Application of α-tricalcium phosphate coatings on titanium subperiosteal orthodontic implants reduces the time for absolute anchorage: a study using rabbit femora

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We are currently developing a small perforated titanium subperiosteal implant specifically for orthodontic therapy, which can be placed anywhere on the bone surface. In the present study, we coated this implant with hydroxyapatite (HA) or α-tricalcium phosphate (α-TCP) in an attempt to shorten the initial stabilization period relative to the few months that is usually required. The coated implants were placed beneath the periosteum in rabbit femora. The implants were observed by radiographically and histologically, and measured the tensile strength of the bone–implant interface.

Two weeks after placement, the volume of new bone formed in the perforations of the implant was significantly greater for the α-TCP-coated implants than for the HA-coated implants.

Our findings indicate that new bone is formed faster in the surrounding area with α-TCP- and HA-coated subperiosteal implants than with uncoated implants, and that α-TCP is a particularly effective stimulator of new bone formation.

Keywords: Orthodontics, Subperiosteal implant, α-tricalcium phosphate

INTRODUCTION

A variety of orthodontic implants are now in common clinical use; however, most of these implants are designed to be placed in the bone, and must be placed in such a way that they do not damage adjacent teeth or their roots, and do not impede the subsequent movement of the teeth. For this reason, the range of sites in which orthodontic implants can be placed is severely constrained by factors including the condition and position of teeth and their roots, the intended direction or extent of tooth movement, and the size of the alveolar bone. Because orthodontic implants need to be removed after therapy is complete, there is no avoiding the necessity for two invasive procedures, on placement and removal. The perforated subperiosteal implants described by Ogawa et al.10 solved these problems, because they are placed on the surface of the bone rather than being inserted into the bone, but have the drawback that a non-weight bearing period of at least one month is required after placement, to ensure that they are supported by the newly formed bone.

In the present study, in an attempt to reduce, as much as possible, the non-weight bearing period necessary to ensure initial stabilization and to improve the stability of the implant by establishing a robust bond with the bone, we coated the implants with α-tricalcium phosphate (α-TCP) and hydroxyapatite (HA), which are osteoconductive materials that have a high affinity for osseous tissue, and examined the impact of the implants on the surrounding area.

MATERIALS AND METHODS

1. Preparation of implants
We manufactured the subperiosteal implants for the present study using pure titanium (Japanese Industrial Standards II, Nilaco, Tokyo, Japan). Discs of 5 mm diameter were cut from a 0.25 mm-thick titanium sheet, and each disc was perforated with 28 holes, each of which was 0.5 mm in diameter, placed at 0.75 mm intervals. A hook was constructed on the center of each disc, using a 0.76 mm-diameter piece of titanium wire that was bent into a loop and welded in place. The bone-contacting surfaces of the implants were coated with α-TCP using the plasma spray method2-9. The HA coating was created by hydrothermally processing the α-TCP-coated surface (procedure performed by Advance, Tokyo, Japan). Uncoated implants were used for controls. (Figs 1, 2 and 3)

Before implantation, three implants from each group (α-TCP- or HA-coated, and control) were analyzed by using two methods. First, their surfaces were examined by scanning electron microscopy (SEM) at magnifications of 20×, 100× and 5000×. Second, they were qualitatively analyzed using powder X-ray diffraction (RINT 2000; Rigaku, Tokyo, Japan). PSPC (curved form) was used as a detector and Cu (Kα) was used as the radiation source. The angle used for measurement (2θ) was 10-100°, and Joint Committee on Powder Diffraction Standards (JCPDS) data was used for reference (cards 9-432 and 9-348 for HA and α-TCP, respectively).

2. Placement of implants
All animal experiments were approved by the animal care and use committee of the School of Dentistry,
Aichi Gakuin University. Twenty-one Japanese white rabbits weighing 2.5–3.0 kg were divided into the α-TCP, HA and control groups (n = 7 in each). The placement procedure was performed under general anesthesia induced using 0.5 ml/kg pentobarbital (Nembutal; Dainippon Sumitomo Pharma, Osaka, Japan) injected into the auricular vein and infiltration anesthesia of the incision site using 1.8 ml lidocaine (Xilocaine; AstraZeneca, Osaka, Japan). An intermuscular space was expanded to allow visualization of the femur, then the periosteum was carefully incised and separated from the bone. Implants that had been sterilized in an autoclave at 120ºC for 20 minutes before placement were placed on the femur surface. After placement, implants were tightly fixed to the bone by a ligature created using 0.3 mm-diameter stainless steel wire. Three implants were placed on each of the right and left femora (six implants per rabbit) at approximately 10 mm intervals. After placement, the implants were immediately covered by
Fig. 3  Scanning electron micrograph of surfaces of uncoated (control) and coated implants.

Fig. 4  Schematic illustration of the method used for implantation.
the periosteum and then the fascia was sutured (Fig. 4). For each implant type, 14 implants (2 implants per rabbit) were placed for three different placement periods, one, two and three weeks.

3. Removal and radiographic examination of implants and tissue

Rabbits were sacrificed by injection of a lethal dose of pentobarbital one, two or three weeks after placement of the implants ($n = 7$ for each of the three different placement periods). The implants, femora and other surrounding tissues were removed and stored in physiological saline until examination. After as much soft tissue as possible was quickly removed from the bone surface, the area was subjected to radiography (25 kV, 2.5 mA, 20 sec; OMC 403; Ohmicron, Newtown, PA, USA).

Radiographic examination was performed on all 126 implants that had been placed in rabbits during the present study. Within each placement period, 14 implants of each type were placed in each group of rabbits (i.e. a total of 126 implants). Among these 14 implants placed in each group of rabbits, 7 implants were randomly selected and used for pull test, as well as another randomly selected 3 implants were used for histological study.

4. Measurement of the bone–implant bond strength

Following radiography, pull tests were performed using the method of Ban et al.\(^\text{10}\) with a universal material tester (EZ-test; Shimadzu, Kyoto, Japan; cross head speed of 5 mm/min) to measure the tensile strength of the bond between the subperiosteal implant and rabbit femur (Fig. 5). Ten implants for each placement period, in each of the $\alpha$-TCP, HA and control groups (i.e. a total of 90 implants), were examined. The significance of differences between different placement periods and groups was assessed using analysis of variance (ANOVA).

5. Histological study

Implants with surrounding femur were fixed by immersion in 10% phosphate buffered formalin. Undecalciﬁed ground sections that were perpendicular to the long axis of the femur, and that included the interface between the implant and bone, were prepared using the standard method. The sections were stained with toluidine blue and observed using a light microscope. Three implants for each placement period, in each of the $\alpha$-TCP, HA and control groups (i.e. a total of 27 implants), were examined using this method.

6. Electron microscopic examination of the implant–bone interface

In subperiosteal implants that had been in place for two weeks, new bone that had formed in the perforations of the implant was observed using SEM (Fig. 6). The maximum cross-sectional area of new bone was measured using the non-contact surface roughness test (VF7500; Keyence, Osaka, Japan) and the results were statistically analyzed using ANOVA ($n = 28$). Ten implants in each of the $\alpha$-TCP, HA and control groups (i.e. a total of 30 implants), were examined (Fig. 7).
RESULTS

1. Properties of coating materials
SEM revealed that in the control (uncoated) implants, the bone-contacting surface contained fine concavities and convexities due to the sandblasting treatment during manufacture. At the higher magnification, the $\alpha$-TCP- and HA-coated surfaces were markedly dissimilar. The $\alpha$-TCP-coated surface had a fused glass-like appearance and the HA-coated surface had an acicular crystal structure. X-ray diffraction data for the $\alpha$-TCP-coated surface was consistent with that on the JCPDS card for $\alpha$-TCP. X-ray diffraction data for the HA-coated surface was largely consistent with that on the JCPDS card for HA; Although the card listed a peak for $\alpha$-TCP ($d$ value = 2.905), in our data the peak was very weak, which confirmed that most of the $\alpha$-TCP had been converted into HA (Fig. 8).

2. Macroscopic and radiographic observations
Macroscopically, for implants of all types that were removed after one week, connective tissue was seen to cover the area surrounding the subperiosteal implant, and no hard osteoid tissue was present. For implants of all types that were left in place for two or three weeks, the area surrounding the implant was rich in hard osteoid tissue, such that only the hook of the implant could be seen (Fig. 9).

For all implants of all types that were removed after one week, radiographic imaging revealed no opacity in the area surrounding the implant. For implants left in place for two weeks, opacity was evident in the $\alpha$-TCP and HA groups, whereas opacity in the control group was minimal. For implants left in

Fig. 7  Diagram showing how the new bone area was calculated by counting pixels in cross-sectional images of new bone protuberances.

Fig. 8  X-ray diffraction patterns of uncoated (control), $\alpha$-TCP-coated and HA-coated implants.
Fig. 9 Macroscopic views of uncoated (control) and coated implants at the time of removal.

Fig. 10 Radio graphic views of uncoated (control) and coated implants in situ.
place for three weeks, opacity was evident around the implants for all implant types (Fig. 10).

3. Strength of implant–bone bond

Figure 11 shows the results of tests in which the tensile strength of the bond between the implant and the rabbit femur was measured one, two and three weeks after placement for each of the three groups. After one week, the mean tensile strength was 0.006 MPa in the control group, 0.003 MPa in the α-TCP group and 0.019 MPa in the HA group. There was no significant difference among the three groups after one week of placement. After two weeks, mean values were higher in all groups: 0.365 MPa in the control group, 1.004 MPa in the α-TCP group and 1.063 MPa in the HA group. At this time, tensile strength was greater in the α-TCP and HA groups than in the control group ($p < 0.01$ and $< 0.05$, respectively), but there was no significant difference between the α-TCP and the HA groups. After three weeks, values were higher again: 2.846 MPa in the control group, 3.686 MPa in the α-TCP group and 3.161 MPa in the HA group. At this time, there were again no significant differences among the three groups. In all groups, the tensile strength tended to increase with the duration of placement.

4. Histological findings

Figure 12 shows histological sections of the rabbit femora and the areas surrounding the implant for each placement period. After two and three weeks of placement, in all three groups, there was extensive formation of new bone around the implants, along the surface of the femora. Myeloid tissue and osteocytes were found in the new bone.
Properties of the femoral surface after implant removal
In the α-TCP and HA groups, SEM examination (×20) of the bone surface under the implants after two weeks of placement revealed a number of protuberances of new bone that had grown above the femoral bone surface into the perforations in the implants. When the metal surfaces of the implants that had been in contact with bone were examined, there was no evidence suggesting peeling or absence of the coating layer for either the α-TCP and HA coatings.

Higher-magnification SEM examination (×100) revealed that the new bone in the perforations comprised large protuberances with a trapezoidal cross-section in the axis perpendicular to the surface of the bone. Pixels in cross-sectional images of the new bone protuberances were counted to determine the area of new bone.

The area of new bone was significantly larger in the α-TCP and HA groups than in the control group (p < 0.01), and significantly larger in the α-TCP group than in the HA group (p < 0.01) (Fig. 13 and 14).

DISCUSSION
1. Orthodontic implants
The application of implants as anchorages in orthodontic therapy has been attempted since around 1980. At the early stage, the root-form implants intended for dental prosthetic treatment were used for such application. Currently, mini-screw implants, mini-plate implants stabilized by screws, and subperiosteal implants are used for this purpose.

Screw implants are mainly deployed in alveolar part. However, in many cases of orthodontic therapy, teeth are crowded or there are no missing teeth, and therefore the roots of the teeth are likely to be located close to each other. Moreover, implants must be placed such that they do not constrain the intended movement of the teeth. For this reason, the range of sites in which implants can be placed are very much limited depending on the intended direction or extent of tooth movement and the size or shape of alveolar bone. Depending on their location, direction of insertion, and their length, screws can also damage the nerves and blood vessels in the maxillary sinus or inside the bones. The large surgical sites required for the placement of screw implants are associated with a high level of surgical invasiveness and pain.

2. Application of subperiosteal implants
Use of subperiosteal orthodontic implants was first attempted using large implants (approximately 10 mm) specifically for the palate. These implants were coated with HA and did not have undercut notches on the surface to bind to the bone. They required a surgical template to be in place for two weeks to achieve initial stabilization, and then a non-weight bearing period of five months.

Inspired by reports of the formation of new bone between the periosteum and bone surface, Ogawa et
*al. developed a small subperiosteal implant specifically for orthodontic therapy. However, in order to use the implant they developed as an anchor, a non-weight bearing period of at least one month was required until new bone had formed to support the implant.

In the present study, we considered the use of α-TCP and HA, which are known to have high levels of osteoconductivity, to coat subperiosteal implants. In the present study we aimed to accelerate the formation of new bone and make earlier weight-bearing possible by using α-TCP or HA coating at the interface between a titanium mini-subperiosteal implant and the bone.

In the present study, we placed 5 mm pure titanium subperiosteal implants beneath the femoral periosteum in rabbits in order to examine the performance of α-TCP or HA coatings on the implants with a view to using the implants as anchors for orthodontic therapy. Implants were coated on the bone-contacting surface, and an uncoated implant was used as a control. At one, two and three weeks after implant placement, the implant sites were observed radiographically and histologically, and the strength of the bone–implant bond was measured, with the following results:

1. Two weeks after the placement, radiography and histological examination revealed excellent formation of new osseous tissue around the subperiosteal implants coated with α-TCP or HA.
2. After one and three weeks, the bone–implant bond strength was similar among all three groups, but after two weeks, the bond strength was significantly greater in the α-TCP and HA groups than in the control group.
3. At two weeks, significantly larger volume of new bone had formed in the perforations of the implants in the α-TCP group than in the HA group.

These findings indicate that perforated titanium implants coated with α-TCP facilitate early osseointegration and can be used as subperiosteal anchors for orthodontic therapy soon after placement.

**CONCLUSION**

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