Bone augmentation using tetrapod-shaped artificial bone in rat tibia

Jun Iwata

Nihon University Graduate School of Dentistry,

Major in Oral and Maxillofacial Surgery

(Director: Prof. Yoshiyuki Yonehara)

Table of Contents

The following article and new unpublished data (Fig. 8 and 9) are part of this doctoral thesis: Augmentation of bone area using tetrapod-shaped artificial bone in rats by Iwata J, Namaki S, Mashimo T, Chung UI, Honda K, Yonehara Y, Journal of Hard Tissue Biology, 24(1): 69-76, 2015

Abstract

The aims of this study were to compare bone augmentation height and mechanical strength after implantation of tetrapod-shaped granular artificial bone (Tetrabone® [TB]), hydroxyapatite granules [HA], α-tricalcium phosphate paste [Pα] or autogenous onlay bone grafts (veneer graft [VN]) and to evaluate the long term effect of bone augmentation by TB.

Forty-eight 6-week-old male Wistar rats were used. The graft materials were implanted in the left tibial subperiosteal pocket. Bone augmentation was evaluated by computed tomography (CT), mechanical testing and histological observation immediately after surgery (0 weeks) and 4, 8, and 12 weeks later. The augmentation height was measured in CT images and the proportion of bone augmentation was calculated as the ratio of 12 weeks/0 weeks. In addition, the long-term evaluation of bone augmentation by TB was performed in radiological and histological observations. CT imaging was carried out at 0, 4, 8 and 48 weeks after implantation. After 48 weeks, rats were sacrificed and histologically observed.

TB aggregates were smooth in shape compared with HA aggregates. The augmentation height was significantly higher in the TB group than in the VN group. At 12 weeks, bone stiffness was significantly higher in the TB group than in the HA and Pα groups, and very similar to that in the VN group. TB remained even at 48 weeks after implantation, and the thickness of augmented bone was more than that of 8-week-bone in TB group. Bone remodeling was observed in the bone marrow side.

TB presented good shape stability and mechanical strength, and served as a scaffold for promoting bone formation and maintaining osteoconduction until 48 weeks. Therefore, TB could be useful for bone augmentation on flat bone surface, when compared with other currently used artificial graft materials.

Introduction

The vertical height of the alveolar ridge decreases with ageing and upon trauma, inflammation and tumor resection. An atrophied alveolar ridge cannot sustain the support of dentures or dental implant. Autogenous bone grafting is the standard method for patients who need bone augmentation before prosthetic treatment. However, the technique is invasive and the graft bone is limited in amount and will be absorbed with time.

Artificial bone has excellent bone conductivity and there are no limits on supply. Hydroxyapatite (HA) granules[1-3)] and α-tricalcium phosphate paste (Pα)[4-6)] are widely used as bone substitutes in patients with fractures, bone defects or absorption of the alveolar ridge[7,8)]. On the other hand, animal studies have led to the developments of new techniques, including autogenous bone onlay grafting[9-12)] and artificial bone block[3, 6, 13-18)].

In the present study, tetrapod-shaped artificial bone made of α-tricalcium phosphate granules of uniform size[19)] was used. Previous studies using so-called Tetrabone® (TB) have been successful in the repair of bone defects[19-23)], indicating that TB is osteoconductive and biocompatible. This study is based on the hypothesis that TB is useful in order to form new bone ridge at the bone flat surface before dental prosthesis and implant. Therefore, this experiment was designed to compare bone augmentation height after implantation of TB or other artificial bone grafts (HA and Pα). Autogenous veneer bone (VN) grafting was used as positive control. Additionally, the long-term effect of bone augmentation by TB was observed. Radiological and histological observations were carried out at 4, 8 and 48 weeks after implantation. After 48 weeks, rats were sacrificed and observed.

Materials and Methods

Graft materials

TB is a form of tetrapod-shaped granular artificial bone developed by injection molding of α-tricalcium phosphate microparticles[19)]. TB granule is 1mm in size (Fig. 1). For comparison purposes, also Neobone® (MMT Co. Ltd., Osaka, Japan) and Biopex-R® (Pentax Co., Tokyo, Japan) were used. Neobone® is composed of hydroxyapatite (HA) granules of 1-3 mm in size. Biopex-R® is a α-tricalcium phosphate paste (Pα). These materials are commonly used in dental treatment. Each material was used about 25 mg.

Animals

Six-week-old male Wistar rats (n = 48) weighting 0.10-0.15 kg were purchased from Sankyo Labo Service Corporation (Tokyo, Japan). The animals were kept in standard metal cages in an experimental animal room (22°C, 55% humidity, 1 atm, 12/12-h light/dark cycle) and fed a standard laboratory diet and water. This study was approved by Nihon University Animal Care and Use Committee (AP10D020).

Operation

General anesthesia was administered by intraperitoneal injection of pentobarbital sodium (50 mg/kg; Kyoritsu, Seiyaku Co., Tokyo, Japan). Local anesthesia using lidocaine (Xylocaine Injection 2% with Epinephrine; AstraZeneca, Osaka, Japan) was used during all surgical procedures. The left shin area of each rat was shaved and made a 20 mm skin incision using a scissors. Then, 15 mm was incised along the bone surface of the medial aspect (about 5 mm down from the proximal metaphysis of the tibia) using a scalpel. The fascia and periosteum were also incised and the subperiosteal lateral aspect of the tibia was exposed. Subsequently, TB, HA and Pα (each, n = 12) were implanted on exposed bone surface subperiosteal space for bone augmentation. In the same way, the lateral aspect of the right tibia was exposed. A piece of cortex (2.0 mm × 8.0 mm) was harvested using a diamond disk bar from the right tibia of each rat for veneer grafting (hereafter referred to as VN) of autogenous bone. Then the graft material was implanted on the left tibia of the same animal (n = 9) as a VN. After implantation, soft tissue was finally returned to its original position and sutured with 5-0 nylon threads. All animals were maintained on a soft diet during the perioperative period.

CT imaging

The left tibia of each rat was observed using *in vivo* micro-computed tomography (R_mCT® system; Rigaku Co., Tokyo, Japan) [24]. Before the imaging procedure, the rats were anesthetized with isoflurane (DS Pharma Animal Health Co. Ltd., Osaka, Japan). Radioscopy was performed in the horizontal and vertical directions, and the tibia was confirmed to be at the center of the field of the vision. The exposure conditions were 17 seconds at 4.0 magnification power, and 90 kV/100 mA. The same monitor was used to create uniform brightness at the time of image evaluation. The images were obtained immediately after surgery (0 weeks) and 4, 8 and 12 weeks later. These images were constructed using image reconstruction software (i-VIEW-R; Morita Co., Kyoto, Japan).

Amount of bone augmentation

Three distances (Fig. 2) at the bone cross section that was increased most were measured immediately after surgery (0 weeks) and 12 weeks later. Proportion of bone augmentation was calculated as the ratio of 12 weeks to 0 weeks. The data are expressed as the average ± standard deviation (SD).

Mechanical test

A schematic drawing of the mechanical test setup of a universal testing machine (Type 5567; Instron Corp., Canton, MA, USA) is shown in Fig. 3. The tibia was excised, and the surrounding tissue was removed at 4, 8 or 12 weeks after implantation. The VN group was used as the positive control. The specimens were set so that the grafting area was exposed on the top surface. A 2-mm rod was preloaded onto the surface of the implantation area or bone graft site at 1 kN force. The specimen was loaded at 3 mm/min, and stopped when displacement reached a depth of 0.35 mm to avoid destruction of the specimen. Force-displacement changes in the bone augmentation site were observed and mechanical strength (MPa) was calculated from the slope of the linear region of the resulting force-displacement curve. Measurements were performed at three points. First point was at the center of the grafting site, and other two points were both ends of first point. Distance between points was each 2 mm. The average value of the three points was considered the representative value of each sample. The sample size was $n = 3$ per group. The data are expressed as the average ± SD.

Histological observation

After mechanical testing at 4, 8, and 12 weeks, the specimens were resected and fixed in 4% paraformaldehyde phosphate buffer solution. The specimens were large enough to contain the implantation site. The specimens were decalcified in 10% EDTA (Dojindo, Kumamoto, Japan) for 14 days at 4°C, and then dehydrated in a graded series of ethanol and embedded in paraffin. The embedded specimens were sectioned transversely at a thickness of 8 μm. The sections were stained with hematoxylin and eosin. Histological observation was performed under a light microscope equipped with a morphometric system connected to a personal computer.

Statistical analysis

Comparisons between groups were performed using one-way analysis of variance (ANOVA). Multiple comparisons were performed using Scheffe's method. A value of $p < 0.05$ was considered statistically significant.

Long term observation

Long-term effect of bone augmentation was observed (n = 9). Radiological and histological observations were performed as described above. CT imaging was taken at 0, 4, 8 and 48 weeks after implantation. After 48 weeks, rats were sacrificed and histologically observed.

Results

Observation of CT images

Multiple tetrapod-shaped opaque images were observed in the TB group immediately after surgery (0 weeks) (Fig. 4). The position of the TB granules was almost unchanged. At 4 weeks, a radiopaque area of new bone formation was observed; the area surrounded the TB granules under the periosteum. At 8 weeks, newly formed bone had increased in size and covered the outside surface of TB. At 12 weeks, the tibia cross-sectional diameter had increased.

Immediately after surgery, the position of the HA granules was unchanged. At 4 weeks, the radiopaque area was also observed to surround the HA granules. At 12 weeks, the tibia cross-section diameter increased. The surface of HA aggregates was not smooth as compared with that of TB aggregates.

In the Pα group, the radiopaque area was observed under the periosteum at 4 weeks. Part of Pα had been absorbed. At 8 weeks, Pα was connected with the cortical bone of the implantation site. At 12 weeks, the surface of Pα appeared as smooth as that of TB aggregates.

VN graft was integrated into the existing bone at 4 weeks. However, at 12 weeks, the bone graft appeared to have been absorbed to some extent. The surface of VN appeared as smooth as that of TB aggregates.

Ratio of augmented bone height

The ratio of augmented bone height (h1) of TB, HA, Pα and VN groups was 1.03 ± 0.03, 0.91 ± 0.06, 0.82 ± 0.15 and 0.54 ± 0.01, respectively (Fig. 5). The ratio of VN was significantly lower as compared with TB ($p = 0.026$). There were no significant differences between other groups.

Ratio of bone marrow height

The ratio bone marrow height (h2) of TB, HA, Pα and VN groups was 0.94 ± 0.12, 1.03 ± 0.19, 0.91 ± 0.05 and 0.90 ± 0.20, respectively (Fig. 5). There were no significant differences between groups.

Ratio of the sum of the tibial outer diameter and the graft's height

The ratio of sum of the tibial outer diameter and the graft's height (h3) of TB, HA, Pα and VN groups was 1.15 ± 0.09, 1.04 ± 0.08, 0.97 ± 0.04 and 0.83 ± 0.16, respectively (Fig. 5). The ratio of the TB group was significantly larger than that of the

VN groups ($p = 0.007$). There were no other differences between groups.

Ratio of the value of subtracting h1 and h2 from h3 (h3-h2-h1)

This ratio indicated the proportion of existing bone increase. The ratio of TB, HA, Pα and VN groups was 1.76 ± 0.45, 1.37 ± 0.45, 1.11 ± 0.02, 1.28 ± 0.16, respectively (Fig. 5). Compared with other graft materials, TB tended to increase the height of the existing bone at 12 weeks, but the difference was not statistically significant.

Bone stiffness

The bone stiffness of TB and HA was increased gradually in 12 weeks (Table 1, Fig. 6). Pα group was increased at 8 weeks, and VN maintained high stiffness. At 4 weeks, the stiffness of VN was significantly higher than that of TB, HA and Pα (each $p = 0.001$). The stiffness of TB was also significantly higher than that of HA ($p = 0.002$) and Pα ($p = 0.001$). At 8 weeks, the stiffness of VN was significantly higher than that of HA ($p = 0.023$), but there were no other significant differences. At 12 weeks, compared with HA and Pα, the stiffness was significantly higher in TB (HA; $p = 0.004$, Pα; $p = 0.002$) and VN (HA; $p = 0.006$, Pα; $p = 0.004$).

Histological observation

Histological observation was performed at 4, 8 and 12 weeks after surgery on rat tibia (Fig. 7). In the TB group, immature bone tissue (arrow) was formed around TB at 4 weeks. At 8 and 12 weeks, TB granules were surrounded with dense bone tissue. In the HA group, connective tissue and new bone invaded HA granules at 4 weeks. New bone was increased at 8 and 12 weeks (arrow). In the Pα group, immature bone tissue (arrow) was formed around Pα at 4 weeks. At 8 weeks, Pα was partially replaced by new bone. In the VN group, absorption of VN started at 4 weeks. At 8 weeks, bone remodeling was similar to that of Pα.

Long term observation

At 48 weeks after implantation, CT image showed increases in the tibia cross-sectional diameter and in the thickness of augmented bone (Fig. 8). The existing cortical bone was absorbed and new cortical bone which contains TB aggregate was formed. Histologically, TB was remained in the implant site. New bone had formed around TB, and absorption of the existing cortical bone was observed after 48 weeks (Fig. 9).

Discussion

This study demonstrated infection-free bone augmentation using TB in rats and showed biocompatibility and osteoconductive capacity similar to those of previous studies[19-23, 25]. Most studies of artificial bones of granular or paste type have examined bone conduction and focused on filling bone defects. But this study was focused on bone augmentation at flat bone area. Studies of bone augmentation using periosteal distraction device[26] or titanium screws[17] have already been reported in animal experiments. Shape stability of bone grafting is easy to obtain by using a surgical plate or surgical mesh in clinical case. The evaluation of subperiosteal bone augmentation by bone material alone without depending on them was performed. Some differences in the process of bone formation were found among different types of graft material.

TB granules remained at the graft site by forming aggregates. The surface of the top of the transplantation area was considerably smooth. Conversely, HA granules showed a tendency to scatter and the surface of the transplantation area lacked continuity. There is a difference in the shape and size of granules of TB and HA. TB granules are tetrapod-shaped and uniform in size. TB granules built connected inter-granular pores of 100-300 μm size as an effective scaffold for cells and vascular invasion and promoted more new bone formation[19]. The particle size of HA is about 0.5-1.0 mm and shape is irregular. HA has 75% porosity, with macropores of 50-300 μm[2]. Tamai *et al.*[1] reported that new bone formation with HA was seen in all pores at 6 weeks in the case of drill hall defect. Repairing of bone defects with TB also led to new bone ingrowth[20-23]. In this study, TB showed new bone growth into the intergranular pores at 4 weeks, but HA showed bone growth both into the intragranular and intergranular pores at 8 weeks. These results indicate that new bone ingrowth in intergranular or intragranular pores at early stage was required to maintain shape stability. In addition, both groups had h3-h2-h1 > 1.0. This result showed that both groups keep the height of bone amplification at 12 weeks, but raised the possibility that existing bone becomes thicker by implanting TB rather than HA. It is considered that if granules would have uniform morphology[19], it would reduce risk of their mound collapse caused by an external or mechanical force in the case of bone augmentation at flat site. Throughout the 12 weeks of the study, TB recorded greater rigidity than HA. From these, it is concluded that TB has excellent stability at the site where a large load is applied compared to HA, which is currently used in the clinic.

This results of Pα are in line with previous research[6] which had showed bone replacement seen in the vicinity of the boundary to the host bone. In addition, the surface of Pα implantation area was regular and was confirmed good bone conductivity similar to TB. However, the h1 value of Pα was below 1.0. These data showed that Pα was absorbed to some extent at 12 weeks after implantation. They also suggest that it is difficult for Pα to maintain the height of bone augmentation in a flat area compared to TB.

In VN, the surface of transplantation area was regular in shape. In the case of onlay graft, Donos *et al.*[27)] reported that the height of the graft as control group was reduced and active absorption had occurred at 60 days. Phillips *et al.*[28)] reported that completely or partially bone absorption occurred when they implanted veneer graft without screw fixation. In this study, the height of grafting of VN became approximately half of its original value at 12 weeks, and was significant lower than the ratio of TB. These data reveal that it is hard to keep the amount of augmentation using VN in 12 weeks. The stiffness of TB almost reached that of VN. These findings raise the possibility that TB can be used as a substitute for autogenous bone graft.

TB remained at the subperiosteal space after 48 weeks, and new bone were formed around TB. The thickness of augmented bone increased in the period between 8 and 48 weeks after implantation. Bone remodeling was performed in the bone marrow side. Existing cortical bone was absorbed, and new cortical bone was formed. TB became a scaffold for promoting bone formation and maintaining osteoconduction until 48 weeks. In the experiment of rabbit bone defect which formed by surgery drilling, it was reported that TB was gradually absorbed until 26 weeks after implantation[25)]. In this study, the absorption of TB was not observed. It is speculated that it would require further time for the absorption of TB in the flat area of cortical bone.

Conclusions

In oral and maxillofacial surgery, autogenous bone grafts or bone substitutes are required in order to augment alveolar ridge and sinus floor before treating dental implant or dentures. The possibility of TB as a tissue scaffold for augmentation of alveolar ridge, however, has never been revealed well before. In this result, TB showed good potential to shape stability of bone augmentation compared to other groups, and it has the mechanical strength at the comparable level to VN at 12 weeks in transplantation area. Also TB maintained osteoconduction until 48 weeks. Because TB alone has a limitation at huge flat bone area which requires continuous height, the next step will be to pursue valid scaffolding material which can be used in combination with TB in order to assure the enough amount of bone augmentation.

TB showed advantages over other commonly used artificial graft materials. These findings support the clinical application of TB to treat bone augmentation.

Acknowledgments

Gratitude is expressed to Dr. Nobuyuki Shimohata for supplying of materials and Dr. Yoshinori Arai and Dr. Masahiro Kaketani for technical assistance. This work was supported in part by Grants-in-Aid for Scientific Research (C) (24593063) from the Japan Society for the Promotion of Science (JSPS), Sato Fund, Uemura Fund, Supported by Dental Research Center and Grant from Dental Research Center, Nihon University School of Dentistry.

References

1. Tamai N, Myoui A, Tomita T, Nakase T, Tanaka J, Ochi T and Yoshikawa H (2002) Novel hydroxyapatite ceramics with an interconnective porous structure exhibit superior osteoconduction *in vivo.* J Biomed Mater Res 59, 110-117

2. Yamasaki N, Hirao M, Nanno K, Sugiyasu K, Tamai N, Hashimoto N, Yoshikawa H and Myoui A (2009) A comparative assessment of synthetic ceramic bone substitutes with different composition and microstructure in rabbit femoral condyle model. J Biomed Mater Res Part B Appl Biomater 91B, 788-798

3. Minami M, Takechi M, Ohta K, Ohta A, Ninomiya Y, Takamoto M, Fukui A, Tada M and Kamata N (2013) Bone formation and osseointegration with titanium implant using granular- and block-type porous hydroxyapatite ceramics (IP-CHA). Dent Mater J 32, 753-760

4. Kurashina K, Kurita H, Hirano M, de Blieck JMA, Klein CPAT and de Groot K (1995) Calcium phosphate cement: *in vitro* and *in vivo* studies of the α-tricalcium phosphate-dicalcium phosphate dibasic-tetracalcium phosphate monoxide system. J Mater Sci Mater Med 6, 340-347

5. Musha Y, Umeda T, Yoshizawa S, Shigemitsu T, Mizutani K and Itatani K. (2010) Effects of blood on bone cement made of calcium phosphate: problems and advantages. J Biomed Mater Res Part B Appl Biomater 92B, 95-101

6. Marukawa K, Ueki K, Okabe K, Nakagawa K and Yamamoto E (2011) Use of self-setting α-tricalcium phosphate for maxillary sinus augmentation in rabbit. Clin Oral Impl Res 22, 606-612

7. Uckan S, Deniz K, Dayangac E, Araz K and Ozdemir BH (2010) Early implant survival in posterior maxilla with or without β-tricalcium phosphate sinus floor graft. J Oral Maxillofac Surg 68, 1642-1645

8. Shigeishi H, Takechi M, Nishimura M, Takamoto M, Minami M, Ohta K and Kamata N (2012) Clinical evaluation of novel interconnected porous hydroxyapatite ceramics (IP-CHA) in a maxillary sinus floor augmentation procedure. Dent Mater J 31, 54-60

9. Sullivan WG and Szwajkun PR (1991) Revascularization of cranial versus iliac crest bone grafts in the rat. Plast Reconstr Surg 87, 1105-1109

10. Chen NT, Glowacki J, Bucky LP, Hong HZ, Kim WK and Yaremchuk MJ (1994) The roles of revascularization and resorption on endurance of craniofacial onlay bone grafts in the rabbit. Plast Reconstr Surg 93, 714-722

11. Buchman SR and Ozaki W. The ultrastructure and resorptive pattern of cancellous onlay bone grafts in the craniofacial skeleton (1999) Ann Plast Surg 43, 49-56

12. Ozaki W, Buchman SR, Goldstein SA and Fyhrie DP (1999) A comparative analysis of the microarchitecture of cortical membranous and cortical endochondral onlay bone grafts in the craniofacial skeleton. Plast Reconstr Surg 104, 139-147

13. Araújo MG, Sonohara M, Hayacibara R, Cardaropoli G and Lindhe J (2002) Lateral ridge augmentation by the use of grafts comprised of autologous bone or a biomaterial. An experiment in the dog. J Clin Periodontol 29, 1121-1131

14. Tamura K, Sato S, Kishida M, Asano S, Murai M and Ito K (2007) The use of porous β-tricalcium phosphate blocks with platelet-rich plasma as an onlay bone graft biomaterial. J Periodontol 78, 315-321

15. Wang S, Zhang Z, Zhao J, Zhang X, Sun X, Xia L, Chang Q, Ye D and Jiang X (2009) Vertical alveolar ridge augmentation with beta-tricalcium phosphate and autologous osteoblasts in canine mandible. Biomaterials 30, 2489-2498

16. Yamauchi K, Takahashi T, Funaki K, Miyamoto I and Yamashita Y (2009) Implant placement for periosteal expansion osteogenesis using β-tricalcium phosphate block: an experimental study in dogs. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 108, 861-866

17. Yamauchi K, Takahashi T, Funaki K, Hamada Y and Yamashita Y (2010) Histological and histomorphometrical comparative study of β-tricalcium phosphate block grafts and periosteal expansion osteogenesis for alveolar bone augmentation. Int J Oral Maxillofac Surg 39, 1000-1006

18. Kim SJ, Shin HS and Shin SW (2010) Effect of bone block graft with rhBMP-2 on vertical bone augmentation. Int J Oral Maxillofac Surg 39, 883-888

19. Choi S, Liu IL, Yamamoto K, Igawa K, Mochizuki M, Sakai T, Echigo R, Honnami M, Suzuki S, Chung UI and Sasaki N (2012) Development and evaluation of tetrapod-shaped granular artificial bones. Acta Biomater 8, 2340-2347

20. Maeda Y, Hojo H, Shimohata N, Choi S, Yamamoto K, Takato T, Chung UI and Ohba S (2013) Bone healing by sterilizable calcium phosphate tetrapods eluting osteogenic molecules. Biomaterials 30, 1-8

21. Honnami M, Choi S, Liu IL, Kamimura W, Taguchi T, Hojo H, Shimohata N, Ohba S, Koyama H, Nishimura R, Chung UI, Sasaki N and Mochizuki M (2013) Repair of rabbit segmental femoral defects by using a combination of tetrapod-shaped calcium phosphate granules and basic fibroblast growth factor-binding ion complex gel. Biomaterials 34, 9056-9062

22. Choi S, Liu IL, Yamamoto K, Honnami M, Sakai T, Ohba S, Echigo R, Suzuki S, Nishimura R, Chung UI, Sasaki N and Mochizuki M (2014) Implantation of tetrapod-shaped granular artificial bones or β-tricalcium phosphate granules in a canine large bone-defect model. J Vet Med Sci 76, 229-235

23. Elgali I, Igawa K, Palmquist A, Lennerås M, Xia W, Choi S, Chung UI, Omar O and Thomsen P (2014) Molecular and structural patterns of bone regeneration in surgically created defects containing bone substitutes. Biomaterials 35, 3229-3242

24. Mashimo T, Saito T, Shiratsuchi H, Iwata J, Uryu T, Tamagawa T, Namaki S, Matsumoto K, Kawashima S, Mori Y, Arai Y, Honda K and Yonehara Y (2013) Assessment of the bone regenerative process from fibular periosteum by *in vivo* micro computed tomography. J Hard Tissue Biol 22, 391-400

25. Choi S, Liu IL, Yamamoto K, Honnami M, Ohba S, Echigo R, Sakai T, Igawa K, Suzuki S, Nishimura R, Chung UI, Sasaki N and Mochizuki M (2014) Comparison of the long-term effects on rabbit bone defects between Tetrabone® and β-tricalcium phosphate granules implantation. J Artif Organs 17, 344-351

26. Inoue H, Kuroiwa Y, Naitoh M, Ariji E, Sugita Y, Maeda H and Kurita K (2014) Image analysis of lateral alveolar ridge augmentation using periosteal distraction osteogenesis. J Hard Tissue Biol 23, 125-130

27. Donos N, Kostopoulos L and Karring T (2002) Augmentation of the mandible with GTR and onlay cortical bone grafting. Clin Oral Implants Res 13, 175-184

28. Phillips JH and Rahn BA (1988) Fixation effects on membranous and endochondral onlay bone-graft resorption. Plast Reconstr Surg 5, 872-877

Table 1. Bone stiffness value (MPa) at 4, 8, and 12 weeks after surgery.

	4 weeks	8 weeks	12 weeks
TB	173.5 ± 21.0	194.6 ± 24.1	277.0 ± 8.5
HA	26.1 ± 3.6	105.6 ± 25.5	117.6 ± 14.3
Pα	78.2 ± 6.7	147.9 ± 1.3	111.0 ± 1.9
VN	296.1 ± 39.6	278.3 ± 95.9	264.4 ± 43.6

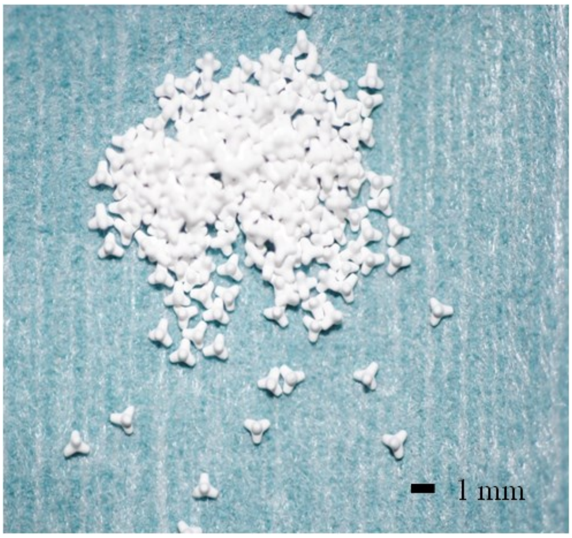

Fig.1. Photograph of the TB granules.

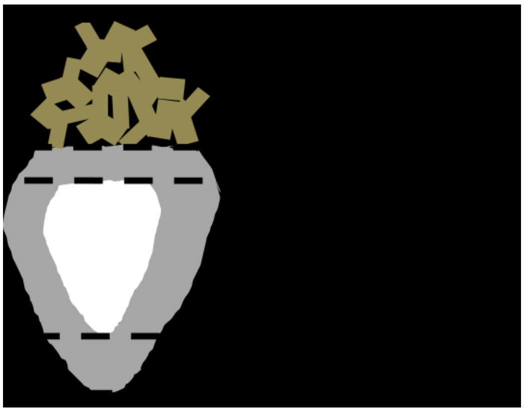

Fig. 2. Definition of landmarks for measuring the amount of bone augmentation. The distances represent the height of the augmented bone (h1), the height of the bone marrow (h2) and the sum of the tibial outer diameter and the graft's height (h3).

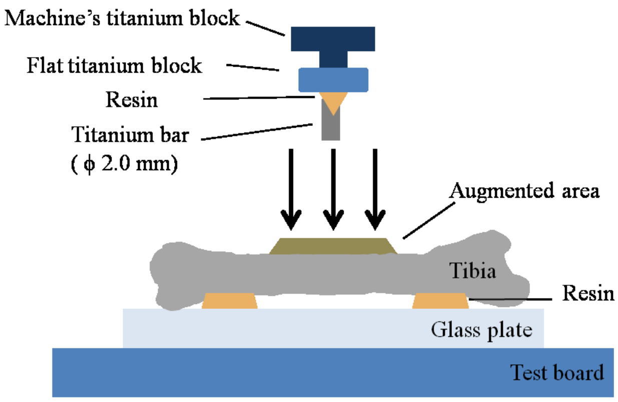

Fig. 3. The tibia was fixed with resin cement to a glass plate on the test board of a universal testing machine (Type5567; Instron Corp., Canton, MA, USA).

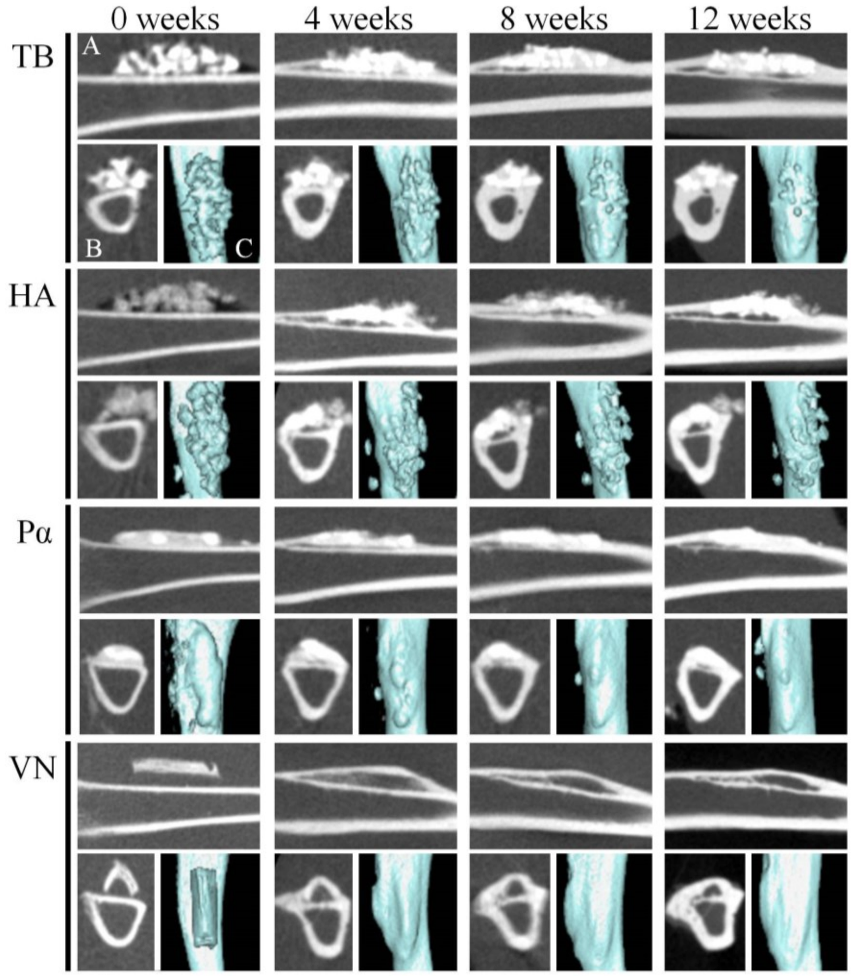

Fig. 4. Representative sagittal plane (A), cross-sectional plane (B) and reconstructed 3D (C) CT images of bone graft sites. Newly formed bone (opaque images) extending to the cortical bone from both ends of each graft material is observed at 4 weeks after implantation.

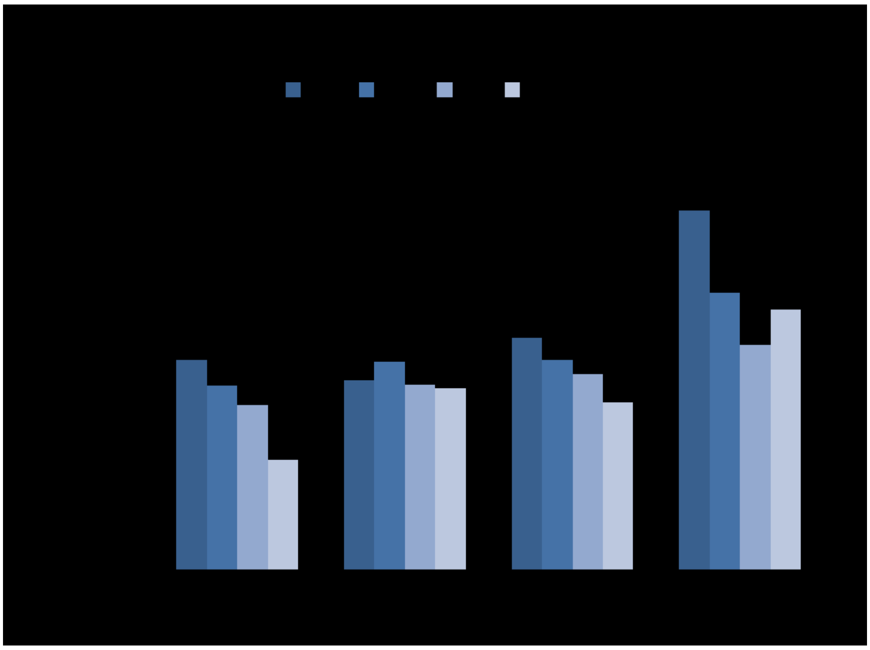

Fig. 5. The 12 weeks/0 weeks ratio of bone augmentation height. *p < 0.05

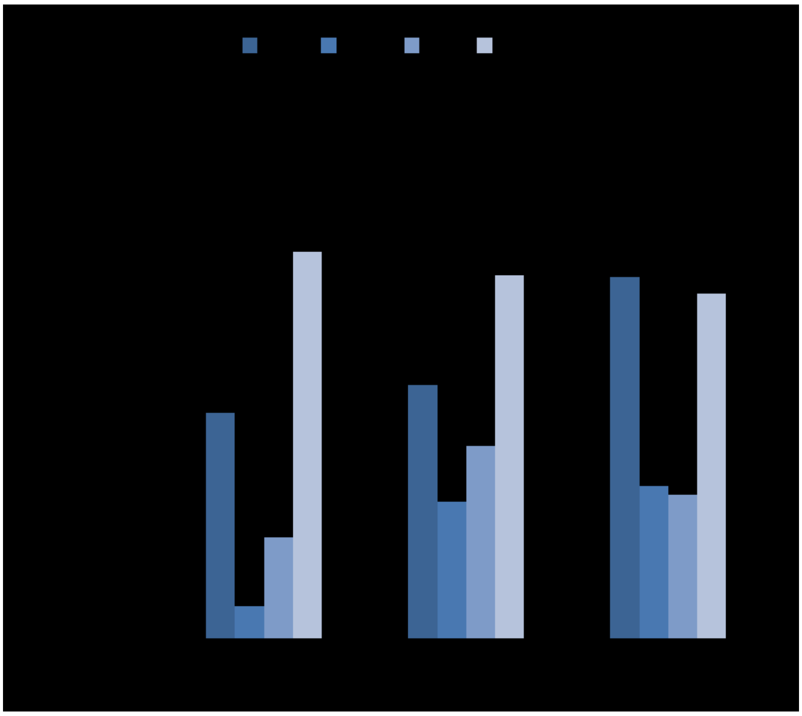

Fig. 6. Bone stiffness as assessed by mechanical test at 4, 8 and 12 weeks after surgery. *p < 0.05

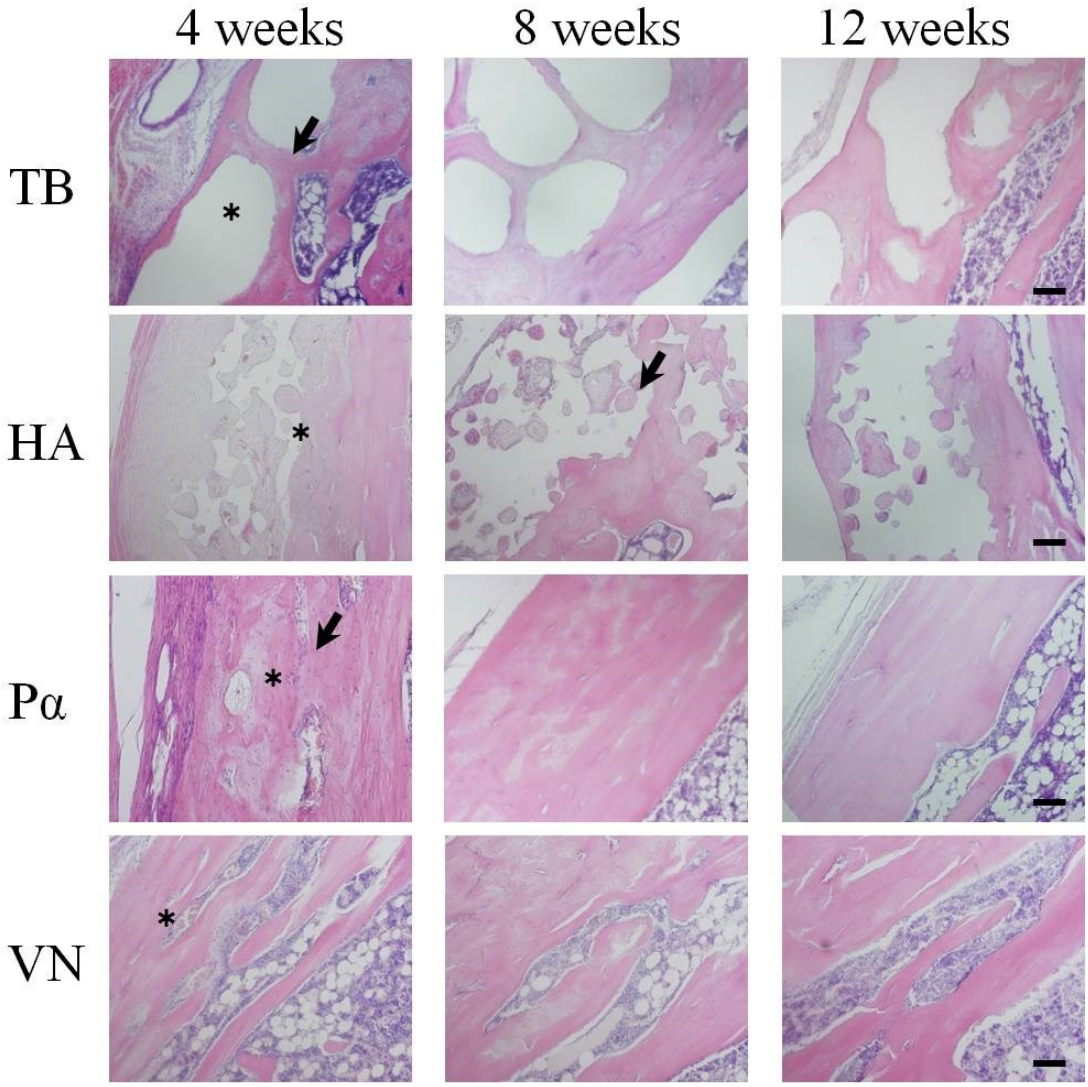

Fig. 7. Hematoxylin and eosin-stained sections of grafting sites. The asterisks indicate the artificial and autogenous grafts. The arrows indicate new bone formation. Scale bar: 200μm.

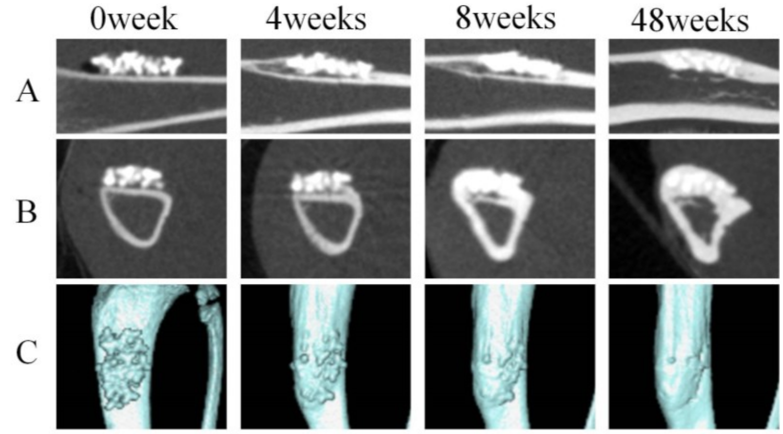

Fig. 8. The sagittal plane (A), cross-sectional plane (B) and reconstructed 3D (C) CT images of the identical rat until 48 week.

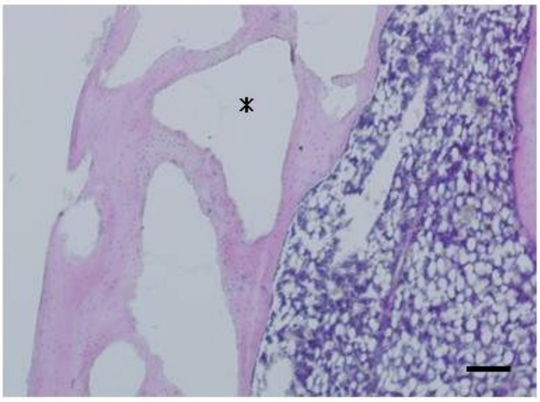

Fig. 9. Hematoxylin and eosin-stained section of TB implant sites at 48 weeks. The asterisk indicates a TB granule. Scale bar: 200μm.