A stability evaluation of a novel titanium dental implant/interconnected porous hydroxyapatite complex under functional loading conditions

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The aim of this study was to evaluate the stability of implant/interconnected porous calcium hydroxyapatite complex (implant/IP-CHA-complex) under functional loading. Implant/IP-CHA-complexes were placed into the mandibles of four Beagle-Labrador hybrid dogs (complex-group). On the other side, an implant was placed directly (control-group). To subject the loading, the animals were fed a hard diet throughout the loading phase of 5 months. The implant stability quotients (ISQs) and bone implant contact (BIC), and histological evaluations were performed. The ISQs of implant/IP-CHA-complex was significantly lower at placement than that of the control-implant. On the other hand, there was no significant difference between in the groups during loading. The BIC measurements, there was no significantly difference between in both groups. Histologically, newly formed bone was observed in contact with most of the implant surface in the complex-group. An IP-CHA/implant-complex would be able to achieve both bone reconstruction and implant stability under functional loading conditions.

Keywords: Bone to implant contact, Functional loading, Histology, Implant stability

INTRODUCTION

Dental implant therapy has been applied for the rehabilitation of missing teeth. The success of implant therapy depends on the presence of favorable bone quantity and quality at the placement site because the implant needs to undergo osseointegration to create implant stability1). However, implant placement might be difficult when a large bone defect caused by injury, tumor, or a misplaced implant is present. In such clinical cases, reconstructive surgery with a bone graft is required before implant placement2). Reconstructive surgery with bone grafting is applied to restore the lost bone shape and to stabilize the implant for occlusal function. However, bone grafts can have unpredictable prognoses, long healing period and require multiple surgeries with a consequently increased risk of morbidity for patients. Block-type bone graft materials, iliac crest bone blocks, or calvaria autologous bone are used as bone substitutes before implant placement2-8). Although beneficial outcomes have been reported after implant placement into grafted sites with autologous bone blocks6-7), autologous grafts present some drawbacks such as insufficient harvesting persistent pain, nerve damage, fracture, and cosmetic defects at the donor site7,8). Therefore, novel approaches are expected to be developed to simultaneously restore bone defects and place implants. Interconnected porous calcium hydroxyapatite (IP-CHA), which is characterized by uniform, spherical, interconnecting pores, was reported to successfully used as a biomaterial in the field of tissue engineering8-12). According to the specific characteristics of IP-CHA, a novel implant/IP-CHA complex to be used as a graft material has been developed. Specifically, the implant/IP-CHA complex consists of a dental implant fixture surrounded by a block of IP-CHA. Our previous study results demonstrated that an implant/IP-CHA complex could simultaneously achieve superior osteoconduction and implant stability13). Osseointegration could be detected at the bone-implant interface with the implant/IP-CHA complex after 6 months. Additionally, it was observed that the implant/IP-CHA complex achieved better implant stability, evaluated by implant stability quotient (ISQ), and bone implant contact (BIC) as well as control implant that was placed in the pre-existing bone site. However, these evaluations were performed in a femur without functional loading, and implant stability under loading conditions has not been assessed yet. Therefore, this study aimed to evaluate the stability of an implant/IP-CHA complex in the mandibles of dogs under functional loading conditions.

MATERIALS AND METHODS

Materials
The hollow IP-CHA cylinders (outer diameter: 5 mm, inner diameter: 3 mm, height: 10.0 mm) used in this study were custom fabricated (NEOBONE®, Covalent Materials, Tokyo, Japan). IP-CHA had a porous structure with 75% porosity, a mean porous diameter of 150 μm, and all pores were interconnected by pores...
Fabrication of the implant/IP-CHA complex

The implant/IP-CHA complex was fabricated in accordance with a previous study. Briefly, the implant screw thread was prepared at the inner surface of a hollow cylinder of IP-CHA using a special electric engine (Nobel Biocare Japan, Tokyo, Japan) with serial cutting drills and a screw tap; then, countersinking was performed in the upper section. After preparation of the IP-CHA cylinder, the titanium implant was incorporated into it to fabricate the complex (Fig. 1).

Observation of the implant/IP-CHA complex

One implant/IP-CHA complex was dehydrated using increasing concentrations of ethanol and then embedded in light-polymerized polyester resin (Technovit 7200VLC, Kulzer, Wehrheim, Germany). To achieve complete polymerization of the resin block, photo polymerization equipment was used (BS5000, EXAKT APARATEBAU, Hamburg, Germany). The resin block was cut using a diamond saw system at the center of the implant/IP-CHA complex, and the cross-section was scanned (ES10000G, EPSON, Tokyo, Japan).

Animal experiments

The animal research protocol was in accordance with the current version of the Japan Law on the Protection of Animals. This study was approved by the Research Facilities Committee for Laboratory Animal Science at the Hiroshima University School of Medicine, Hiroshima, Japan (A11-5-5). All surgery was performed under general anesthesia, and all efforts were made to minimize suffering during the experimental period.

The study design of the animal procedure was shown in Fig. 2. Four male Beagle-Labrador hybrid dogs (each weighing 20–23 kg and 18–20 months of age) were fed in their cages for 1 month to allow them to acclimate to the environment. After 1 month, the mandibular premolars
(P4) were extracted bilaterally, and the sites were allowed to heal for 3 months prior to implant placement. The surgical operations were performed under general anesthesia with sodium pentobarbital (10 mg/kg) and local infiltration anesthesia with 2% lidocaine and 1:80,000 noradrenaline. Implant placement was performed as follows. A mucoperiosteal flap was raised carefully around the edentulous site to minimize damage to the underlying periosteum. The residual bone ridge was flattened out using a grafting blade to place the implant shoulder at the buccolingual crestal bone level. A bone defect (5 mm in width and 10.0 mm in depth) was made in one side of the edentulous mandible using a surgical motor system (Fig. 3a). Then, the test implant/IP-CHA complex was placed into the bone defect and covered by an expanded polytetrafluoroethylene membrane (Gore-Tex, W.L. Gore & Associates, Flagstaff, AZ, USA) (Fig. 3b). The mucoperiosteal flap was closed in layers using 4-0 non-absorbable thread (Gore suture, W.L. Gore & Associates). On the other side of the edentulous mandible, an insertion socket was prepared by drilling and tap preparation of the same size as the implant, and a control implant was placed in accordance with the manual Brånemark® system (Fig. 3c).

**Setting the functional loading**

Six months after the implant placement, secondary surgery was performed, and the ISQ values on each side were measured using an Osstell® device (Osstell AB, Gothenburg, Sweden) and a resonance frequency analysis (RFA) (Fig. 4a). After ISQ measurements, 6-mm superstructures (non-splinted healing abutments; diameter: 3.5 mm, length: 5.0 mm) were set on the bilateral implants (Fig. 4b). To subject the implants to loading, the animals were fed a hard diet (pellet shape, diameter: 8.0 mm, compressive strength: 0.65 MPa) throughout the 5-month functional loading phase.

**Histological evaluation**

After 5 months of functional loading, the animals were euthanized using a sodium pentobarbital injection, and the tissue block sections containing the implants were immediately fixed in 10% buffered formalin.
and processed to obtain thin ground sections. Tissue blocks containing implant/IP-CHA complex and control implants were dehydrated using ascending concentrations of ethanol, cleared with a styrene monomer, and then embedded in light-polymerized polyester resin (Technovit 7200 VLC, Kulzer). To achieve complete polymerization of the resin block, photo polymerization equipment was used (BS5000, EXAKT APPARATEBAU). After polymerization, the specimens were sectioned with a high-precision diamond disc to produce a 200-µm-thick cross-section. The undecalcified specimens were ground to approximately 70-µm-thick sections (MG5000, EXAKT APPARATEBAU), which were stained with toluidine blue. A light microscope (BZ-9000, Keyence, Osaka, Japan) was used for histological examination of both the implant/IP-CHA complex and control specimens.

**Measurement of the ISQ**
The ISQ values of implants were measured repeatedly every month from the start of functional loading. Measurements were performed three times from two different directions, and the values obtained for each implant were averaged. The ISQ measurements were carried out according to previous studies 13,14.

**Measurement of the BIC ratio**
The BIC ratio was measured as the ratio of the contact length of the newly formed bone in total length from the implant portion. The regions of interest for calculation of the BIC ratio are total length from the bottom to the top of implant shoulder part beside on both sides of mesial and distal. BIC ratios were calculated using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

**Statistical analysis**
The data obtained are expressed as means±standard deviations. The ISQ values were statistically analyzed using a one-way analysis of variance and Fisher's test for multiple comparisons, with the significance level set at 5% (Bell Curve software, Social Survey Research Information, Tokyo, Japan). The BIC values were statistically analyzed using Mann-Whitney U tests with the significance level set at 5%.

**RESULTS**

**Cross-section of the implant/IP-CHA complex**
Figure 5 shows a cross-section of the implant/IP-CHA complex. The implant was placed inside of hollow IP-CHA, and a slight space was seen at the interface between the screw threads and IP-CHA.

**Histological evaluation**
Figure 6a shows histological images of a control site; bone formation and osseointegration are mainly detected in the upper portion of the implant (Fig. 6b). The aspects could be observed in cortical bone; however, it was not observed much in the bottom portion in the cancellous bone and bone marrow area (×40).
bone and bone marrow areas.

Figure 7a shows histological images of the implant/IP-CHA complex site; newly formed bone was seen in the pores of the IP-CHA portion of the complex, and the bone formation reached the implant surface (×10). (b) Osteoconduction from the pre-existing bone appeared not only in the upper portion, but also in the middle and bottom portions of the IP-CHA, and resulted in bone contact with the implant surface. New bone formation and osseointegration could be observed in the cortical bone, cancellous bone, and bone marrow areas.

Measurement of the ISQ
Figure 8 shows a comparison of ISQ values between the test group and control group. The ISQ value of the test group at placement was significantly lower than that of the control group (p<0.05, n=4). On the other hand, there was no significant difference between the test group and control group under loading conditions (n=4). In the test group, the ISQ of the complex at placement was significantly lower than those at 3 and 5 months after loading (Table 1). In contrast, there was no significant difference in the control group (Table 2).

Measurement of the BIC ratio
The BIC values were 56.6±6.9 in the control and 49.1±19.6 in the test group group (Table 3). The BIC values were not significantly different between the groups (n=4).

Table 1 Comparison of Mean ISQ for Implant/IP-CHA complex groups

|                        | Mean ISQ (SD) | Fisher test |
|------------------------|--------------|-------------|
| Complex before loading | 59.71 (20.49)| —           |
| Complex 3 months loading | 73.75 (5.04) | *p=0.0378*  |
| Complex 5 months loading | 76.79 (3.66) | **p=0.0138** |

* between Complex before loading and Complex 3M
** between Complex before loading and Complex 5M
SD; standard deviation

Table 2 Comparison of Mean ISQ for control Implant groups

|                        | Mean ISQ (SD) |
|------------------------|--------------|
| Control before loading | 73.24 (2.06) |
| Control 3 months loading | 76.70 (2.49) |
| Control 5 months loading | 77.20 (1.22) |

SD; standard deviation

Table 3 The rate of BIC

|                        | BIC% (SD) |
|------------------------|-----------|
| Control                | 56.6 (6.9) |
| Implant/IP-CHA complex | 49.1 (19.6) |

SD; standard deviation
DISCUSSION

In the present study, the implant/IP-CHA complex achieved both bone regeneration and implant stability under functional loading conditions. Regarding bone regeneration, the biomaterial requirements are biocompatibility, osteoconduct, mechanical strength, and space availability. Generally, pore sizes more than 10 μm in diameter are required to permit osteoconduct because the size of the nucleus in mammalian cells is greater than 10 μm\textsuperscript{15}. Conventional porous hydroxyapatite (HA) has been used in the orthopedic and dental fields, although bone ingrowth into conventional porous HA without interconnected pores penetrated less than 300 μm from the HA surface in a clinical case\textsuperscript{16}. In contrast, IP-CHA has a structure characterized by interconnected pores with an average diameter of 40 μm. The pore size could allow the efficient migration of osteoblasts and mesenchymal stem cells from pore to pore and colonization by blood vessels, which are essential for bone regeneration. For these reasons, the implant stability of the implant/IP-CHA complex could be obtained by osteoconduct from the host bone. To evaluate the osseointegration of the implant/IP-CHA complex, implant stability and the BIC ratio were measured. A previous study demonstrated that placing an implant/IP-CHA complex in the femur resulted in ISQ and BIC values as successful as those of the control implants that were placed in the pre-existing bone\textsuperscript{15}. ISQ measurements using a RFA have been shown to be an effective method of measuring implant stability\textsuperscript{17-19}. The ISQs of successfully stabilized implants are reported to range from 57 to 82\textsuperscript{17}. In the present results, the ISQ of an implant/IP-CHA complex placed in a mandible after 6 months of healing was over 60. The values are compatible with those in a previous study\textsuperscript{19}. On the other hand, those in the control implant group were observed to be 70 or more, and significant differences were observed with respect to the test group. It should be considered that the control implants were placed in sufficient bone, and primary stability was obtained rather than from the implant/IP-CHA complex. The ISQ is considered to increase in proportion to the bone stiffness surrounding the implant surface\textsuperscript{20,21}. The ISQ of the implant/IP-CHA complex gradually increased after loading. Finally, there was no significant difference between the complex and control groups. In the present study, functional loading conditions were set up with the following methods. Healing abutments as super structures were set on the implant bodies of the implant/IP-CHA complexes and control implants, and then the animals were fed a hard diet throughout the functional loading phase of 5 months. The edentulous of the implant placement was prepared by mandibular premolars (P4) extraction. Hard diet pellets are masticated mainly in first molar and premolars, because dog’ occlusion style is scissor-like bite. Therefore, functional loading was given to the super structure portions.

This method was referenced by another study that examined functional loading with the same procedure\textsuperscript{22}. From cross-sectional observation, a slight space was detected at the interface between implant and IP-CHA, where stabilization of the implant and IP-CHA was very poor during the initial stages. ISQ measurement cannot be performed before and immediately after placement. However, stabilization gradually increased after placement in the bone due to bone formation approaching the implant surface in the limited space. Moreover, the formed bone is expected to become functional as bone remodeling occurs in the space of interface. The ISQ correlates with the micro-movement of the placed implant. A low ISQ value indicates a large amount of micro-movement; conversely, a high ISQ value indicates a small amount of micro-movement. Vandamme et al. reported that functional loading with well-controlled micro-motion positively influenced bone formation at the interface of a dental implant\textsuperscript{23}. Therefore, to ingest solid food that has a compressive strength of 65 MPa, it was considered that optimal functional loading occurs around the implants. Therefore, the ISQ values of the implant/IP-CHA complexes and control implants increased over time after functional loading. Histologically, osteoconduct of the complex was detected not only in the cortical area, but also in the cancellous bone area surrounding the periphery. Bone formation was observed on the implant thread surface and osseointegration was maintained during the functional loading phase. In the control group, limited bone formation was observed in the cortical bone area, which was located at the upper portion of the implant, and not much bone formation was detected in the cancellous bone area. Probably when functional loading was applied to the superstructure, the mechanical stimulus was transmitted to the bone tissue in the IP-CHA, and bone remodeling was activated. However, the BIC results were not significantly different between the groups. The reason for the lack of a significant difference in BIC values is as follows. Because the placed complex contained IP-CHA and formed bone, it resulted in less bone contact with the implant surface in the upper portion than in the controls. However, the attached IP-CHA at the implant surface was not calculated as bone contact in the BIC measurement. Thus, bone formation occurred in the cancellous bone area in the complex group, but not in the control group. Therefore, a significant difference in BIC values was not detected between the groups. Blanco et al. showed that there was a positive correlation between increased ISQ and BIC values\textsuperscript{24}. Additionally, histomorphological studies suggest that bone-to-implant ratio and RFA values are related\textsuperscript{25}. According to the results of BIC and ISQ, there was no significant difference between the implant/IP-CHA complex and control implant. ISQ and BIC values were similar, indicating implant stability in both groups. On the basis of the present study, the implant/IP-CHA complex exhibited osteoconduct and osseointegration.
CONCLUSIONS

Our findings indicate that placement of an implant/IP-CHA complex achieved osseointegration and implant stability after undergoing functional loading in the canine mandible. Moreover, the implant/IP-CHA complex may be expected to modify bone quality by its osteoconduction ability. The results of this study may also contribute to increased knowledge and the development of more predictable bone graft materials.

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