ABSTRACT

Objectives: Porous titanium is used for the reconstruction of large bone defects due to its excellent mechanical strength. The quality of osseointegration of implants placed in bone reconstructed with porous titanium is unknown. The purpose of this in vivo study was to evaluate the osseointegration of implants at sites reconstructed using porous titanium.

Material and Methods: Hollow porous titanium (Ti) (outer-diameter 6 mm, inner-diameter 2 mm, length 4 mm, 85% porosity) and similar-sized porous hydroxyapatite (porous HA: 75% porosity) samples were prepared and implanted in 6 New Zealand white rabbit femurs. Four weeks later, an implant bed was created to receive a Ti implant (diameter 2 mm, length 4 mm). An implant placed at a pristine bone site served as the control. Four weeks later, histological and histomorphometric evaluations of the test and control sites were conducted.

Results: Osseointegration was observed in all groups. There was no significant difference in the bone formation ratio and bone-implant contact (BIC) ratio across all groups for the whole area. At the cancellous bone area of the bone defect, superior bone formation ratio and BIC ratio were observed with porous Ti and porous HA compared to the control (bone formation ratio: control 1.8 [SD 3]% I HA 23 [SD 3]%, Ti 23.6 [SD 5]% BIC ratio: control 5.4 [SD 5.3]% HA 28.9 [SD 10.7]%, Ti 41.6 [SD 14]%). Porous Ti demonstrated good osteoconduction and osseointegration abilities, similar to porous HA.

Conclusions: To our knowledge, this is the first report of implant treatment after preliminary bone reconstruction using a titanium biomaterial. Porous titanium is a suitable material for bone reconstruction before implant treatment in load-bearing areas that allow subsequent prosthetic treatment.

Keywords: biocompatible material; dental implants; dental implantation; osseointegration; titanium.
INTRODUCTION

Successful predictable implant therapy depends on the bone quantity and quality at the placement site [1]. Large bone defects due to injury or tumours can complicate implant rehabilitation. In such situations, implant treatment is performed after bone reconstruction using autologous or artificial bone grafts [2,3]. Autografts do not carry a risk of inflammation and have excellent osteoconductivity properties; therefore, they are the gold standard for bone graft materials. However, there are several problems with the limited amount of autologous bone being harvested [4,5]. Hydroxyapatite (HA), an artificial bone substitute, has been used as a graft material due to its excellent biocompatibility and osteoconduction properties. Furthermore, HA, with its porous structure, facilitates implant placement after reconstruction due to the ingrowth of bone within the grafted material [6-8].

Our previous studies [9,10] demonstrated osseointegration of implants after the reconstruction of the preliminary bone site with interconnected porous HA. Despite the high biocompatibility and excellent osteoconduction ability of HA, it is not suitable for the reconstruction of large-size bone defects due to its insufficient mechanical strength. Therefore, novel materials with excellent mechanical strength and osteoconduction properties are required. Currently, autologous bone or titanium (Ti) plates are used as reconstruction materials for large defects. The Ti plate is quite popular as it does not require invasive bone harvesting procedures; however, this treatment does not lead to bone formation at the defect site. On the other hand, porous Ti is a popular alternative in the field of orthopaedics and dentistry due to its biocompatibility and ability to avoid stress-shielding compared to bulk Ti [11,12].

Our previous study showed that reconstruction with porous Ti allows defect sites to be filled with bone due to its interconnected porous structure and osteoconduction properties [13]. Porous Ti, fabricated by the resin-impregnated substitution technique, has a three-dimensional structure where each pore is interconnected. Moreover, it has superior mechanical strength as compared to porous HA. Therefore, implant placement can be used to establish osseointegration in the preliminary bone-reconstructed site using porous Ti. To the best of our knowledge, there are no reports on the implant placement at the preliminary bone-reconstructed site using Ti biomaterials.

The purpose of this in vivo study was to evaluate the osseointegration of implants at sites reconstructed using porous titanium.

MATERIAL AND METHODS

Materials

Hollow pure Ti samples with an 85% interconnected porous structure (outer diameter 6 mm, inner diameter 2 mm, length 4 mm, and pore size 300 µm) were fabricated using the resin-impregnated Ti substitution method and used in this study (Figure 1) [13]. The inner hole was made with high-speed drilling with irrigation. Similar-sized porous HA samples (porosity 75%, 150 µm pore size, NEOBONE®, Aimedic MMT Co., Ltd, Materials, Tokyo, Japan) were also prepared. An inner hole was set at the central portion of the porous material. A custom-fabricated pure Ti implant of diameter 2 mm and height 4 mm (Grade 3 - Nishimura Metal Co., Ltd, Fukui, Japan) was also used (Figure 2). The implant had no threads, and the surface was acid treated by 48% H₂SO₄ (60 °C, 1 h).

![Figure 1. Schematic representation of the resin-impregnated titanium (Ti) substitution fabrication technique.](http://www.ejomr.org/JOMR/archives/2021/3/e4/v12n3e4ht.htm)
Evaluation of the material structure

The porous Ti and HA was evaluated. All samples were sputtered with platinum-palladium to make the surface conductive, set on the sample stage using carbonate adhesive tape, and examined with scanning electron microscopy (JSM-7200F; JEOL Ltd., Tokyo, Japan) at an original magnification x100.

In vivo study

The study was approved by the Research Facilities Committee for Laboratory Animal Science, Hiroshima University School of Medicine (approval no. A16-3). The animal procedure is shown in Figure 3. Six New Zealand white rabbits (male, 17 weeks old, and weight of 3 to 3.5 kg) were used. Prior to any experiment, animals were quarantined in individual cages for a week. All animal experiments were conducted in compliance with the rules of animal experiment in Hiroshima University. Animals were housed in a temperature- and humidity- and air renewal-controlled room and accommodated under a 12 h light-dark cycle. Animals were fed standard dried diet and water ad libitum.

The surgical procedures were performed under general anaesthesia with sodium pentobarbital (10 mg/kg) and local infiltration anaesthesia with 2% lidocaine with 1 : 80,000 noradrenaline. Muscle and periosteal flaps were made on the left and right femurs, and a bone defect (diameter: 6 mm, length: 4 mm) was made using a trephine 1200 rpm with irrigation. Each porous sample was placed in the bone defect. Four weeks later, in a similar operation, the placed porous samples were exposed. The implant was placed at the central portion of the porous sample: the hole was made using a 2 mm diameter pilot drill 1200 rpm with irrigation (HA group, Ti group). At this stage, implants were placed in the existing bone to act as the control. The implants were fixed by press-fit implantation and tight sutures.

Four weeks after implantation (i.e., 8 weeks after the first operation), the rabbits were anaesthetized and perfused with 10% neutral formalin through the aorta. Femurs were harvested and fixed in 10% neutral formalin for 1 week.

Histological evaluation

Tissue blocks including the grafted samples and implants were cut, dehydrated with ethanol, and then embedded in resin (Technovit® 7200VLC - Kulzer, Germany).
The resin blocks were cut at the centre of the implant and ground to a thickness of approximately 60 µm. The specimens were stained with toluidine blue, and the images exhibiting bone regeneration were digitized.

Histomorphometric evaluation

The specimens were histomorphometrically analysed using the ImageJ® software (Fiji distribution; National Institute of Health, Bethesda, Maryland, USA). The region of interest was set as a 1 mm (width) × 4 mm (length) area along the implant surface and divided into the upper portion (cortical bone area [BA]) and the lower portion (cancellous BA) (Figure 4). The newly formed BA was calculated as the ratio of the bone tissue area to the total tissue area in the pore. The bone-implant contact (BIC) was measured as the ratio of BIC length to the implant length.

Statistical analysis

Statistical analysis was conducted using the one-way ANOVA Kruskal-Wallis test followed by Tukey’s honestly significant difference test for multiple comparisons (n = 4). Statistical significance was set at P < 0.05. Parametric data were expressed as mean and standard deviation (M [SD]).

RESULTS

Material structure

Both porous HA and porous Ti had three-dimensional interconnected porous structures. The pore sizes were 150 µm in porous HA and 300 µm in porous Ti (Figure 2 D, E, original magnification x50).

General characteristics

None of the implants showed mobility or loss. No inflammation was observed in the operation site.

Histological observation

The Ti implant exhibited osseointegration in the control group. The bone formation was limited to half of the upper portion, and the lower portion exhibited almost no bone formation or osseointegration (Figure 5). In porous HA and porous Ti samples, bone formation into the material was observed. Bone formation and
osseointegration were achieved along the porous materials of the bone scaffolds, both in the upper and lower portions (Figures 6 and 7).

**Histomorphometric analysis**

No significant differences were observed between the groups for BA and BIC of the total area (BA: control 34.9 [7]%; HA 34.9 [5.6]%; Ti 42.5 [3.5]%; BIC: control 38.1 [6.6]%, HA 43 [9.2]%, Ti 49.3 [9.2]%).

In the HA group, BA in the upper portion (46.6 [8]%) was lower compared to that in the control group (67.9 [12.2]%). At the lower portion, the Ti (23.6 [5]%) and HA groups (23 [3]%) had increased bone formation compared to the control (1.8 [3]%)(Figure 8). The BIC in the upper portion demonstrated no significant differences among the groups; however, in the lower portion, the Ti (41.6 [14]%) and HA groups (28.9 [10.7]%) had higher BIC values than the control group (5.4 [5.3]%)(Figure 9).
DISCUSSION

The present study shows that implants placed at sites reconstructed with porous Ti can achieve osseointegration. Both the porous Ti and porous HA possessed good osteoconduction properties due to an optimal pore size. Through SEM evaluation, the pores were found interconnected in both the samples, with the pore sizes being approximately 200 to 300 µm in porous Ti and 150 µm in porous HA. These pore sizes of 200 to 500 µm are optimal for the colonization of osteoblasts and fibroblasts, or for vascularization within the biomaterial [14].

Failure of materials with low strength is seen when used to reconstruct large bone defects in and load-bearing sites [15-17]. In our previous study [13], the porous Ti showed higher mechanical strength despite its increased porosity (85%), compared to the porous HA, and achieved adequate bone reconstruction at a critical-sized bone defect. In this study, we used hollow porous Ti as a bone reconstruction material. The inner hole was used for implant placement because it was not possible to prepare an implant bed in porous Ti, which is harder than bone, using the normal drilling procedure; a high-speed drill is required. Eriksson et al. [18] reported that when the frictional heat during drilling exceeded 47 °C, bone healing was impaired; therefore, low-speed drilling with irrigation is crucial. To overcome this limitation, we prepared the implant bed in the porous Ti samples before their placement at the bone reconstruction sites. De Santis et al. [19] reported that BIC was superior in bone sites reconstructed prior to implantation compared to when grafting and implantation were performed simultaneously. These results suggest that implant placement after preliminary bone reconstruction would give superior predictability at sites with large bone defects.

Because it was not possible to prepare an implant bed in porous Ti, which is harder than bone, using the normal drilling procedure; a high-speed drill is required. Eriksson et al. [18] reported that when the frictional heat during drilling exceeded 47 °C, bone healing was impaired; therefore, low-speed drilling with irrigation is crucial. To overcome this limitation, we prepared the implant bed in the porous Ti samples before their placement at the bone reconstruction sites. De Santis et al. [19] reported that BIC was superior in bone sites reconstructed prior to implantation compared to when grafting and implantation were performed simultaneously. These results suggest that implant placement after preliminary bone reconstruction would give superior predictability at sites with large bone defects. The bone support around an implant body is essential for successful implant treatment. Bone support was assessed at the upper and lower portions alongside the implant. For BA and BIC, no significant differences were observed between the samples for the total BA. Reconstruction with porous Ti resulted in good implant osseointegration, similar to that with porous HA and at the pristine bone site.
Moreover, BA and BIC values for porous Ti were not significantly different from the pristine bone site in the upper portion. The upper portion was surrounded by pristine bone tissue and is favourable for bone formation within both porous Ti and porous HA. In our previous study, we showed that porous Ti and porous HA can achieve sufficient bone formation using a similar rabbit bone defect model. In contrast, the BA and BIC values in the lower portion were significantly higher for porous Ti and porous HA than for the pristine bone site. This may be attributed to the central part of the femur of a rabbit being primarily occupied by cancellous bone and bone marrow tissue \[20\]. These higher BA and BIC values in the lower portion suggest that porous biomaterials with excellent osteoconduction can achieve bone ingrowth from the surrounding pristine bone even in the cancellous BA and the bone marrow tissue. These results are consistent with that of our previous studies \[10,21\] where bone formation was detected not only in the cortical bone portion of the porous HA but also inside the part that was located in the bone cancellous BA. Furthermore, we placed the implants in the pristine bone sites and reconstructed them using porous HA sites. The bone tissue surrounding the implant threads was then observed. As a result, the bone formation was not detected in the cancellous bone portion of the lower part of the pristine bone site; instead, the bone formation was detected in the cancellous bone portion inside the implant thread of the porous HA site and the bone tissue, which likely supports the placed implant. Based on these results, the BIC at the lower portion demonstrates increased bone formation with scaffolds such as porous Ti or porous HA. The results confirm the good osteoconduction ability of porous Ti, similar to porous HA.

CONCLUSIONS

In this study, porous titanium achieved suitable osteoconduction for preliminary bone reconstruction before implant treatment, equivalent to that of porous hydroxyapatite, and implant support. To our knowledge, this is the first study to evaluate implant treatment after preliminary bone reconstruction using titanium biomaterial. Porous titanium is a suitable material for bone reconstruction before implant treatment in load-bearing situations that will allow for subsequent prosthetic treatments.

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