ORAL AND MAXILLOFACIAL IMPLANTS

Implant placement for periosteal expansion osteogenesis using β-tricalcium phosphate block: An experimental study in dogs

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Objectives. This study was performed to evaluate the clinical outcome of periosteal expansion osteogenesis for correction of a horizontally deficient alveolar ridge, stability of dental implants placed in the expanded areas, and osteocompatibility of β-tricalcium phosphate (β-TCP) block areas.
Study design. The mandibular premolars were extracted and buccal corticotomy was performed in 5 female dogs. Narrow alveolar ridge models were produced at 10 weeks. The β-TCP block was placed at the lateral surface of the mandibular bone and 2 titanium screws were inserted from the lingual aspect to push the block to the buccal side. After a latency period of 8 days, during which time primary wound healing occurred, the lingual screws were advanced by approximately 0.5 mm/day for 6 days. The expansion areas were left untreated for 8 weeks. Then implants (diameter 3.5 mm, length 9 mm) were inserted into the gap between the β-TCP block and the original alveolar bone. We evaluated the changes in alveolar width, resonance frequency analysis of implants, and histomorphometric analysis of the β-TCP block.
Results. No problems with the materials were observed at any of the sites of intervention before, during, or at the end of the experimental period. The width increased after expansion and showed stable results on week 8 from the end of expansion. Implants were placed in the expansion area and showed sufficient stability with slight increases in the implant stability quotient value until 8 weeks after implant placement. The amount of remaining β-TCP decreased significantly compared with the original amount of material inserted. The mean values remaining inside the block were 44.6 ± 8.2% and 32.1 ± 12.0% at 8 and 16 weeks of consolidation, respectively, whereas newly formed bone comprised 20.2 ± 7.2% and 33.5 ± 9.5%, respectively.
Conclusions. Newly formed bone could be acquired by periosteal expansion osteogenesis using a β-TCP block for implant placement in a dog model. However, the bone volume was not stable after implant placement despite sufficient implant stability for 8 weeks. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108:861-866)**

Alveolar bone augmentation is one of the standard treatments for dental implantation when alveolar bone volume is insufficient. A relevant vertical and/or horizontal defect of the alveolar ridge is still a challenge for appropriate implant placement and predictable long-term results. Such volume insufficiency can be overcome by augmentation procedures such as inlay or onlay bone grafting, guided bone regeneration (GBR) techniques,[1,2] and alveolar distraction osteogenesis (DO).[3,4] Autografts are considered to be the gold standard for maxillofacial bone reconstruction.[5] However, autogenous bone grafting may be associated with donor site morbidity and resorption of the grafted bone, and this technique cannot be used for simultaneous soft tissue augmentation.[2,6] Therefore, the amount of bone augmentation is usually limited. In contrast, DO is an alternative method that uses a biologic process in which new bone formation occurs between segments that are gradually separated.[3,7] This gradual traction of pedicled bone fragments is followed by simultaneous osteogenesis (bone) and histogenesis (functional soft tissue matrix).[3] However, disagreement exists regarding the various treatment parameters, such as surgical technique, type of distraction device, and minimal bone height and width necessary to perform the distraction.

Recently, osteogenesis by periosteal distraction or elevation without corticotomy for bone augmentation has been suggested. This method is based on the con-

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cept that tensile strain on the periosteum, which causes tenting of the subperiosteal capsule, is sufficient to produce bone formation without corticotomy or local harvesting of the bone.[8-12] Earlier studies indicated a new technical aspect of DO or tissue expansion, with the controlled guided formation of new bone. Previously, we investigated the utility of periosteal expansion osteogenesis (PEO), the same concept as periosteal DO or elevation, using a highly purified β-tricalcium phosphate (β-TCP) block instead of titanium devices in a dog model.[13] We found newly formed bone at the gap between the original bone and β-TCP block, and the β-TCP block acted as a space maker under the periosteum. However, few reports have described implant placement at the augmented bone area via periosteal activation.

The present study was performed to evaluate the clinical outcome of PEO for correction of a horizontally deficient alveolar ridge, the stability of dental implants placed in the expanded areas, and the osteocompatibility of β-TCP block areas.

MATERIALS AND METHODS

Preparation of the β-TCP block

Beta-TCP (Osferion) was obtained from Olympus Terumo Biomaterials (Tokyo, Japan), and fine β-TCP powder was synthesized by wet milling (a mechanochemical method). Calcium-deficient hydroxyapatite (HA) was obtained by milling dibasic calcium phosphate dihydrate and calcium carbonate at a molar ratio of 2:1 with pure water and zirconium beads, followed by drying at 80°C. This crystalline solid was converted to β-TCP by calcination at 750°C for 1 hour. Upon sintering the β-TCP powder at 1,050°C for 1 hour, a porous β-TCP block was obtained, which was then characterized by assessing the surface area and pore size distribution of the porous structure. The porous blocks (15 × 10 × 3 mm) were manufactured at high purity.

Surgical protocol

The protocol and guidelines for this study were approved by the Institutional Animal Care and Use Review Committee of Kyushu Dental College, Kitakyushu, Japan. Five female beagle dogs (weighing 10-15 kg) were used. The animals were anesthetized by intramuscular administration of ketamine hydrochloride (50 mg/kg), followed by diazepam (5 mg) and atropine sulfate (0.5 mg), without endotracheal intubation. Before the operation, 10 mg/kg pentobarbital sodium was injected intravenously. Immediately after the operation, the dogs received cefazolin sodium (20 mg/kg) subcutaneously, which was continued until postoperative day 3. The operation was performed under standard sterile conditions, and local anesthesia using 2% lidocaine with epinephrine was used during all surgical procedures. The mandibular premolars were extracted and a buccal corticotomy was performed in each dog. During 10 weeks of healing, a horizontal incision was made around the mucogingival junction and the mucoperiosteal flap was reflected, which exposed the lateral surface of the mandible. The β-TCP block was placed at the bone surface, and two titanium screws were inserted at the inferior border of the block to avoid inferior displacement. Another 2 titanium screws were inserted from the lingual aspect to push the block to the buccal side (Fig. 1). After checking the movement of the block, the screws were returned to the initial position. The wound was closed using 5-0 nylon. After a latency period of 8 days, during which time primary wound healing occurred, the lingual screws were activated to push the β-TCP block laterally and expand the lateral soft tissue by 0.5 mm/day for 6 days. Activation of the lingual screws was done under sedation using intramuscular administration of ketamine hydrochloride.

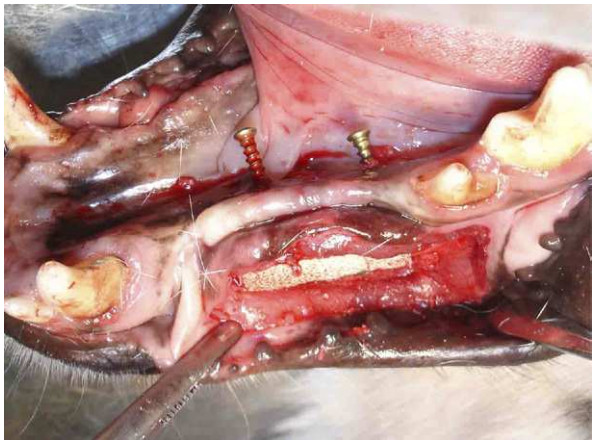

Fig. 1. Intraoperative photograph. The block was placed under the mucoperiosteum. Two titanium screws were inserted from the lingual aspect to push the block to the buccal side.

The expansion areas were left untreated for 8 weeks. Then mucoperiosteal flaps were raised, and the superior aspects of β-TCP and expanded areas were exposed. After the lingual screws were removed, implants were placed at the midpoint of the β-TCP block, and osteotomies for 1 implant were drilled according to the manufacturer's protocols. Implants were inserted such that the implant shoulder was located at the gap between the β-TCP block and original alveolar bone (3.5 mm in diameter, 9 mm in length; Astra Tech, Mölndal, Sweden) (Fig. 2). The flaps were sutured and the implants were left submerged with their cover screws in place for 8 weeks. Sutures were removed after 1 week.

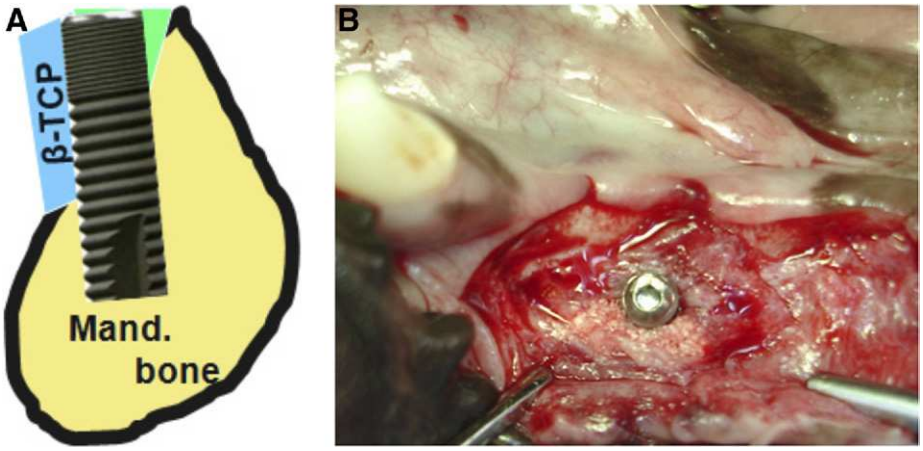

Fig. 2. **A,** Diagram showing that implants were inserted such that the implant shoulder was located in the gap between the β-TCP block and the original alveolar bone. **B,** Intraoperative photograph. The implant was placed in the gap between the block and original bone.

The dogs were killed by intramuscular administration of midazolam and ketamine and intravenous injection of pentobarbital sodium. Both mandibular experimental areas, including peripheral soft tissues and the β-TCP blocks, were carefully removed.

Evaluation of changes in alveolar bone width

Alveolar width was measured using bone calipers (YDM, Tokyo, Japan). The measurement point was 3 mm inferior to the top of the alveolar ridge and the midpoint of the β-TCP block. These measurements were made before and immediately after the operation, after expansion, 8 weeks after expansion at implant placement, and 16 weeks after expansion before the animals were killed.

Implant stability measurement

Resonance frequency analysis (RFA) was conducted for all implants at the time of placement and at 8 weeks after placement using Osstell Mentor (Integration Diagnostics, Savadaled, Sweden). Stability of the implants was measured based on detection of vibration with a resonance frequency measurement probe (SmartPeg Type 5; Integration Diagnostics; Fig. 3). This probe has a magnetic material in the upper part of the instrument that forms a magnetic field with the Osstell Mentor used to detect the vibration. This probe was screwed into the implant body in accordance with the manufacturer's recommendations. The RF value is represented by a quantitative unit called the implant stability quotient (ISQ), which ranges from 1 to 100. An elevated ISQ indicates increased stability, and a decreased value indicates a reduction in implant stability. The measurements were also performed before the animals were killed.

Fig. 3. The resonance frequency value was measured using Osstell Mentor at the time of implant placement and at 8 weeks after implant placement.

Tissue preparation and histologic evaluation

Bone biopsy was performed with a trephine bur 3.5 mm in diameter from the lateral surface of the β-TCP block. The most medial site of the block was chosen for first biopsy at implant placement, and the most distal site of the block was chosen for second biopsy at the time of death. All biopsy specimens were placed immediately in containers filled with 70% ethanol and fixed for at least 5 days. The specimens were fixed without decalcification and then immersed in Villanueva bone stain solution (Maruto, Tokyo, Japan). The specimens were then dehydrated through a graded ethanol series, embedded in methylmethacrylate (Wako Pure Chemical Industries, Osaka, Japan), and cut into sections 5 μm thick.

Table I. Changes in alveolar width (mm)

Pre-op	*Pre-exp*	*Post-exp*	*Post-exp 8 wk: implant placement*	*Post-exp 16 wk*
2.7 (±0.4)	6.3 (±0.9)	10.7 (±1.1)	10.0 (±1.3)	7.8 (±1.5)

exp, Expansion.

Table II. Changes in implant stability quotient at the time of implant placement and 8 weeks after implant placement

T1	*T2*
64.4 (±4.4)	65.2 (±5.8)

T1, Implant placement; *T2,* 8 weeks after implant placement.

Histomorphometry

Histomorphometric measurements were performed to calculate the percentage of newly formed bone and remaining β-TCP material that formed inside the block: area of newly formed bone (or remaining β-TCP material)/total area × 100.

Histologic and histomorphometric analyses were performed under a light microscope with a digital camera (model DP12; Olympus, Tokyo, Japan) and digital image editing software (Photoshop CS2; Adobe, San Jose, CA). Morphometric measurements were made on 3 vertical sections per specimen closest to the center of the β-TCP area. The area of an unused β-TCP block was measured as a control.

Statistical analyses

Nonparametric analyses were performed, because blocks were used on the same individual and because the experimental groups were not large enough for parametric analyses. The Wilcoxon *t* test was used for intergroup analyses, and $P < .05$ was taken to indicate statistical significance.

RESULTS

Neither complications related to the materials used at the sites of intervention, before, during, or at the end of the experimental phase nor infections within or around the β-TCP block were observed. The alveolar form at the experimental region changed dramatically after lateral expansion with the β-TCP block, and the amount of augmentation was significant.

Changes in alveolar width are shown in Table I. Alveolar width increased, followed by lateral expansion and was maintained until implant insertion. However, the width decreased by about 2.2 mm after implant placement.

The RFA conducted immediately after implant placement gave a mean ISQ value of 64.4 ± 4.41 (range

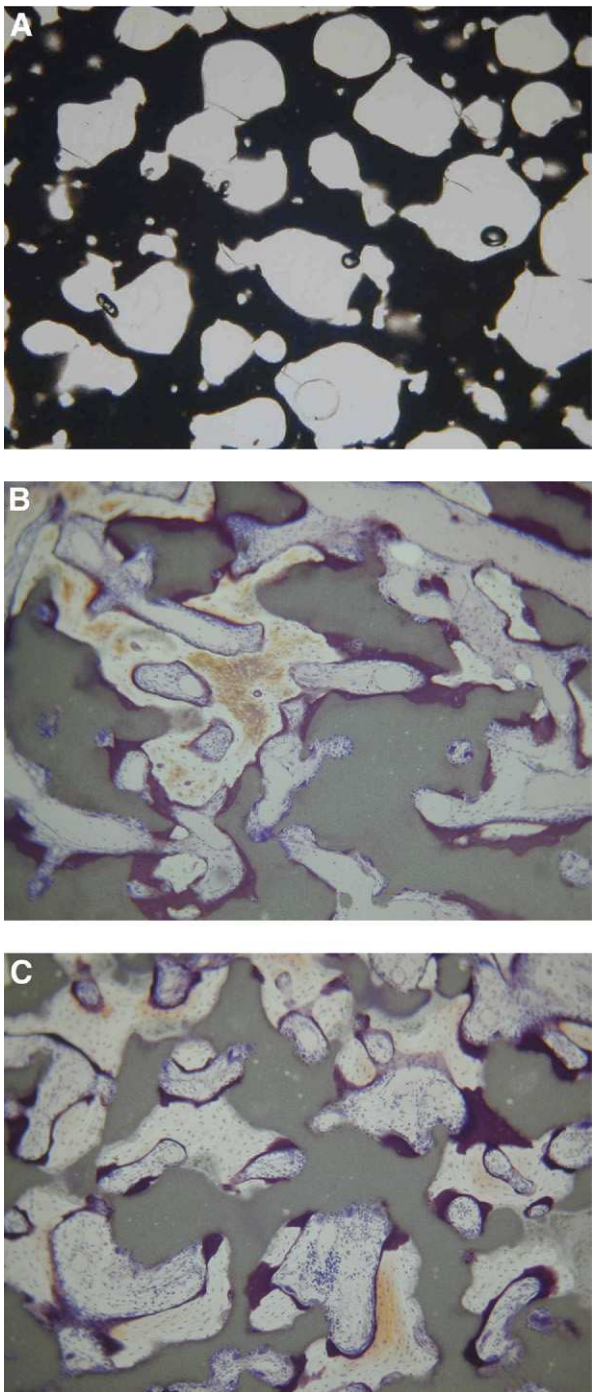

Fig. 4. Histomorphometric analysis was performed using specimens from the inside of the block. **A,** The area of the original β-TCP that was not used for the experiment was measured as a control. Undecalcified specimen visualized with Villanueva bone stain at the time of implant placement (**B**) and 8 weeks after implant placement (**C**). Newly formed bone including the osteoid was seen inside the β-TCP block, and β-TCP material remained as well as fibrous tissue. Original magnification ×100.

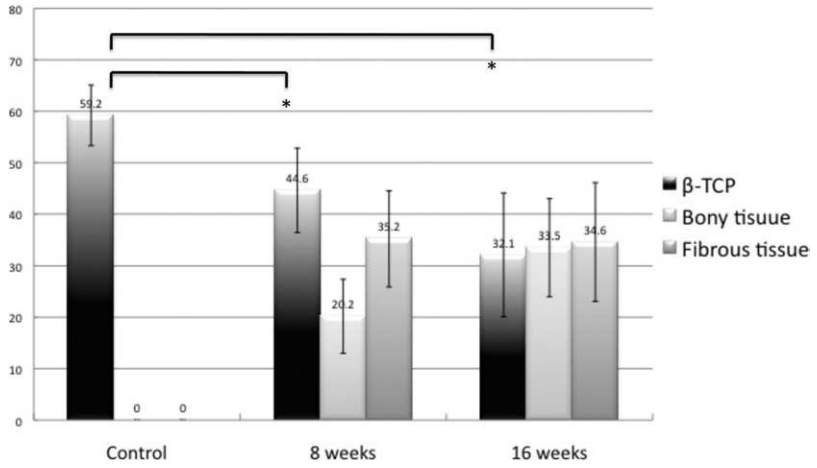

Fig. 5. Histomorphometric analysis. Changes in the amounts of materials or tissues remaining inside the β-TCP block. *$P < .05$ vs. original β-TCP.

58-70). In addition, the mean ISQ value at 8 weeks after implant placement was 65.2 ± 5.78 (range 56-71) (Table II).

The mean value of the original β-TCP block before it was used for the experiment (Fig. 4, *A*) was 59.2 ± 5.5%. All specimens evaluated by histomorphometric analyses were treated with Villanueva bone stain (Fig. 4, *B* and *C*). Changes in the amount of remaining material are shown in Fig. 5. The amount of remaining β-TCP decreased significantly compared with the original amount of material inserted. The mean values remaining inside the block were 44.6 ± 8.2% and 32.1 ± 12.0% at 8 and 16 weeks of consolidation, respectively, whereas newly formed bone comprised 20.2 ± 7.2% and 33.5 ± 9.5%, respectively.

DISCUSSION

Dental rehabilitation of partially or completely edentulous patients with dental implants has become common practice over the last several decades.[14,15] However, vertically and/or horizontally atrophic conditions of the alveolar ridge may provide insufficient bone volume for the placement of implants.[16] Distraction osteogenesis can yield natural bone formation between the distracted segment and basal bone, thus avoiding the necessity of autogenous bone harvesting from intraoral or extraoral sites, as occurs with use of other surgical alternatives, such as GBR or onlay/inlay bone grafting.[4] However, creating distracted segments is sometimes difficult in highly atrophic alveolar cases and results in increased mental and physical burden for the patient at surgery. Recently, periosteal distraction and dynamic periosteal elevation founded on the DO concept that osteogenesis after distraction involves the periosteum without distracted bone segments have been reported.[9,11,12] Based on these studies, we investigated the feasibility of PEO, which is also based on the concept of DO, using a highly purified β-TCP block instead of an original bone segment in a dog model.[13] This technique may help avoid donor-site morbidity, corticotomy, osteotomy, and secondary operations to remove devices.

At 8 weeks after periosteal expansion, the β-TCP blocks were completely integrated into new bone filling the area created under the periosteum, allowing identification of the gap between the original bone and β-TCP material. The width of the alveolar ridge increased, followed by lateral expansion of the β-TCP block and showed sufficient stability until implant placement. However, the width had decreased 8 weeks after implant placement. The trauma from the second operation for implant placement, especially flap formation or drilling, has been suggested as being responsible for the observed bone resorption. At the drilling procedure, the bone in the hybrid area of the β-TCP including newly formed bone was much stiffer than the original bone. Therefore, heating trauma may occur around the drilling area of the β-TCP block. Few reports have described the osseointegration of titanium implants under hybrid conditions, i.e., newly formed bone tissue in the bone-substitute biomaterial. Further studies are required to advance clinical applications of this method.

Our histomorphometric analyses indicated that β-TCP had been absorbed by about 24.7% on week 8 and 44.8% on week 16 after completion of expansion. These results indicated that the β-TCP area decreased over time and that the percentage of newly formed bone increased gradually from 20.2% to 33.5%. Kondo et

al.[17] suggested that the micropores of β-TCP play an important role as storage spaces for extracellular matrix components, providing ideal conditions for osteoinductivity. Our data indicated prominent bone conduction and material absorption inside the β-TCP block. Thus, prominent osteoconductive activity and biodegradability of β-TCP in the alveolar bone of dogs were demonstrated 16 weeks after placement under the periosteum. These results also showed that inclusion of a sufficient space under the periosteum induces new bone formation. The highly purified β-TCP block acted as a space maker, inducing an osteoblastic response.

Until recently, no reliable and reproducible standardized methods existed for measuring implant stability, although RFA is now a reliable and validated method for this purpose. The RFA values are converted into an index known as the ISQ,[18,19] and changes in the ISQ value are related to alterations of implant stiffness in the surrounding tissues. The ISQ values, which may range from 0 to 100, can be used to determine different healing phases and the stability of implants. Successfully integrated implants have ISQ values >40.[20,21] In the present study, the mean ISQ values were >60, and the mean value 8 weeks after implant placement showed stabilization or even a slight increase compared with immediately after implant placement. Although the width decreased after implant placement, the ISQ values were stable until 8 weeks after implant placement, perhaps because the osseointegration progressed with original mandibular bone at the inferior parts of implants.

In conclusion, newly formed bone could be acquired by periosteal expansion osteogenesis using a β-TCP block for implant placement in a dog model. However, the bone volume was not stable after implant placement despite sufficient implant stability for 8 weeks. Further research is needed to examine the mechanisms and progress of PEO and the use of biomaterial blocks for alveolar ridge augmentation.

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