxygen bonds releases silicic acid, which forms a negatively charged gel at the particle surface. In several hours, calcium phosphate is produced in the gel, which then crystallizes into a

consistent with active bone formation that may still extend beyond the 5- to 6-month healing period allowed in this study. Many areas of new bone demonstrated haversian canals with well-developed

new surface apatite layer. This aids in the production of a direct chemical bond with the host bone.[21,22] The apatite layer helps in the stimulation of osteoprogenitor cells to produce transforming growth factor by the release of silicon from the glass surface.[22] Results of histologic analysis revealed a high level of bone formation within the implanted material. This was evidenced by new bone formation, including mature trabecular bone with osteocytes in the 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 following a similar time frame.[23,24]

Several publications have demonstrated the efficiency of CPS putty to regenerate bone in human extraction sockets. A recent histologic study on 20 sockets treated with CPS putty revealed a mean vital bone content of 49.5% (± 20.7%). A residual graft content of 4.3% (± 7.8%) was observed following a healing time of 4.9 (± 0.8) months.[12] A separate histologic study evaluated the performance of CPS putty in 22 extraction sockets, showing similar results (mean vital bone content: 48.2% ± 6.8%) after 5 to 6 months of healing.[25] These results are in accordance with the present findings of a small fraction of residual biomaterial in the healed sites, leading to the conclusion that CPS is an alloplastic bone substitute that presents a high and timely absorption rate.

Table 1	Vital bone, marrow, and residual graft as evaluated histomorphometrically for each bone specimen		
Specimen no.	Vital bone (%)	Marrow (%)	Residual graft (%)
1	50	45	5
2	55	43	2
3	39	58	3
4	54	44	2
5	17	58	25
6	13	59	28
7	18	60	22
8	39	54	7
9	19	61	20
10	32	64	4
11	40	54	6
12	29	63	8
13	30	50	20
14	47	50	3
15	14	76	10
16	25	66	9
17	19	61	21

No difference in the percentage of new bone growth was noted between sockets treated with either of the two techniques employed for socket preservation in this study. As reported by Kotsakis et al, the socket plug technique is efficient for socket preservation in sites that do not exhibit significant destruction of the socket walls following extraction.[15] When the bony walls are severely damaged, a barrier membrane should be employed for space maintenance and to prevent soft tissue from occupying part of the socket.[15] With the exclusion of type III extraction sockets[14] from this study, sockets with significant destruction of the alveolar socket walls were not included. As expected, both techniques yielded similar outcomes in this study for the percentage of new bone growth in each socket.

Results on the survival of implants placed in sites grafted with this type of bone graft are satisfactory. A longitudinal study with an observation period of up to 44 months postloading recorded the success rate for implants placed in healed sockets that were grafted with CPS alloplastic bone substitute.[26]
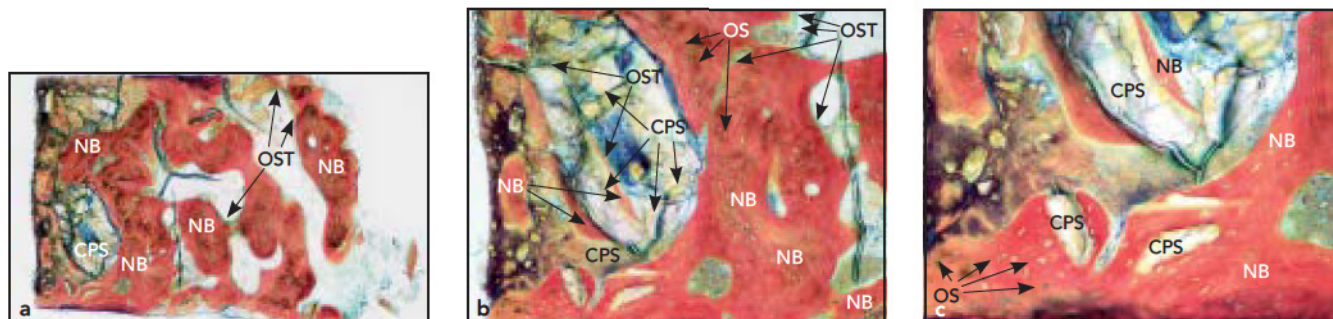
Fig 4 *Histomicrographs of a core obtained 162 days postextraction from a 49-year-old male patient. The coronal side of the core is located on the right of the histomicrophotograph. CPS = calcium phosphosilicate, NB = new bone, OST = osteoid, OS = osteocytes. (a) A high percentage of vital bone (50%) with some residual particles can be seen (5%). Note the good trabecular pattern with healthy marrow tissue. Differentiation can be made between new bone formation and small incorporated graft particles (Stevenel's blue and Van Gieson's picro fuchsin; original magnification ×40). (b) Image clearly demonstrates bone forming over the surface of a residual CPS graft particle. Osteoid along with osteoblasts (blue color cells) can be visualized at the surface of particles, indicative of new bone formation at the particle surface. Mature osteocytes are embedded in the newly formed bone (Stevenel's blue and Van Gieson's picro fuchsin; original magnification ×100). (c) Image shows osteoid bone at the surface of the graft particle with immature osteocytes (Stevenel's blue and Van Gieson's picro fuchsin; original magnification ×200).*

Fig 5 *Histomicrographs from a core obtained 5 months postextraction from a 64-year-old male patient. The coronal side of the core is located on the left of the histomicrophotograph. CPS = calcium phosphosilicate, NB = new bone, OST = osteoid, OS = osteocytes, OB = osteoblasts. (a) Image shows cancellous network with well-spaced graft particles. A thick seam of green-staining osteoid can be seen on the periphery of the newly formed bone. Photomicrograph suggests new bone has formed on the surfaces of the residual graft particles (Stevenel's blue and Van Gieson's picro fuchsin; original magnification ×20). (b) Newly formed cancellous bone with osteoid and osteoblasts around residual CPS particles can be better appreciated. The remainder of the core shows healthy marrow spaces, more residual graft particles, and connective tissue (Stevenel's blue and Van Gieson's picro fuchsin; original magnification ×40). (c) Bone forming along the surface of the residual particles embedded inside bone can be clearly visualized in this high-magnification histomicrograph (Stevenel's blue and Van Gieson's picro fuchsin; original magnification ×200).*

The cumulative success rate was 96.8% for patients with a noncontributory medical history.

In the present study, all 24 treated sites healed without complications. No incidence of alveolar osteitis was reported in any of the treated sockets, including 12 mandibular molars. This may be attributed to the stabilization of the blood clot in the postextraction socket by the hydrophilic characteristics of the CPS putty, supporting the findings of other researchers who have suggested that graft placement in the postextraction sockets may decrease the incidence of alveolar osteitis.[27]

A limitation of this study is the multicenter design. Even though a calibration session was provided to minimize any clinician-related variations, the relatively high standard deviation values noted in new bone formation may be attributed to the multiple operators located in different settings.

Conclusion

Within the limitations of this study, it can be concluded that the osteo-stimulative properties of CPS putty lead to high amounts of regenerated bone at 5 to 6 months posthealing. The high percentage of vital bone content in the healed sites in combination with the biomaterial's timely absorption rate suggest that CPS putty can be a reliable choice for osseous regeneration in ridge preservation procedures and implant-related surgeries.

Acknowledgments

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