Histopathologic reaction of a calcium phosphate cement for alveolar ridge augmentation

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Abstract: The objective of the present study was to evaluate the feasibility of using a calcium phosphate cement (CPC) in the reconstruction of a defective alveolar ridge in conjunction with implant placement. The CPC consisted of an equimolar amount of tetracalcium phosphate and dicalcium phosphate anhydrous. At the beginning of the experiment, all mandibular premolar teeth of mature beagle dogs were extracted. After 1 month of healing, alveolar bone was reduced to make a space for a CPC block that was prefabricated from a CPC mixed with water at a powder/liquid ratio of 5 g/mL. After an additional month, 8-mm long hydroxyapatite-coated titanium implants were placed in such a way that the apical half was embedded into alveolar bone and the coronal half in the preformed CPC block. The dogs were sacrificed and biopsies were obtained at 1, 3, and 6 months after surgery. Sections that included implants were evaluated for integration of the CPC block to the alveolar bone and of the implant to the alveolar bone. Additional sections without the implants served as controls. The results obtained from this study show that the CPC ridge augmentation gradually is replaced by natural bone. Six months after surgery, histopathologic features of the augmentation area were quite similar to those of natural alveolar bone. The coronal half of the implants, previously surrounded by the CPC block, was firmly fixed by natural bone. Therefore, this method may be useful for increasing the height of the alveolar ridge. © 2002 Wiley Periodicals, Inc.* J Biomed Mater Res 61: 47–52, 2002

Key words: histopathologic reaction; alveolar ridge augmentation; calcium phosphate cement (CPC); CPC block; implant

INTRODUCTION

The quality of alveolar bone at the desired location is one of the most important prerequisites for an ideally functional implant. Reconstructive surgery prior to an implant procedure usually is required if alveolar

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deficiencies are present. Reconstructive surgery can be performed with a number of techniques, including guided bone regeneration (GBR),^{1–3} in which an occlusive barrier membrane is placed between the connective tissues and the residual alveolar bone to create a space for the formation of new bone. These osseous defects can be treated with either the barrier membrane alone or in combination with bone-graft materials, such as autogenous bone,^{4,5} allogenic bone materials,⁶ and calcium phosphate alloplastic materials.⁷

In general, with the use of autogenous bone graft successful osseous regeneration occurs in a most predictable manner. However, it would be quite difficult to reconstruct a large defect with intraoral autogenous bone-graft materials.^{4,5} Implant placement should not be performed until there is normal bone formation at the surgical site. When autogenous bone successfully achieves ridge augmentation, it takes 6 to 8 months^{8,9} for the necessary substantial reconstructive bone healing before implantation can be undertaken. Obviously, it would be highly desirable if ridge augmentation and implant placement could be performed in the same procedure.

Previous studies have reported that the chemical reaction of a calcium phosphate cement (CPC) consisting of $Ca_4(PO_4)_2O$ and $CaHPO_4$ proceeded in the pH range from 7.4 to 9 until the hardening^{10–12} and that the CPC was nearly completely converted to hydroxyapatite (HA) within 24 h in an aqueous environment. The setting reaction of CPC can be expressed by the equation

$$\begin{array}{c} H_2O\\ Ca_4(PO_4)_2O + CaHPO_4 \xrightarrow{I} Ca_5(PO_4)_3OH \end{array}$$

The set CPC consisted mostly of rod-like crystals that were extremely small (~50 nm in width and ~1000 nm in length). The volume occupied by water contains essentially no calcium phosphate materials. As a result, the set CPC has a high pore volume with microscopic pores (mean pore diameter = 2 nm).¹⁰

An increase of alkaline phosphatase activity, which is closely related to new bone formation,^{13–15} indicated enhanced osteoconductivity in the presence of CPC.¹⁶ CPC was shown to be highly biocompatible and osteoconductive when used to fill artificially formed bone pockets or periodontal pockets in dog jaws.¹⁷⁻¹⁹ The CPC that was used to fill periodontal bone defects in a dog model was completely converted to natural bone within 6 months.²⁰ Friedman et al.²¹ reported that CPC used for human frontal cranial defects (maximum size of 25 cm²) showed successful reconstruction at 24 months. Because of its self-hardening and in vivo resorption properties, CPC has the potential to be useful in a number of clinical applications, including alveolar ridge augmentation. The objective of the present study was to evaluate the feasibility of using CPC in the reconstruction of a defective alveolar ridge in conjunction with implant placement.

MATERIALS AND METHODS

The study protocol was reviewed and approved by the institution review board. Mature (4 to 5 years old) beagle dogs were used in this study. The outline of the study is illustrated in Figure 1. Materials used in this study are shown in Table I.

Surgical anesthesia was induced by injecting pentobarbital (Nembutal, Abbott Laboratories, North Chicago, IL; 0.5 mL/kg of body weight), supplemented with the local administration of lidocaine-HCl (2% Xylocaine, Astra Japan Ltd., Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan) to reduce hemorrhage in the surgical sites. At the beginning of the experiment, all mandibular premolar teeth were extracted. One month after the extraction, mucoperiosteal flaps



Figure 1. Experimental design.

were turned over, and then osteoplasty or ostectomy procedures were performed to further reduce the alveolar ridge [Fig. 2(a)].

After taking an impression of the exposed alveolar bone using an alginate impression material, a plaster cast of each exposed alveolar bone was formed from the impression. Mucoperiosteal flaps were replaced in their preoperative position and secured with black braided 4-0 silk suture (Ethicon Inc., New Jersey). A self-cured resin block with a maximum height of 4 mm was prepared on the plaster model and used as the reference model for the ridge augmentation and implant procedures [Fig. 2(b)]. This block (20-25 mm of mesidistal and 10–15 mm of buccolingual lengths) had holes (3.5–4 mm in diameter) that would be used as reference points for the implant drilling procedure [Fig. 2(b)]. A CPC block was duplicated from the resin block by using a silicon impression material (Examixfine; GC Co., Tokyo, Japan) after pouring CPC paste, prepared at a powder-to-liquid ratio of 5 g/mL, into an impression model of the resin block. The CPC paste was kept in the impression model for 24 h in 100% humidity at 23°C, and the hardened block then was

TABLE I Materials Used in This Study

Material	Acronym	Source
Tetracalcium phosphate (Ca ₄ (PO ₄) ₂ O)	TTCP	ADAHF, NIST Gaithersburg, MD
Dicalcium phosphate anhydrous (CaHPO ₄)	DCPA	J. T. Baker, Inc. Phillipsburg, NJ
Equimolar amounts of TTCP and DCPA	CPC	ADAHF, NIST Gaithersburg, MD



Figure 2. (a) Occlusal view of surgical region (above) and the eliminated alveolar bone (below); (b) schematic drawing of CPC block and placement on the eliminated alveolar bone; and (c) correct implant placement (above), CPC block (middle), and CPC block placement on the residual alveolar bone (below).

removed from the impression model. The CPC block was kept in a desiccator at 23°C and sterilized by ethylene oxide gas (E.O.G., Ikiken Co., Ltd., Tokyo, Japan) just before the surgery.

One month after osteoplasty, mucoperiosteal flaps were reflected again, and the CPC block was placed directly over the previously defined ridge site. Standard-sized holes (3.3 mm in diameter, 4 mm in depth) were drilled into the al-



Figure 3. (a) Histopathologic features of decalcified buccolingual section 1 month after surgery (below) and at high magnification (above). CPC: calcium phosphate cement, AA: augmentation area, RA: residual bone area, TB: trabecular bone, CB: cortical bone, HL: Harversian lamellae, WB: woven bone, FCT: fibrous connective tissue. Hematoxylin–eosin stain. (b) Subperiosteal new bone (SNB) formation with a smooth surface (between arrows) on the outer residual cortical bone (RCB) at 3 months. Hematoxylin–eosin stain. (c) Histopathologic features of decalcified buccolingual section 3 months after surgery (below) and at high magnification (above). CLB: cancellous bone, BMC: bone marrow cavity. Hematoxylin–eosin stain. (d) Histopathologic features of decalcified buccolingual section (above). HL: Harversian lamellae, NB: new bone, ABM: adipose bone marrow. Hematoxylin–eosin stain.

veolar bone through the holes in the CPC block by using a tungsten-carbide bur under saline solution cooling [Fig. 2(c)]. Commercially obtained HA-coated cylindrical implants (8 mm in length, 3.3 mm in diameter; IMZ Twin Plus, Friatec Medical Devices Division, Dental Section, Mannheim, Germany) then were placed in the mandibular alveolar bone. The apical 4-mm length of the implants was inserted into alveolar bone and the coronal 4-mm length of the implants was outside of the alveolar bone and surrounded by the CPC block. The spaces between the CPC block and the implant or the crestal alveolar bone surface were filled with CPC paste. The mucoperiosteal flaps were repositioned and sutured to obtain primary closure of the buccolingual flaps without exposure of the CPC block and the implants [Fig. 2(c)]. No periodontal dressing or antibiotic medication was provided. After surgery, daily plaque control was carried out using cotton balls moistened with saline.

The dogs were sacrificed at 1, 3, and 6 months after surgery,^{22–24} and in each case biopsy was excised *en bloc*. The block specimens, including the implants, were fixed with a volume fraction of 10%-neutralized and buffered formalin and sectioned buccolingually.

The augmentation block without implants was examined for the osseous replacement of the CPC block, which was decalcified with Plank-Rychlo solution (CS-5151; Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan) and embedded in paraffin wax. Subsequently, paraffin-embedded portions of decalcified sections were cut into 6-µm sections and stained with hematoxylin and eosin for histopathologic examinations.

The other block with implant was dehydrated by passage through a series of water–ethanol solutions with ascending ethanol concentrations and then dried with a critical-point dryer and, finally, embedded in methyl methacrylate resin (Nissin EM Co., Ltd., Tokyo, Japan). This portion was cut into the thickness of 100- μ m sections with a microtome, and further ground to 30- μ m thick specimens. These specimens were stained with hematoxylin and eosin and used for the observation of the implants and the surrounding tissues using light microscope (Vanox-S, Olympus, Tokyo, Japan).

RESULTS

Histologic findings of the sections without implants

One month after surgery

Newly formed woven bone (WB), which was immature new alveolar bone, was observed with fewer inflammatory cells in the portion adjacent to the residual bone area (RA) and also at the crestal level of the CPC block augmentation area (AA). The CPC block had been mostly replaced by newly formed WB and fibrous connective tissues (FCT). The augmentation block area also was in complete union with the residual bone surface. Original osseous tissues extended into the AA from the residual alveolar bone. Some sections showed that the outer portion of the AA already was covered with cortical bone (CB) with Harversian lamellae (HL). New trabecular bone (TB) also was formed in the AA. Many activated osteoblastic cells were observed in same area. Osteoclastic resorption and the residual CPC domains, which should have been mostly converted to HA, also were observed. These histologic findings were not found in the residual bone area (RA). The outline of the AA was quite similar to that of the natural bone surface (SNB) [Fig. 3(a)].

In addition of these findings, subperiosteal new bone (SNB) formation with a smooth surface was observed on the residual cortical bone (RCB) surface. This was true in the 3-month [Fig. 3(b)] and the 6-month specimens as well as in the 1-month specimens.

Three months after surgery

The AA had been nearly completely replaced by newly formed TB and FCT and also was remodeled by CB and cancellous bone (CLB). Osteoclastic resorption and residual CPC domains still were found in some areas. CB in the periphery enclosed CLB with welldefined TB. Many activated osteoblastic cells were found in the endosteal surface of the bone-marrow cavity (BMC). The CB of the AA was much thicker than that of the 1-month section [Fig. 3(c)].

Six months after surgery

The augmentation block had been nearly completely replaced with CB and TB. HL were distinctively observed. External and interstitial lamellae also were found. The osteoblastic activity was virtually quiescent in the AA. Adipose bone marrow (ABM) formation was observed among FCT. These histologic features were very similar to those of the normal alveolar bone [Fig. 3(d)].

Histologic findings of the sections including implants

One month after surgery

Residual alveolar bone was in contact with the implant surface in the apical area. Newly formed bone tissues were generated from the RCB. The CPC augmentation block, which was similar in shape to the original bone, was in direct and complete contact with the residual bone and the implant surface. The augmentation block area was distinguishable from the implant and the RA [Fig. 4(a)].

Three months after surgery

The histologic findings were almost the same as those observed at 1 month. The implant was fixed and covered by residual and newly formed bone tissues. The augmentation block had been mostly replaced by normal bone tissues. The new bone formation, appearing to occur from RA, was extended into the AA. The apical half of the implant was completely surrounded by new bone tissues. The coronal half of the implant was surrounded and fixed by the augmentation materials, which already had been replaced by newly formed bone and FCT [Fig. 4(b)].

Six months after surgery

A sagittal section including the implant showed well-formed osseous tissues. The implant was completely covered by normal bone tissues. This was true on the entire surface of the implant. Haversian lamellae predominantly were observed, and relatively thick external lamellae also were found. The CPC augmentation block including the implant had been completely replaced with normal bone tissue [Fig. 4(c)].

DISCUSSION

Firmly anchored and osteointegrated implants may be affected by bone conditions, for example, deformity of morphology, quantity, and quality of normal alveolar bone, etc. A vertical gain of alveolar ridge height was obtained by placing a membrane over the implant and the alveolar bone.^{1,2} However, membrane collapse and lack of soft tissue coverage are likely to occur in the vertical augmentation procedures because of inadequate supply of blood and tissue fluid to the covering soft tissue. As a result, the membrane technique usually is unable to obtain ideal alveolar bone morphology. Reconstructive surgeries using the former graft materials had not been defined clearly for the reconstruction of defective alveolar bone ridge.^{25,26}

The CPC block used in the present study had sufficient strength for alveolar augmentation, and it was porous so that it could be supplied with blood circulation from the periosteal flap and the residual bone for healing or osteoconductivity. The height of the CPC augmentation block used in this study was restricted to within 4 mm because Simion²⁷ demonstrated that human augmentation procedures could not generate alveolar bone over 4 mm in vertical height. The spaces between the CPC block and the implant or the coronal alveolar bone surface were filled with the CPC paste. The paste, used as a grouting material, has the ability to adapt closely to the irregular bone surfaces and to harden uniformly. The results from this study suggest that the CPC filling area is converted to natural bone more rapidly and more uniformly than are the presently used materials such as Apaceram (Asahi Kogaku Kogyo Co., Tokyo, Japan).

The results obtained from this study show that the CPC ridge augmentation block gradually was replaced by natural bone and that the implants, previously surrounded by CPC block, with time were cov-



Figure 4. Histopathologic features of nondecalcified buccolingual section including implant [1 month (a), 3 months (b), and 6 months (c) after surgery]. AA: augmentation area, RA: residual bone area, NB: new bone, IM: implant. Hematoxylin–eosin stain.

ered and firmly fixed by natural bone. CPC block appears to be clinically useful for vertical alveolar ridge augmentation coupled with implantation.

CONCLUSIONS

The CPC augmentation block gradually was replaced by newly formed bone tissues within 1 month after surgery. Six months after surgery, the augmentation area nearly completely had been replaced by natural bone. Osteointegrated implants, surrounded by the CPC block and CPC paste, were completely fixed by natural bone at 6 months after surgery. Therefore, this method using CPC may be useful for increasing the height of the alveolar ridge.

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