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Bone regeneration and graft material resorption in extraction sockets grafted with bioactive silica-calcium phosphate composite versus non-grafted sockets: clinical, radiographic, and histological findings

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Trial Registration

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ABSTRACT

Purpose: The purpose of the present study was to evaluate the effect of silica-calcium phosphate composite (SCPC) granules on bone regeneration in extraction sockets.

Methods: Ten patients were selected for a split-model study. In each patient, bone healing in SCPC-grafted and control ungrafted sockets was analyzed through clinical, radiographic, histomorphometric, and immunohistochemical assessments 6 months postoperatively.

Results: A radiographic assessment using cone-beam computed tomography showed minimal ridge dimension changes in SCPC-grafted sockets, with 0.39 mm and 1.79 mm decreases in height and width, respectively. Core bone biopsy samples were obtained 6 months post-extraction during implant placement and analyzed. The average percent areas occupied by mature bone, woven bone, and remnant particles in the SCPC-grafted sockets were 41.3%±12%, 20.1%±9.5%, and 5.3%±4.4%, respectively. The percent areas of mature bone and woven bone formed in the control ungrafted sockets at the same time point were 31%±14% and 24.1%±9.4%, respectively. Histochemical and immunohistochemical analyses showed dense mineralized bundles of type I collagen with high osteopontin expression intensity in the grafted sockets. The newly formed bone was well vascularized, with numerous active osteoblasts, Haversian systems, and osteocytes indicating maturation. In contrast, the new bone in the control ungrafted sockets was immature, rich in type III collagen, and had a low osteocyte density.

Conclusions: The resorption of SCPC granules in 6 months was coordinated with better new bone formation than was observed in untreated sockets. SCPC is a resorbable bone graft material that enhances bone formation and maturation through its stimulatory effect on bone cell function.

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Author Contributions

Conceptualization: Doaa Adel-Khattab; Formal analysis: Nermeen S. Affi, Shaimaa M. Abu el Sadat; Investigation: Nermeen S. Affi, Mona N. Aboul-Fotouh, Karim Tarek; Methodology: Doaa Adel-Khattab, Mona N. Aboul-Fotouh, Karim Tarek; Supervision: Robert A. Horowitz; Writing - original draft: Doaa Adel-Khattab, Mona N. Aboul-Fotouh, Karim Tarek; Writing - review & editing: Doaa Adel-Khattab, Nermeen S. Affi, Shaimaa M. Abu el Sadat, Robert A. Horowitz.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Trial Registration: ClinicalTrials.gov Identifier: [NCT03897010](https://clinicaltrials.gov/ct2/show/study/NCT03897010)

Keywords: Alveolar ridge augmentation; Cone beam computed tomography; Histological techniques

INTRODUCTION

Alveolar ridge resorption following tooth extraction leads to a 50% loss in bone width over a 1-year period, which corresponds to a loss of 5–7 mm, mainly from the buccal aspect rather than the palatal/lingual aspects, and a 0.7 mm loss in height [1]. Bone resorption takes place in 2 phases. The first involves rapid bundle bone resorption in the buccal aspect of the socket, and the second phase is associated with remodeling of the outer surface of the alveolar bone, leading to horizontal and vertical tissue contraction. The vertical and horizontal dimensional shrinkages are in the ranges of 11%–26% and 29%–63%, respectively, at 6 months post-extraction [2]. Alveolar bone resorption makes ideal implant placement difficult due to the unavailability of enough dense bone to stabilize the implant in the appropriate prosthodontically desired location [3].

Over the last decades, numerous methods were proposed to use socket augmentation to minimize alveolar ridge volumetric contraction. Several biologically derived and synthetic bone grafts have been used, with varying degrees of success [4]. Autografts, xenografts, and allografts are widely used to augment bone defects. The limited bone supply for autografts and the risk of infection at the secondary surgery site have prompted the use of allografts from human cadaveric bone as an alternative. A histological analysis of bone biopsies taken from extraction sockets grafted with mineralized human bone allografts showed no statistically significant differences in the amount of newly formed bone or residual graft particles at 16 and 27 weeks after implantation [5,6]. Moreover, most of the newly formed bone was woven bone, even in the late healing group. A combination of woven and lamellar bone was also reported at 5 to 6 months post-grafting. Bone maturation in the grafted defects is essential for the long-term stability of dental implants.

Synthetic bioactive ceramics have been shown to release chemical cues that stimulate bone cell differentiation, tissue formation, and vascularization in critical-size bone defects [6]. Several materials containing calcium phosphate from bovine and synthetic sources have been used for preservation of the extraction socket, with varying degrees of success [6-8]. Horowitz et al. [8] used tricalcium phosphate bone grafts and reported good bone volume preservation. A biphasic calcium sulfate-hydroxyapatite composite was used alone or mixed with allograft granules to augment human extraction sockets. Eleven months post-augmentation, a comparable percent of new bone formation (31%–33%) was observed in defects grafted with either biphasic ceramic alone or in combination with an allograft [6]. Clinical studies demonstrated that calcium phosphate products derived from bovine bone showed a limited osteoconductive effect, despite having the porous architecture of natural bone [9,10]. A histological analysis demonstrated the encapsulation of the Bio-Oss bovine bone mineral particles in fibrous tissue. Fibrous encapsulation inside a bone defect is undesirable and indicative of an immune reaction. In addition, the study reported that the average percentage of graft material area was 30.8% after 9 months and concluded that the Bio-Oss particles were biocompatible, but their resorbability was not demonstrated [11]. Other studies have showed that bovine bone mineral particles were not resorbed even 5

years after grafting in extraction sockets [12,13]. Biphasic calcium phosphate, made of 60% hydroxyapatite and 40% tricalcium phosphate, has been used to graft extraction sockets. Three years postoperatively, a histological analysis of a bone biopsy from the grafted sockets showed limited bone formation and unresorbed graft material. Histological sections showed normal fibrous tissue with numerous fibroblastic cells, blood vessels, and a small quantity of osteoid tissue between the particles [14]. These limitations indicate that it is insufficient to use a bone graft simply because it is composed of calcium phosphate or its porosity is similar to that of natural bone, as these factors do not guarantee efficient bone formation and graft resorption. There is a need for a new graft design that takes into consideration a more comprehensive range of factors related to the elements of chemical composition that promote osteoblast differentiation, bone formation, and vascularization. In addition, the inappropriate resorption rate of current bioceramic bone grafts necessitates a new approach to engineering the structural parameters of the material to facilitate resorption and stimulate bone regeneration. Together, these properties should enable a new graft material to maximize volume preservation in an extraction socket, while filling the space with vital bone suitable for osseointegration of dental implants.

Data in the literature have repeatedly demonstrated the role of silica in the metabolic activity of bone cells. *In vivo* and *in vitro* studies have shown that silica plays important role in osteoblast differentiation, the synthesis of type I collagen, and mineralized tissue formation [15-17]. Moreover, angiogenesis in newly formed bone in grafted defects has been correlated with the presence of silicate ions [16]. A systematic increase of silica (from 20% to 80%) in calcium phosphate ceramics showed a significant increase in alkaline phosphatase activity and collagenous protein synthesis [15-17]. Silica-calcium phosphate composite (SCPC) has the ability to stimulate rapid bone regeneration and was found to be resorbed when grafted in critical-size saddle-type mandibular defects in dogs [18,19]. The aim of the present study was to evaluate clinically, radiographically, and histologically the effect of SCPC granules on new bone formation qualitatively and quantitatively following human extraction socket augmentation.

MATERIALS AND METHODS

Study design

A case series was performed in 10 participants (2 men and 8 women) who underwent socket augmentation procedures and dental implant placement in a staged approach. All participants were fully informed about the procedures, including the surgery, bone substitute materials, and implants. The study protocol followed the ethical standards outlined in the 1975 Declaration of Helsinki, revised in 2013. The study was approved by the Ain Shams University ethical committee (registry number FDASU-RECR 121812). This clinical trial was registered on ClinicalTrials.gov (NCT03897010). All participants provided written informed consent, and all operations were performed at the Ain Shams University Faculty of Dentistry. In each patient, 1 extraction socket was grafted with SCPC dental bone graft granules and the contralateral socket served as an ungrafted control. At the time of implant placement, bone biopsies were harvested from each socket for histomorphometric, histochemical, and immunohistochemical analyses.

Patient selection

Participants were recruited from the outpatient clinic of the Ain Shams University Faculty of Dentistry. The inclusion criteria encompassed patients requiring extraction of bilateral

or adjacent non-restorable (premolar or anterior) teeth located in the maxillary arch with subsequent implant restoration. Type 1 sockets were selected in this study, in accordance with Elian et al. [20]. In addition, the patients were in good general and oral health, without any active periodontitis. The following categories of patients were also excluded: smokers, patients with remaining root accompanied by an acute periapical infection or sinus tract, and patients with compromised health (American Society of Anesthesiology category III or IV), including drug or alcohol abuse or any significant systemic disease. Ten eligible participants were selected, including 8 women (mean age, 40 years; age range, 30–50 years) and 2 men (mean age, 42 years; age range, 30–55 years). Atraumatic extraction and socket augmentation procedures were planned.

The patients provided written informed consent regarding the surgical procedures. An antibiotic (875 mg+125 mg tablets of Augmentin [GlaxoSmithKline, Brentford, UK]) and analgesic (600 mg Brufen tablet [Abbott, Chicago, IL, USA]) were given to the patient 1 hour before surgery. At the beginning of the procedure, a 1-minute rinse with 0.2% chlorhexidine solution was performed by the patient. After administration of local anesthesia (articaine [Septanest, Septodont, Cedex, France]), atraumatic extraction using periostomes, luxators, and forceps was performed. The socket was debrided with curettes and alveolar spoons, and the granulation tissue was carefully removed. The buccal wall integrity was checked with a periodontal probe (Hu-Friedy PCP UNC 15). The mesio-distal and buccal-lingual diameters of the socket were measured with a periodontal probe, and an elliptical aluminum foil chip with the same dimensions was cut from a sheet that had been previously autoclaved. The chip was placed on the palatal mucosa and used as a guide to obtain a free gingival graft with a #15c surgical blade. The free gingival graft (1.5 to 2 mm thick) was taken from the area between the first and second premolar, 5 mm from the gingival margin. The donor site was covered by a platelet-rich fibrin (PRF) membrane prepared according to Choukroun et al. [21] Briefly, a 10 mL blood sample was taken without anticoagulant, and was immediately centrifuged in a glass tube at 2,700 rpm for 12 minutes (Duo Quattro, Mectron Medical, Vertriebs, Germany). The platelet-poor plasma was removed and the PRF was separated from the erythrocytes using sterile scissors. The PRF was then used as a dressing for the palatal wound area.

Bioactive porous SCPC dental bone graft granules in the size range of 90–710 μm (lot #4, Shefabone Inc., Concord, NC, USA) were hydrated with saline and loosely packed in the extraction sockets according to the manufacturer's instructions (Figure 1). The grafted SCPC granules were covered with a free gingival graft obtained from the palatal tissues and sutured with 4-0 polypropylene (blue monofilament, Assut, Pully-Lausanne, Switzerland) to stabilize the grafting material in place. Sutures were removed 7–10 days postoperatively and patients had follow-up visits scheduled every 2 weeks for the first month, and then once a month until implant placement. In each patient, 1 month after socket augmentation, the contralateral badly decayed tooth was atraumatically extracted and left to heal without grafting as a control. Routine postoperative instructions were given to the patients in a written form including postoperative oral hygiene measures with a 0.12% chlorhexidine mouth rinse twice daily for 10 days.

Dental implant placement and biopsy sampling were carried out under local anesthesia 6 months after extraction. Crestal incisions were made in the ridge, followed by a minimal flap elevation to the buccal and lingual crest of the ridge. The implant size was determined by cone-beam computed tomography measurements 6 months after socket augmentation. The alveolar bone was inspected, and biopsies were taken from both the grafted sockets and

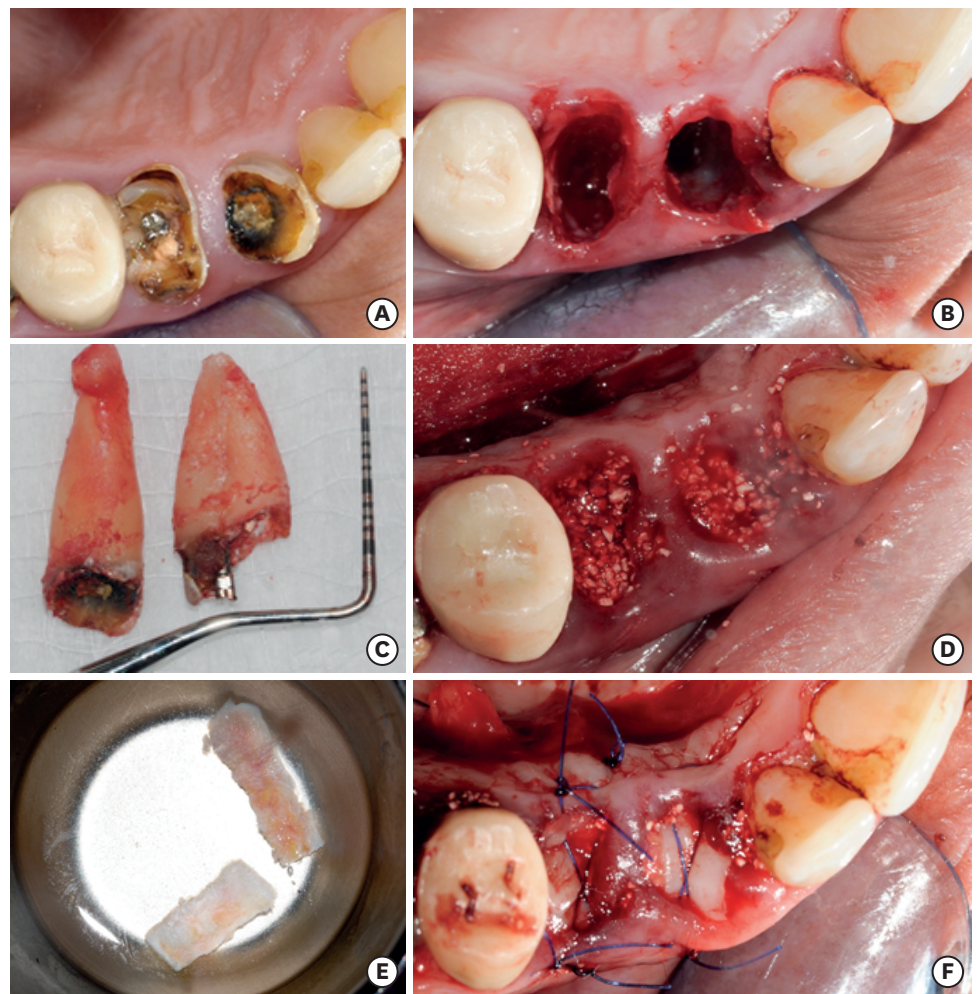


Figure 1. Digital images of (A) teeth before extraction, (B) atraumatic extraction of teeth 23 and 24, (C) extracted teeth, (D) the socket after grafting with SCPC granules, (E) 2 rectangular strips cut from a piece of the palate and used to seal the 2 grafted sockets, and (F) SCPC granules covered with the free gingival graft obtained from the palatal tissues and sutured with 4-0 polypropylene sutures. SCPC: silica-calcium phosphate composite.

the control ungrafted sockets using a trephine bur (2 mm internal diameter, Straumann®, Basel, Switzerland) with copious saline irrigation. The biopsy sample was approximately 5 mm in length. The apical aspect of all biopsies was marked to identify the apico-coronal orientation during the histological analysis. After biopsy specimen removal, osteotomy sites were prepared for Ratioplast® implant placement (Humantech, Steinenbronn, Germany) with an insertion torque of 25 N·cm. This was followed by wound closure, and the implants were allowed to heal subgingivally for 6 months. Figures 2 and 3 demonstrate the implant placement in the newly formed bone 6 months after grafting with SCPC.

Clinical parameters

The clinical parameters of the tooth to be extracted in both the test and control groups were assessed. The width of the keratinized mucosa was measured from gingival margin to the mucogingival junction using a graduated periodontal probe. Buccolingual bone width was measured using a bone caliper 4 mm away from the gingival margins. Buccal gingival thickness was measured using clinical reference points 4 mm away from the gingival margin

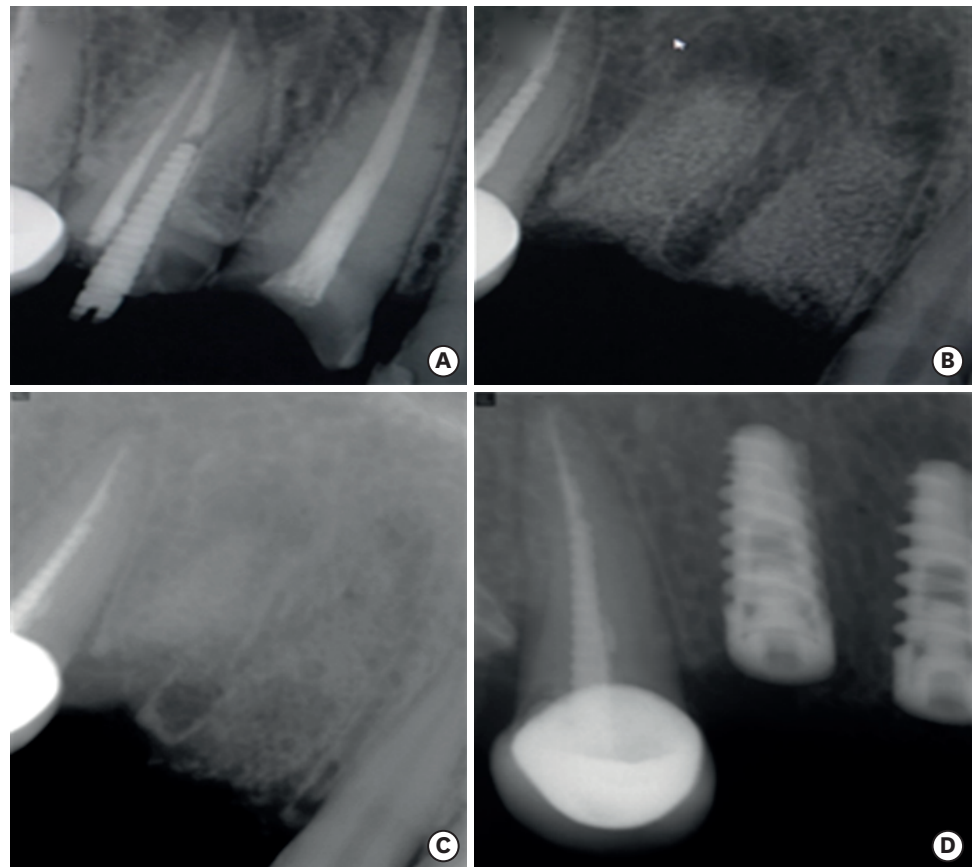


Figure 2. X-ray radiographs of (A) the decayed teeth (number 23 and 24) prior to extraction, and (B) the extraction socket immediately after grafting with the SCPC granules. The SCPC particles were radiopaque and easily visualized inside the socket. (C) Six months postoperatively, new bone formation and graft material resorption were evident. The edges of the grafted sockets were no longer visible due to remodeling of the newly formed bone and graft material resorption. (D) Six months after implant placement in the newly formed bone. SCPC: silica-calcium phosphate composite.

using a graduated periodontal probe. All clinical parameters were assessed preoperatively and 6 months after socket augmentation and in the control group. The standardization of the buccolingual width and gingival thickness was done by an acrylic stent that was custom-made for each patient. The stent covered the occlusal surface of the adjacent teeth, as well as the buccal and palatal aspects. A reference point 4 mm from the gingival margin was made in the stent in order to standardize the measuring points.

Radiographic parameters

Preoperative and 6-month postoperative assessments were conducted using an i-CAT Next Generation device (Imaging Sciences International, Hatfield, PA, USA) with exposure parameters of 120 kVp, 0.2 mm voxel size, a scanning time of 26.9 seconds, and a field of view of 6×16 cm in all cases. Scans were taken preoperatively before tooth extraction and postoperatively 6 months after extraction. Linear measurements were performed for height and buccolingual width in all cases. Control measurements were made for each case. To ensure standardization, the adjacent tooth was used as a reference and the reference planes were adjusted to be perpendicular on the referenced tooth, after which sequential slices were taken at 1-mm intervals in the region of interest.

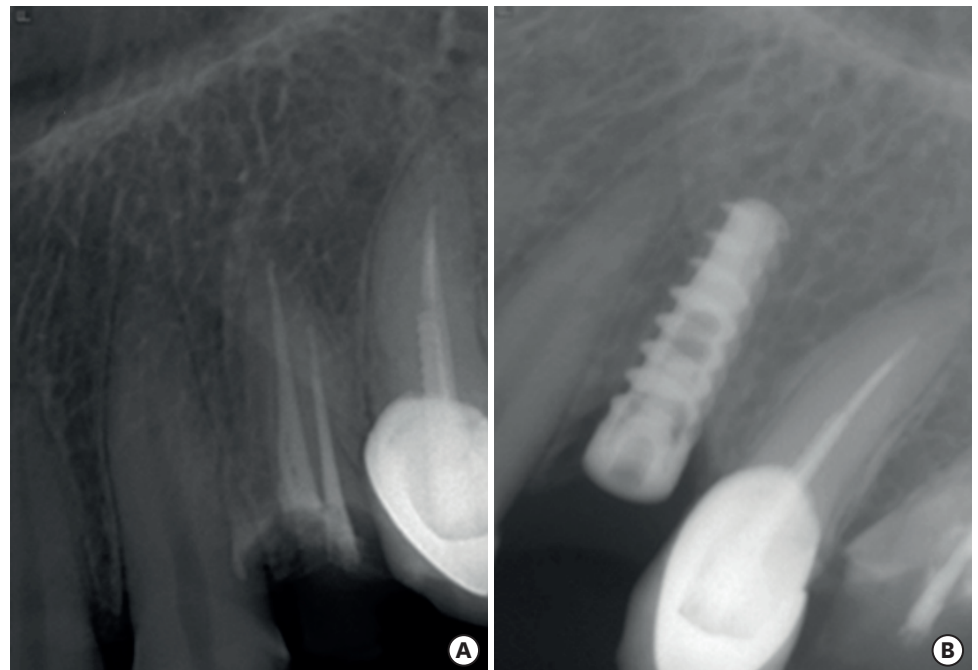


Figure 3. (A) Control decayed tooth 14 preoperatively. The socket was left to heal for 6 months. (B) Periapical X-ray 6 months after implant placement.

The preoperative buccolingual width was measured using a horizontal line at the crest level. The vertical height was measured as the length of a perpendicular line drawn at 90° to the horizontal plane until the nearest anatomical landmark was encountered. Sequential slices (5–9 slices) were taken, the buccolingual width and the vertical height were measured for each, and the average was used to ensure consistency in measurements. The abovementioned adjustments were made for both the control group and the grafted group (Figure 4A and B). The same procedures were performed postoperatively except for the buccolingual measurements, which were acquired at the level of the crest, 1 mm from the crest, and 2 mm from the crest to ensure the consistency of measurements (Figure 4C and D).

Histomorphometric, histochemical, and immunohistochemical assessments

The biopsy samples from the SCPC-grafted and ungrafted (control) sockets were fixed in 10% formalin for 2 days and then decalcified for 14 days in 5% ethylenediaminetetraacetic acid (pH 7.0) and prepared according to standard protocols. The entire core biopsy was embedded into paraffin wax (Shadon Histocentre 3, Thermo Fisher Scientific Inc., Kalamazoo, MI, USA) and oriented top-to-bottom for cross-sectional slicing. All samples were serially sectioned using a microtome (Leica RM2025, Leica, Wetzlar, Germany). Four serial 5- μ m thin sections were collected every 200 μ m for histomorphometric, histochemical, and immunohistochemical analyses. Two thin sections were stained separately with hematoxylin and eosin staining and Masson trichrome and analyzed by light microscopy. The other sections were incubated in a 0.1% sirius red dissolved in aqueous saturated picric acid for 1 hour, washed in acidified water (0.5% hydrogen chloride), dehydrated, and then mounted with DPX mounting medium. The sirius red-stained sections were examined using a polarized light microscope (BX60 supplemented with a U-POT polarizing lens, Olympus, Tokyo, Japan) to analyze the type and quality of mineralized collagen in the newly formed bone. Immunostaining of osteopontin (OPN), a non-collagenous protein

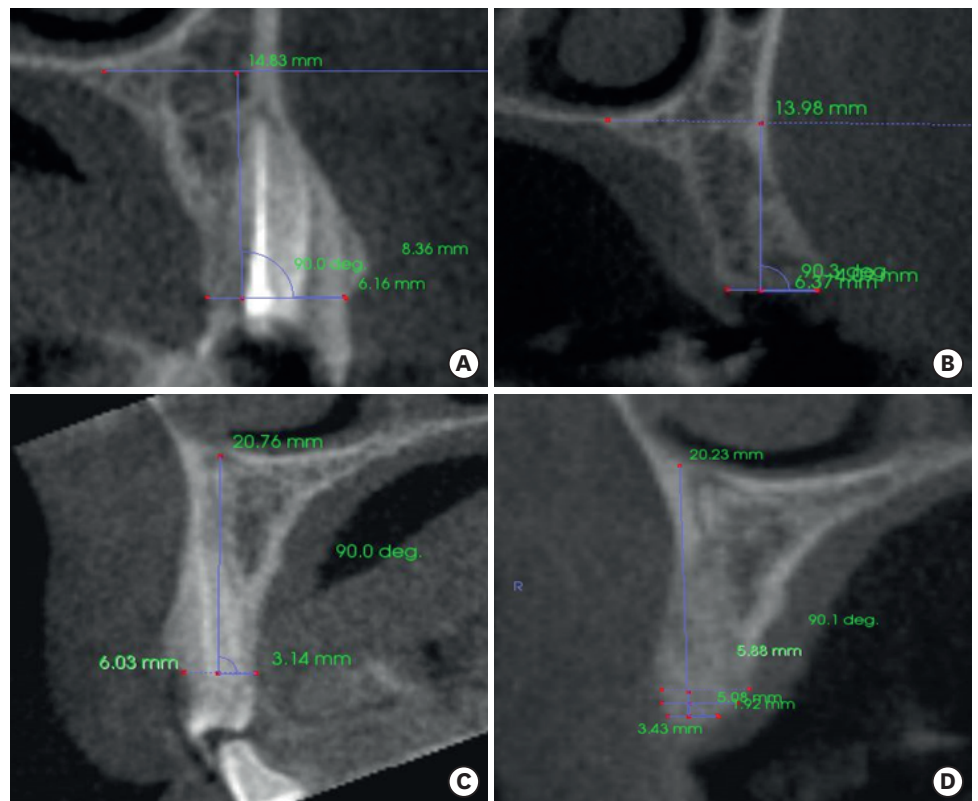


Figure 4. (A) Control group before tooth extraction showing the measured buccolingual width and the measured height. (B) The buccolingual width and height 6 months after extraction.

bone marker, was performed using a universal kit (Lab Vision). Thin sections were cut and mounted on positively charged glass slides, deparaffinized with xylene, rehydrated in graded ethyl alcohol, immersed in citrate buffer solution (pH 4.8), and put in a microwave oven before staining procedures. Hydrogen peroxide (3%) was applied to the sections to block endogenous peroxidase activity. Sections were immunostained using a primary monoclonal lyophilized antibody (clone OP3N) against OPN following the manufacturer's instructions (Visionbiosystems Novocastra™ Laboratories, Ltd, Newcastle Upon Tyne, UK), then incubated overnight at room temperature after rinsing with phosphate-buffered saline (PBS). Sections were then covered by the link antibody, followed by the streptavidin labeling antibody. After rinsing with PBS, diaminobenzidine tetrahydrochloride chromogen was applied to the sections followed by a counterstain; then, the sections were dehydrated in graded alcohol, cleared in xylene, and mounted.

For histological evaluation and histomorphometric analysis, 20 photomicrographs from different sections taken at each 200 μm of each biopsy sample were captured at the original magnification (×10, ×20, and ×40) using a digital camera (C5060, Olympus) mounted by a C-mount to a light microscope (BX60, Olympus). All the steps for the histomorphometric evaluation were carried out using image analysis software (ImageJ version 1.41a, National Institutes of Health, Bethesda, MD, USA). The area fraction of the newly formed mature bone, residual graft, and woven bone was calculated as a percent of the total surface area. The number and size of residual graft particles were also calculated. In the core biopsy samples taken from the SCPC-grafted sockets, the coronal and apical parts were marked, and the apical part was in contact with the native bone.

Statistical analysis

Mean values and standard deviations were calculated for each outcome variable. For each group, the obtained values were compared using the nonparametric Mann-Whitney *U* test to compare the SCPC and control groups. The Wilcoxon signed-rank test was used to compare the histomorphometric bone percent of the coronal and apical parts in the SCPC group. *P* values <0.05 were considered to indicate statistical significance. Statistical analysis was performed using StatsDirect (version 3.0.150, StatsDirect Ltd, Birkenhead, UK).

RESULTS

Clinical results

In all cases, healing was uneventful, with no signs of infection, swelling, or edema. The demographic composition of patients was non-proportional, as the selected patients included 2 men and 8 women. However, since all patients had 2 non-restorable teeth, each patient had a control tooth and a test tooth. Nonetheless, better clinical and radiographic results would have been obtained if there had been an equal distribution of patients according to sex.

Table 1 presents the preoperative and postoperative measurements of buccolingual bone width, keratinized soft tissue width, and gingival thickness. Measurements of clinical buccolingual bone width in SCPC-grafted sockets showed comparable bone width 6 months postoperatively. In contrast, a statistically significant decrease in the buccolingual bone width was observed for the control ungrafted sockets. The measurements of keratinized tissue width demonstrated a slight increase in SCPC group, whereas there was a significant decrease in the control ungrafted sockets. Moreover, while the gingival thickness increased in the SCPC group by about 35%, that of the control group decreased by about 50%.

Radiographic results

The radiopaque SCPC particles were easily recognized inside the grafted sockets by periapical X-rays immediately post-grafting (Figure 2B). Six months later, the cribriform plates of the grafted sockets were no longer recognizable and the radiopacity inside the socket was comparable to that of the host bone (Figure 2C), indicating that graft material resorption and new bone formation had both occurred. The strength of the newly formed bone supported placement and stabilization of the implant (Figure 2D). The control ungrafted sockets showed less radiopacity than the grafted socket (Figure 3). Cone-beam computed tomography showed minimal changes in the ridge dimensions in SCPC-grafted sockets, with decreases of 0.39 mm (2.3%) and 1.79 mm (22.7%) in height and width, respectively (Table 2). The control (ungrafted) group showed statistically significant decreases in height (1.8 mm; 12.3%) and width (3.11 mm; 42.1%). The decrease in bone height and width in the SCPC-

Table 1. Clinical parameters (mean±standard deviation) in the SCPC and control groups

Clinical parameters	Groups	Preoperative	Postoperative	Mean difference	<i>P</i> value
Buccolingual bone width	SCPC	7.7±0.9	7.1±0.5	-0.6±0.8	0.28
	Control	8.5±0.8	6±0.7	-2.5±0.6	0.03 ^{a)}
Keratinized tissue width	SCPC	6.7±1.6	7±1.7	0.3±0.83	0.12
	Control	8.6±1.5	6.7±1.6	-1.9±0.9	0.37
Gingival thickness	SCPC	2±0.6	2.9±0.7	0.9±0.7	0.008 ^{b)}
	Control	2.1±0.9	1.2±0.5	-0.9±0.7	0.06

SCPC: silica-calcium phosphate composite.

^{a)}*P*≤0.05; ^{b)}*P*≤0.01.

Table 2. Measurements of bone height and width in SCPC-grafted and control ungrafted sockets, preoperatively and 6 months postoperatively

Measurements	Group	Preoperative	Postoperative	Mean difference
Bone width	SCPC	7.88±1.66	6.1±0.79	1.79±0.95
	Control	7.4±1.5	4.2±1.7	3.11±1.1
<i>P</i> value				<i>P</i> ≤0.001
Bone height	SCPC	17.1±3.7	16.7±4.2	0.39±1.48
	Control	14.6±0.71	12.8±3	1.8±0.7
<i>P</i> value				<i>P</i> ≤0.001

SCPC: silica-calcium phosphate composite.

grafted group was significantly lower than the corresponding values in the control ungrafted group (*P*<0.001).

Histological analysis

Histomorphometry

The average surface area percentage occupied by new bone in the SCPC-grafted sockets was 41.3%±12% (Figure 5A). A significantly higher percentage (49.4%±9.3%) of new bone was observed in the apical part of the SCPC grafted socket than in the coronal part (33.3%±8.9%) (Figure 5B). In contrast, the average surface area percentage occupied by woven bone in the apical part of the SCPC-graft sockets was 18.6%±9.5% and that in the coronal part was 29.4%±17.3%. The average surface area percentage occupied by residual SCPC granules was 5.3%±4.4% (Figure 5A). The residual SCPC granules accounted for 9.46%±5.3% of the area in the coronal part and only 2.7%±1.5% in the apical part, which was a significant difference (Figure 5B). An analysis of the number and size range of the SCPC particles remaining in the socket showed that there were 1-12 particles ranging in size from 7 to 100 µm. Figure 6 shows a quantitative analysis of the surface area percentage occupied by SCPC granules in serial sections of the entire core biopsy samples from the coronal to apical direction. At a coronal depth of 0.2 mm, there was 13%±6% residual bone graft, which decreased to 1%±0.6% at a depth of 4 mm in the apical direction. At a depth of 4 mm from the socket surface, close to 1% of the surface area was occupied by the SCPC granules, indicating nearly complete resorption of the graft material. SCPC resorption was associated with increased new bone formation, as shown in Figure 6.

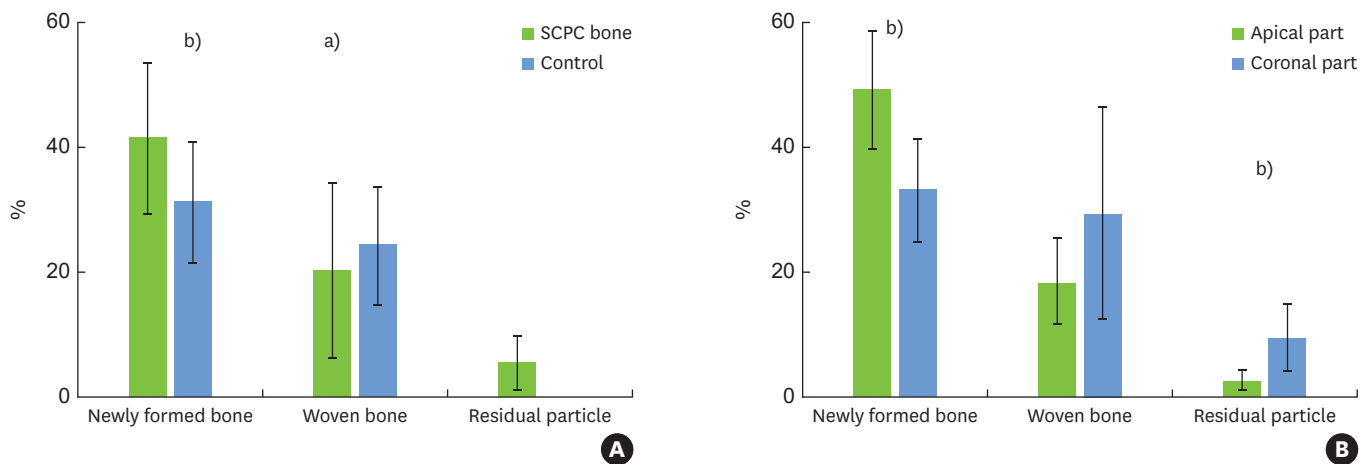


Figure 5. (A) A bar graph showing the mean surface area percentages of newly formed mature bone, woven bone, and residual particles in the SCPC-grafted and control ungrafted sockets. (B) A bar graph showing the mean surface area percentages of new mature bone, woven bone tissue, and residual particles in the coronal and apical part of the SCPC graft.

SCPC: silica-calcium phosphate composite.

^{a)}*P*≤0.01; ^{b)}*P*≤0.001.

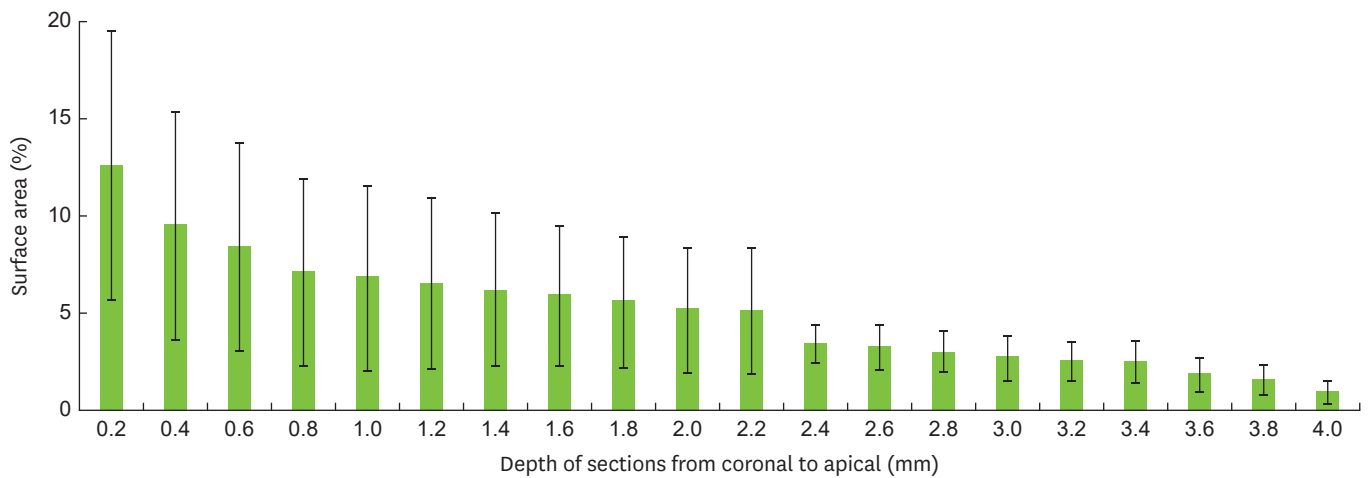


Figure 6. A bar graph showing gradual coronal-to-apical resorption and decrease in the surface area percentage occupied by SCPC granules in serial sections of a core bone biopsy obtained 6 months post-grafting in extraction sockets. A significant drop in the surface area percentage of the residual graft was observed, from 13%±6% within the first 0.2 mm of the coronal part to 1%±0.6% at the apical part of the sample. SCPC: silica-calcium phosphate composite.

Histological, histochemical, and immunohistochemical assessments

In the cross-sectional analysis, light microscopy showed near-complete bone regeneration and graft material resorption inside the grafted sockets (Figure 7). New bone formation was seen both in the core and in the peripheries of the bone biopsy samples. In conjunction with new bone formation, significant graft material resorption was observed. Woven bone and few remnants of SCPC particles were seen in direct contact with the newly formed bone. The black arrows point out the newly formed bone growth in the interspace between the SCPC particles. Areas with woven bone and high levels of cellular activity can also be seen, indicating the progress of bone healing (Figure 8A). Analysis at a higher magnification showed maturation of the newly formed bone, as indicated by the presence of numerous osteocytes (black arrow heads in Figure 8B), Haversian systems, and blood vessels. Osteoblasts (white arrows) can be seen aligned on the mineralization front on the surface of the newly formed bone (Figure 7B and C) and on the surface of the SCPC particles (black arrows in Figure 7C). Figure 7D shows new bone formation within the cracks (red arrows) and in the interspace between the SCPC particles. The control ungrafted sockets (Figure 7E) showed thin bone spicules separated by empty space and woven bone tissues. The newly formed bone appeared porous and contained osteocytes (Figure 7F).

Sirius red-stained sections taken from the core biopsy of the SCPC-grafted sockets examined by polarized light microscopy revealed that the newly formed bone was packed with thick organized mineralized collagen type I (red color) (Figure 8A). In contrast, bone tissue taken from the control ungrafted sockets (Figure 8B) demonstrated less packing of randomly oriented thin collagen I fibrils, <50 nm in width, mixed with collagen type III fibers (green–yellow color). Masson trichrome staining confirmed the maturation of the newly formed bone in the SCPC-grafted socket, where the majority of the newly formed bone stained red, indicating maturation (Figure 8C). In contrast, the blue staining of the new bone in the control ungrafted socket (Figure 8D) was indicative of woven immature bone. Immunohistochemical analysis showed expression of OPN in the newly formed bone in the grafted sockets (Figure 8E) and control ungrafted sockets (Figure 8F). However, the OPN expression was more intense in the grafted samples, with accentuation of the reaction at the periphery of the newly formed bone trabeculae and along the incremental lines (cement

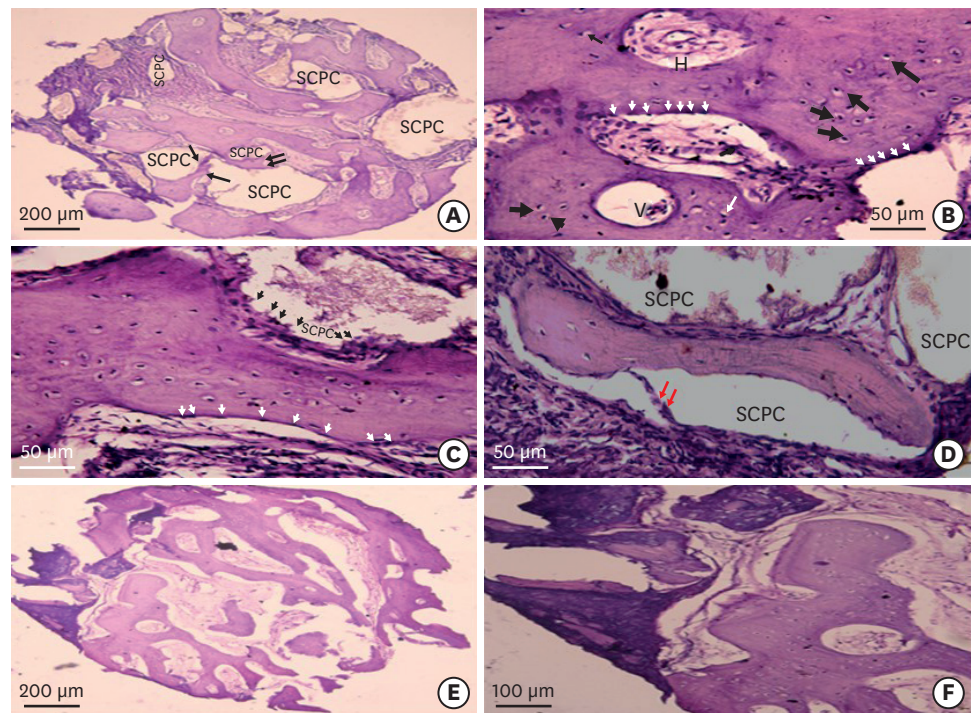


Figure 7. Photomicrographs of hematoxylin and eosin-stained sections of core biopsy samples from sockets grafted with SCPC granules (A-D) and control ungrafted sockets (E, F) after 6 months, showing (A) bone regeneration and SCPC graft resorption. (B) The maturity of the newly formed bone was indicated by the presence of numerous osteocytes, osteoblasts, blood vessels, and Haversian systems. (C) Erosion of the surface of SCPC granules in the presence of osteoblasts attached at the material surface (black arrows), osteoblasts lining the mineralization front of the newly formed bone (white arrows). (D) New bone formation in the interspace between particles and with cracks inside the SCPC particles. (E) Thin bone spicules in newly formed bone in the control ungrafted extraction socket after 6 months. (F) Osteocytes in the newly formed bone surrounded by woven bone tissue in the control ungrafted defect. The darkly stained area indicates fibrous immature connective tissue. SCPC: silica-calcium phosphate composite, H: Haversian systems, V: blood vessels.

lines) of bone. The bone marrow cells and some of the connective tissue cells surrounding the bone trabeculae also showed OPN immunorexpression.

DISCUSSION

Socket augmentation procedures aim to limit the adverse volumetric changes associated with bone resorption during healing following tooth extraction. Such augmentation may lead to further extension of the ridge volume more than was present at the time of extraction [22,23]. Factors that contribute to successful socket augmentation include atraumatic tooth extraction, socket cleaning, use of a resorbable bone bioactive graft, availability of good blood supply, placement of a coronal barrier using a gingival flap or membrane, and a sufficient healing period [24,25]. The present study provided clinical, radiographical, and histological evidence of the successful augmentation of extracted sockets grafted with a resorbable SCPC. Combining socket augmentation with socket-sealing surgery can provide a good volume of soft tissue prior to implant placement [26]. Therefore, in our study we chose to seal the socket tightly with a free gingival graft in order to facilitate soft tissue gain. The free gingival graft seal minimized soft tissue shrinkage and assisted in initial graft containment, while possibly providing some barrier function. The newly formed bone in the SCPC-grafted socket was mature and vascularized, demonstrating bone lamellae packed with thick mineralized

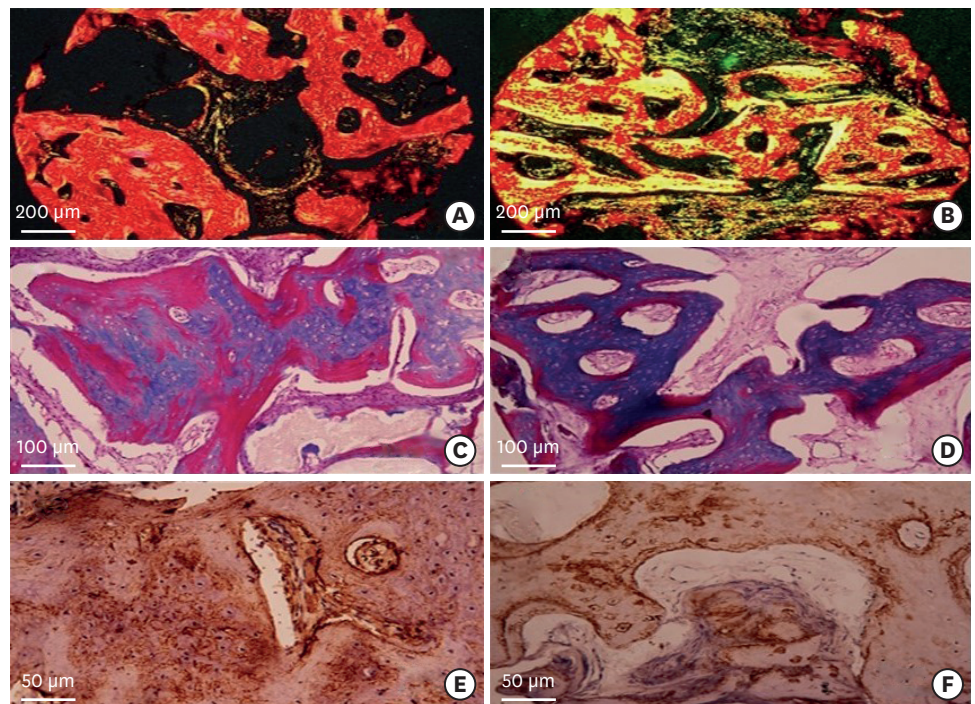


Figure 8. (A, B) Polarized light microscopy of sirius red-stained sections, showing (A) a high density of red-stained newly formed bone and connective tissue, which is indicative of well-packed thick mineralized collagen type I in SCPC-grafted sockets. (B) The predominance of yellow-green is indicative of collagen type III and the limited red staining is indicative of low synthesis of collagen type I in the newly formed bone in the control ungrafted sockets. (C, D) Masson trichrome-stained sections, showing (C) a high density of red-stained newly formed bone, indicative of mature bone formation in the SCPC-grafted socket, and (D) predominance of blue-stained areas in the control ungrafted socket, which is indicative of immature woven bone. (E, F) Osteopontin immunohistochemical staining, showing (E) intense osteopontin immunoreactivity by activated osteoblasts and along the incremental lines of the newly formed bone in SCPC-grafted sockets, and (F) weak osteopontin immunoreactivity in the newly formed bone and osteoblasts in control ungrafted sockets. SCPC: silica-calcium phosphate composite.

type I collagen, osteocytes, Haversian systems, and osteoblasts. In conjunction with bone regeneration, nearly complete resorption of the graft material was observed 6 months postoperatively. The newly formed bone provided successful and sustained volumetric and implant stability. Histomorphometry demonstrated a statistically significant higher quantity of new bone in the SCPC-grafted sockets than was formed in the control ungrafted sockets, which demonstrated immature woven bone rich in type III collagen with a limited amount of type I collagen.

The selection of an SCPC bone graft was based on previous clinical and animal studies, which demonstrated that SCPC had a superior effect on alveolar bone regeneration when used for sinus augmentation treatment compared to a hydroxyapatite ceramic or when used to treat mandibular saddle-type defects [17,27]. The superior bone bioactivity and resorbability properties of SCPC are attributable to the engineered crystalline structure of the 2 phases incorporated in the graft material; beta-rhenanite (β -NaCaPO₄) and alpha cristobalite (SiO₂) solid solutions. The silica phase in SCPC has been shown to stimulate osteogenic gene expression and to provide guided bone tissue growth. Several *in vitro* and *in vivo* studies have shown correlations between silica dissolution from SCPC and enhanced bone formation and cell-mediated resorption compared to 45S5 bioglass [17,28-30].

The application of a free gingival graft preserved the SCPC granules in the sockets and facilitated a significant increase in the thickness of the gingiva and keratinized tissue, as shown in Table 1. The increase in soft tissue thickness can be attributed to remodeling in the graft and revascularization by the plasmatic circulation. This favorable effect was not observed when other resorbable membranes were used. Previous socket preservation using collagenated porcine bone covered with resorbable collagen membrane showed a limited decrease in the contour of the alveolar ridge [31]. The buccolingual bone width decreased on average by 3.87 mm following tooth extraction without augmentation, mainly due to resorption of the surrounding bundle bone [1]. The resorption of bundle bone is enhanced by the loss of blood supply provided by the periodontal ligament of the extracted tooth. This significant decrease in bone volume and density poses risks for the successful placement and stabilization of the dental implant. The slight decrease (0.6 mm) in the buccolingual bone width in the SCPC-grafted sockets was comparable to that reported when allografts were used together with acellular dermal matrix or a polytetrafluoroethylene membrane [32].

Immunohistochemical and histomorphometric analyses of the core bone biopsy samples demonstrated significant regeneration of mature bone in the SCPC-grafted sockets. Bone maturation was confirmed by the homogeneous distribution of healthy osteocytes, as well as the organized Haversian canals and blood vessels in the newly formed mineralized tissue. Of considerable interest was the presence of high levels of osteoblastic activity at the surface of the newly formed bone, indicating the continuing progress of mineralized tissue formation. Moreover, numerous osteoblasts were seen at the interface with the residual SCPC particles, indicating cell-mediated resorption of the graft material during new bone formation. Sirius red and Masson trichrome staining provided evidence for the maturation of bone by demonstrating deep staining of mineralized collagen type I [6]. Further evidence of bone maturation was demonstrated by the increased intensity of OPN in the mineralized tissue and cells present in the SCPC-grafted sockets. OPN is a non-collagenous bone marker that is produced by osteoblastic cells at various stages of differentiation, as well as by osteocytes, and accumulates in the mineralized bone matrix [33,34]. The intense immunoreexpression of OPN at the interface between the remaining grafted particles and tissue is indicative of the stimulatory effect of SCPC on osteoblastic differentiation. Previous cell culture studies have also demonstrated a significant increase of both OPN and osteocalcin expression by osteoblasts incubated with SCPC [15,17]. Clinical studies have reported the expression of osteocalcin in core biopsies 4 months after sinus lifting with SCPC and implant placement [27].

Quantitatively, 41% lamellar bone and 21% woven bone were present in the SCPC-grafted sockets. The formation of a high amount of mature bone observed in our study is superior to that reported in earlier studies on sockets grafted with mineralized freeze-dried bone, which reported a 65% proportion of mineralized tissue, of which 41% was new bone, mostly woven bone, formed 6 months after grafting [35]. The enhanced bone formation and maturation in SCPC-grafted sockets could be attributed to the controlled release of silicate ions, which play an important role in stimulating osteogenic gene expression and bone formation [29,36]. Mineralization with this material is further enhanced by the alkaline pH created when the SCPC bioactive ceramic releases sodium ions into the surrounding tissue fluids. Recent studies have shown that both silica ions and alkaline pH stimulate bone cell activities and downregulate osteoclast function [37,38]. Moreover, the coronal and apical bone percentages in the SCPC-grafted sockets were 33% and 49%; the resulting average of 41% is higher than the 30% average bone formation reported for sockets grafted with biphasic calcium sulfate alone or in combination with an allograft [33]. In addition, the average residual SCPC graft in

our study was 5.3%, which was slightly lower than the 7% residual graft reported for biphasic calcium sulfate-grafted sockets [6]. Data in the literature have shown correlations between the cell-mediated resorption of SCPC and new bone formation [14,39]. Other studies have demonstrated the formation of a biomimetic calcium-deficient hydroxyapatite surface layer similar to the mineral phase of bone on the surface of SCPC after immersion in physiological solution [17,28-30].

In conclusion, clinical, radiographic, and histological analyses confirmed bone regeneration and graft material resorption in extraction sockets grafted with resorbable, bioactive SCPC granules in humans in the desired time frame between extraction and implant placement. The vitality and functionality of the newly formed bone facilitated placement and stabilization of endosseous dental implants. The demographic distribution of the patients was non-proportional, meaning that care is needed in the interpretation of the findings. However, this issue was overcome by the split-mouth design, so that each patient belonged to both the control and study groups. Histomorphometry showed percent areas of 41.3%±12% new mature bone, 20.1%±14% woven bone tissue, and 5.3%±4% residual SCPC granules in the SCPC-grafted sockets. Histology and immunohistochemistry demonstrated maturation of the newly formed bone, as indicated by the presence of osteocytes, Haversian systems, blood vessels, compact mineralized type I collagen, and high immune staining for OPN. The control ungrafted sockets showed a lower quantity of immature woven bone and more volumetric collapse in the apico-coronal and bucco-lingual dimensions. Taken altogether, the present study demonstrates that SCPC is a resorbable bone-grafting material that preserves extraction socket volume and enhances bone formation and maturation, providing favorable support for implant placement.

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