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Stability of beta tricalcium phosphate-coated mini-implants in rat tooth sockets

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We investigated the potential of the osteoconductive material β-tricalcium phosphate to promote bone healing when coated on a titanium alloy screw implanted in the mandibular tooth socket of a healthy rat. Forty-eight 9-week-old male Wistar rats were divided into three groups : the Blast group, which received blast-treated screws ; the βTCP group, which received β-tricalcium phosphate-coated screws ; and the Control group, which received mechanically polished screws. Mandibles were removed at 3 or 9 weeks after screw implantation. Screw removal torques were measured using a handheld torque gauge and compared. At both 3 and 9 weeks after implantation, the screw removal torques were significantly greater in the βTCP group than in the Blast and Control groups ($p < 0.05$). Removal torques did not differ significantly between the Blast and Control groups at either time point. These results suggest that a β-tricalcium phosphate coating on screws accelerates osseous healing around the screw and increases the retentive capacity of the screw. A β-tricalcium phosphate-coated implant fitted into the tooth socket immediately after extraction may therefore provide a favorable environment for early loading of implants. (J Osaka Dent Univ 2011 ; 45 : 135–141)

Key words : Removal torque ; Rat ; Mini-implants ; Beta-tricalcium phosphate ; Primary stability ; Tooth socket

INTRODUCTION

Since Branemark *et al*.[1–4] first reported osseointegration of titanium, researchers have sought to improve the practicality of oral implants. However, oral implants require about 6 months in the maxilla and 3 months in the mandible before they can withstand occlusal force.[5] The development of materials that can reduce the treatment period until fitting of the implant superstructure or can provide greater osseointegrative capacity and faster integration is therefore an important clinical issue. There has been wide-ranging research into implant surface modifications and treatment methods.[6]

Implants coated with hydroxyapatite (HAP) are currently used to shorten the period until osseointegration,[7–9] since HAP shows high bioaffinity and promotes osteogenesis in the surrounding area.[10, 11] However, because HAP treated at high temperatures has poor bioabsorption and remains in the bone for a long period,[12–14] attention has turned to β-tricalcium phosphate (βTCP), which offers excellent osteoconductivity and is fully absorbed following bone healing.[15, 16]

Although these characteristics suggest that βTCP implants could offer efficient bone regeneration in tooth sockets, βTCP coatings have been reported to have almost no effect[17, 18] or to attenuate cell growth.[19] Further research is therefore needed to clarify the utility of βTCP. As part of research into biological responses to βTCP, we extracted anterior mandibular teeth from rats, implanted a titanium screw coated with βTCP into the socket, and used biomechanical methods to observe hard tissue formation and the healing process in the tooth socket in the presence or absence of βTCP.

MATERIALS AND METHODS

Experimental animals

Forty-eight 9-week-old male Wistar rats (Shimizu Laboratory Supplies, Kyoto, Japan) were used. Animals had ad libitum access to solid feed and water throughout the experimental period. Growth was monitored by weighing each animal at each intervention until sacrifice. All study protocols were approved by the animal research committee of our institution, and followed our institutional guidelines for animal research.

Screws

We used titanium alloy screws (Ti-6 Al-4 V, SNK Screwpost Titan®; Dentsply-Sankin, Tokyo, Japan) with a diameter of 1.2 mm and a length of 17.0 mm. Screws were either blast-treated, blast-treated and coated with βTCP, or mechanically polished.

βTCP surface

The fine surface structure of βTCP coated on the screw was measured for roughness using a Precision Talysurf CLI 2000 surface profiler (Taylor Hobson, Leicester, UK) in contact mode, and was observed under scanning electron microscopy (SEM) (Figs. 1 and 2). The βTCP coating surface showed a very rough, porous structure with deep valleys and concentrated peaks. We also performed X-ray photoelectron spectroscopy (XPS) using a Kratos Axis Ultra DLD Spectrometer (Kratos Analytical, Manchester, UK) and a single-color AlK$_\alpha$ X-Ray

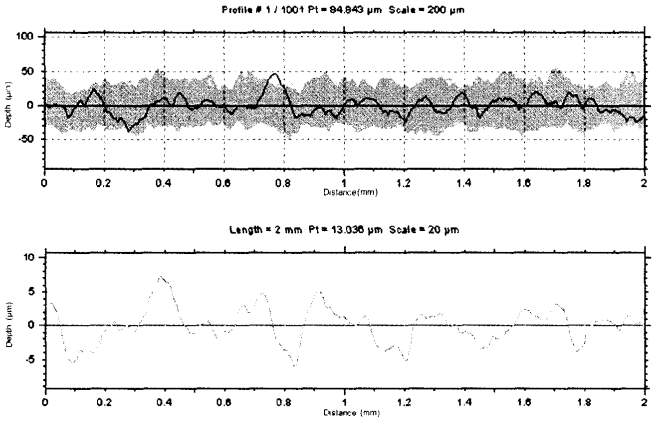
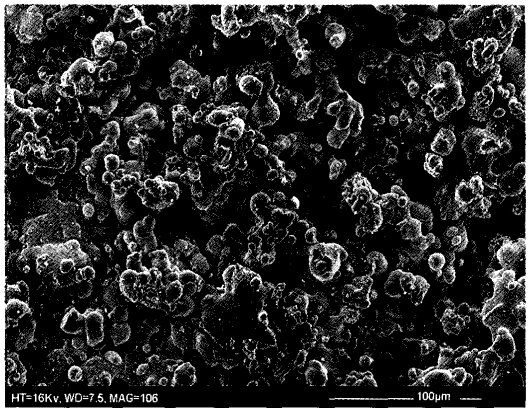
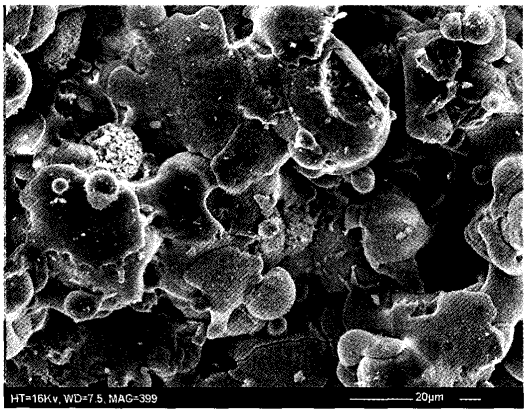


Fig. 1 Surface roughness measured by surface profiler. The fine surface structure of bTCP coated on the screw was measured for roughness using a Precision Talysurf CLI 2000 surface profiler in contact mode.



A



B

Fig. 2 SEM images of the βTCP coated screw. (A) Low magnification. (B) High magnification.

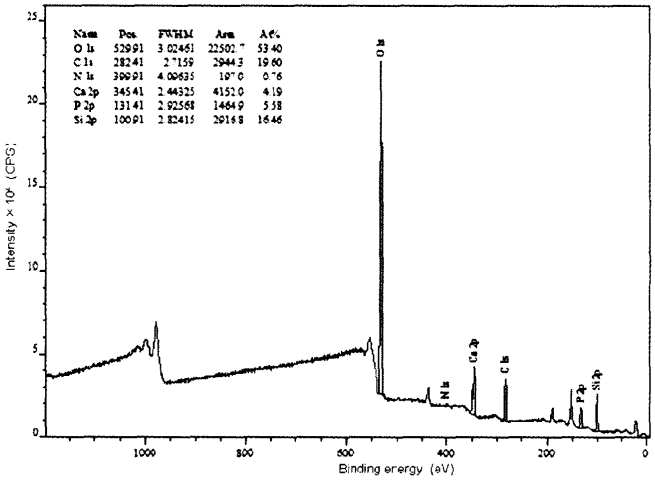


Fig. 3 X-ray analysis chart of βTCP. We performed X-ray photoelectron spectroscopy (XPS) using a Kratos Axis Ultra DLD Spectrometer and a single-color AlK$_\alpha$ X-Ray source (75–150 W) to examine the basic structure of the βTCP coated onto the titanium surface.

source (75–150 W) to examine the basic structure of βTCP coated onto the titanium surface (Fig. 3). The significant levels of calcium phosphate evident in XPS analysis (Fig. 3) indicated that the βTCP-coated surface is covered with calcium phosphate.

Extraction of rat left mandibular incisor

Following the method of Sato *et al*.,[20] the left mandibular incisor of 48 rats was cut down to the height of the interdental papillae as a pre-extraction intervention. This was performed three times (days 11, 7 and 4 before extraction) using a dental turbine, with the rat held supine in a rat restraint under light anesthesia (Isoflurane Rhodia®; Nissan Chemical Industries, Tokyo, Japan), taking care not to injure the interdental papilla and adjacent tooth (right mandibular incisor). This procedure was carried out three times because the trimmed incisor erupts to its original position after 3 or 4 days. The tip of the left maxillary incisor was also trimmed to avoid occlusion.

The left mandibular incisors were then removed from the rats at 11 weeks of age. The animals were initially anesthetized with isoflurane, then administered general anesthetic by intraperitoneal injection of sodium pentobarbital (Nembutal®; Dainippon Pharmaceutical, Osaka, Japan). The tooth was completely luxated with an elevator modified for rats, then extracted by tugging along the long axis of the tooth using a needle-holder, taking care not to rotate the tooth. Animals were reared on normal solid feed after extraction, with the right mandibular incisor kept intact to allow food consumption.

Screw implantation

Immediately after extraction of the left mandibular incisor, the screw was implanted in the tooth socket with several parts of the screw, including the tip, in contact with the tooth socket wall, to obtain initial fixation (Figs. 4 and 5). Three groups (16 rats each) were implanted with one screw per rat. The Blast group received blast-treated screws, the βTCP group received screws coated with βTCP after blast-treatment, and mechanically polished screws were implanted in the Control group.

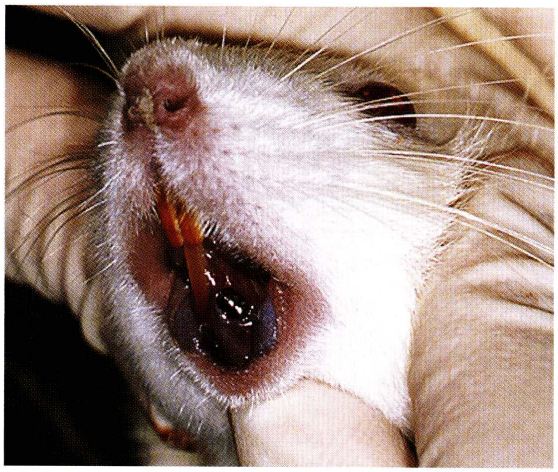


Fig. 4 Screw implanted in rat.

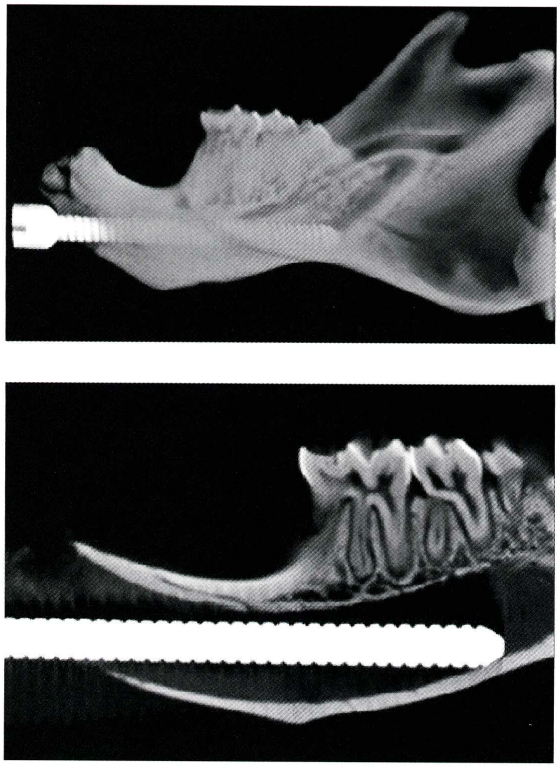


Fig. 5 X-rays of implanted screw.

Collection of samples and measurement of removal torque

At 3 or 9 weeks after screw implantation 8 rats in each group were euthanized by intraperitoneal overdose of sodium pentobarbital 30 min after intraperitoneal injection of the anticoagulant sodium heparin (Novo-Heparin Injection 1000®; Mochida Pharmaceutical, Tokyo, Japan).

After perfusion fixation with 10% neutral-buffered

formalin according to standard methods, the entire mandible was removed and dissected centrally into left and right halves with a surgical scalpel. The left side was retained as the sample. Holding the mandible in the fingers, the torque required to remove the implanted screw was measured using an HTG-2 N handheld torque gauge (HTG 2-200 Nc ; Imada, Aichi, Japan) with screwdriver attached.

Statistical analysis

Removal torques in each group at 3 and 9 weeks after screw implantation were compared by two-factor analysis of variance. Intergroup comparisons were performed using the Turkey-Kramer multiple comparison test. SPSS 12.0 J Base System software (SPSS Japan, Tokyo, Japan) was used for all statistical analyses. Values of $p<0.05$ were considered statistically significant.

RESULTS

Changes in body weight

All rats were weighed each time the teeth were cut, when the screw was implanted immediately after extraction, and when the removal torque was measured. Throughout the experimental period, body weight increased consistently in all groups, with no weight decreases. Control and experimental groups showed no differences in nutritional condition (Fig. 6).

Removal torque

At both 3 and 9 weeks after implantation, the screw

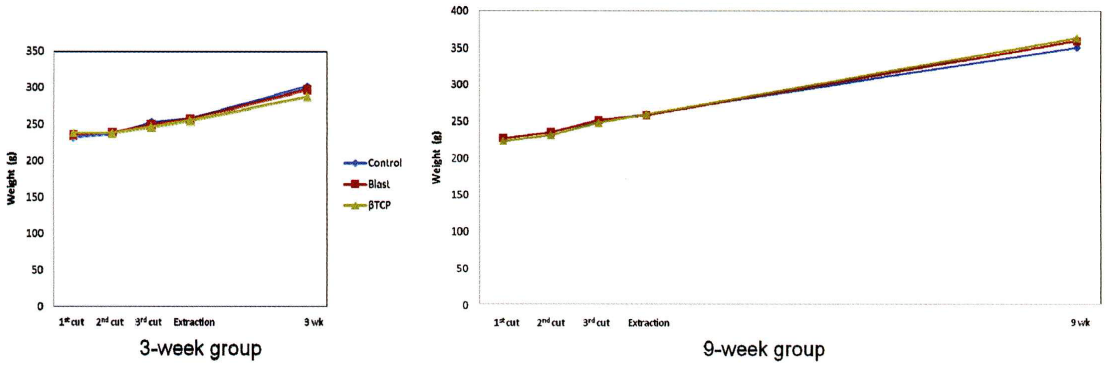


Fig. 6 Changes in rat body weight at 3 and 9 weeks.

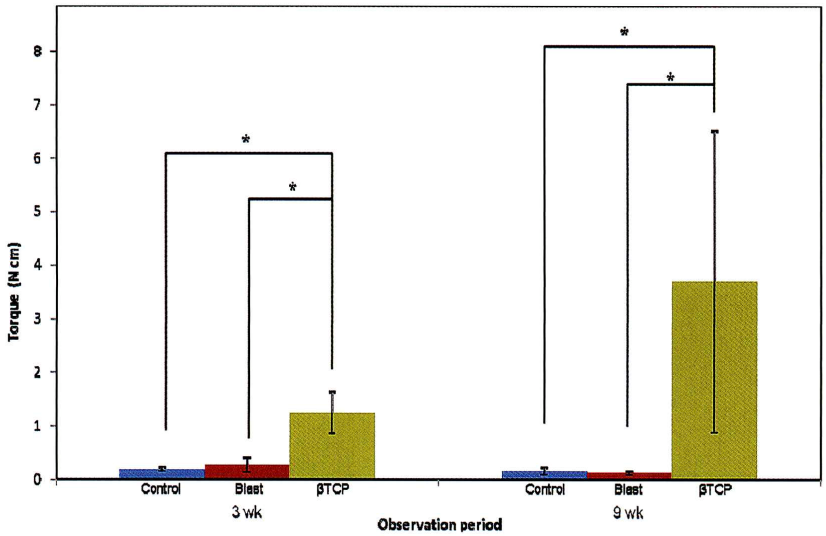


Fig. 7 Removal torque.
Mean ± SD, *$p<0.05$.

removal torques were significantly greater in the βTCP group than in the Blast or Control groups ($p < 0.05$). Removal torques did not differ significantly between the Blast and Control groups at either time point (Fig. 7).

DISCUSSION

Carlsson *et al*.[21] demonstrated the effectiveness of measuring removal torque as a biomechanical method for evaluating bone implants. Because this method is well established for evaluating the strength of the union between bone and dental implants, we used it to analyze healing dynamics of the tooth socket in rats. Most torque measurement studies have used the femur and tibia of rats and rabbits.[22–25] Because the tooth-bearing mandible represents a different situation, we extracted rat mandibular incisors and implanted screws following the methods of Sakai *et al*.[26] to investigate the effectiveness of the βTCP coating.

Rat mandibular incisors are described as rootless and differ somewhat from human teeth. Enamel forms on the labial side of the tooth even before it exits the alveolar bone, and the teeth lack a periodontal membrane.[27] Nevertheless, rats provide a useful model for investigating extraction-site healing in humans, since the healing process in human sockets and in rat mandibular incisor sockets is broadly similar.[28]

Compared to titanium, which is the most biocompatible metallic biomaterial, βTCP and other calcium phosphates have the advantages of encouraging faster osteogenesis and directly integrating with bone.[29, 30] This is because osteoblasts in contact with βTCP acquire high levels of alkaline phosphatase activity, osteocalcin production and parathyroid hormone response, secreting large quantities of extracellular matrix and proteins contributing to osteogenesis.[31, 32] The secreted bone protein osteocalcin then increases osteoblast migration, and osteopontin binds with integrins (transmembrane proteins on osteoblasts), further improving adhesion between calcium phosphate and cells. Also, as hydroxy apatite (HAP) is the main component of the bone produced by cells, this bone forms a crystallographic continuity with HAP precipitated by the calcium phosphate, which bonds directly with the bone.

We found that screw removal torques were significantly greater in the βTCP group than in the Blast and Control groups at both 3 and 9 weeks after implantation. This stronger union with βTCP seems likely to result from the high adsorption capacity and porous surface structure of βTCP, as inferred from the SEM images of βTCP. This facilitates the advance of blood components from the tooth socket, together with fibroblasts and osteoblasts, over the βTCP surface, which is followed by the entry of collagen fibers and new bone into the pores. Torque values were probably also higher because the porous βTCP naturally provided a greater surface area than a mechanically polished or blast-treated titanium surface.

Bhanskar *et al*.[29] implanted βTCP pieces into the medullary cavities of rat tibias to investigate the bioaffinity and absorption properties of βTCP. Within 4 days, connective tissue had penetrated the βTCP pores, and bone tissue began forming from the host bone around the areas of penetration in the first week. At about 2 weeks, vacuoles of various sizes appeared in osteoclast-like cytoplasm ad-

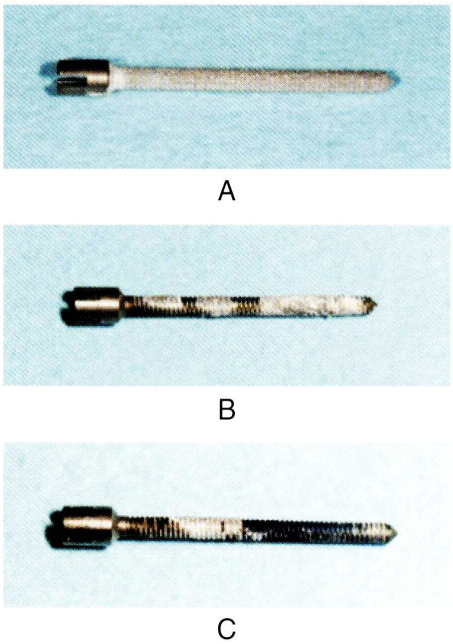


Fig. 8 Removed screws. (A) Before implantation, (B) 3 weeks after implantation and (C) 9 weeks after implantation.

hering around βTCP, and mesenchymal cells began phagocytosis of βTCP.

In a study of high-purity βTCP in beagle dogs, Ozawa *et al.*[33] reported that βTCP had almost completely dissolved and had been replaced with bone by 24 weeks after implantation. Similarly, although the screws in our study were completely coated with βTCP before implantation, the coating was partially peeled off by removal at 3 weeks, and the titanium screw surface was exposed at 9 weeks (Fig. 8). This is probably because osteoconduction had occurred on the βTCP by 3 weeks after implantation, and βTCP had been dissolved by adherent osteoclast-like cytoplasm by 9 weeks after implantation.

These results suggest that βTCP-coated implants may provide a favorable environment for early loading of implants. However, further detailed investigations of the merits of βTCP in clinical settings are needed, given that the titanium alloy screws used in this experiment differ slightly from those used in clinical practice, the roots of rat anterior teeth differ slightly from those of humans, and implantation in this experiment was performed immediately after extraction.

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