Original Article

Fixation of an orthodontic anchor screw using beta-tricalcium phosphate in a screw-loosening model in rats

Kie Nishioka-Sakamoto[a]; Hitoshi Hotokezaka[b]; Yuka Hotokezaka[c]; Yukako Nashiro[a]; Mariko Funaki[a]; Seigo Ohba[d]; Noriaki Yoshida[e]

ABSTRACT

Objectives: To create an orthodontic anchor screw (OAS)–loosening model and to investigate whether filling the bone hole with beta-tricalcium phosphate (β-TCP) can fix the OAS against orthodontic force.

Materials and Methods: Bone holes with different diameters (1.6, 2.1, or 2.5 mm) were drilled in the tibias of 11-week-old male Wistar rats, and an OAS (3.0 mm in length and 1.2 mm in diameter) was inserted. After a healing period of 2 or 4 weeks, orthodontic force was applied, and the diameter of the bone hole appropriate for the loosening model was determined. Subsequently, under the loosening model, the bone hole was filled with β-TCP, orthodontic force was applied, and movement of the OAS and surrounding tissue changes were evaluated by micro–computed tomography images and histological specimen analysis.

Results: The bone hole of 1.6 mm in diameter was employed as the OAS-loosening model. When β-TCP was inserted into the bone hole, the linear distance and mesial tipping angle of the OAS movement decreased markedly. Furthermore, the values of bone morphometry significantly increased with β-TCP filling.

Conclusions: An OAS-loosening model was established in rats and demonstrated that the loosening OAS was stabilized by β-TCP filling through bone formation. β-TCP may be useful for fixation of a loosening OAS. (*Angle Orthod.* 0000;00:000–000.)

KEY WORDS: Orthodontic anchor screw; Beta-tricalcium phosphate; Fixation; Loosening model; Rat; Bone morphometry

INTRODUCTION

Orthodontic anchor screws (OASs) are commonly used as temporary anchorage devices because of the simple surgical procedure required for insertion and the performance they offer in terms of absolute anchorage.[1] However, loosening and loss of OAS occasionally occur and interfere with the planned treatment.[2]

During OAS insertion, insertion sites are often restricted because of anatomical limitations.[3] Then, once the site becomes loose, reinserting an OAS is considered a challenge. When an OAS is reinserted during orthodontic treatment, it may be inserted in the same location where it was originally placed or it may be inserted in another appropriate location. Because repair of the bone defect at the site must occur, reinsertion of the OAS at the same site requires a healing period. Additionally, this OAS loosening often forces a change in the treatment plan and could include changes in the location for reinsertion and the length and diameter of the OAS.[2] In fact, Uesugi et al.[2]

---

[a] Postgraduate Student, Department of Orthodontics and Dentofacial Orthopedics, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan.

[b] Associate Professor, Department of Orthodontics and Dentofacial Orthopedics, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan.

[c] Senior Assistant Professor, Department of Orthodontics and Dentofacial Orthopedics, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan.

[d] Associate Professor, Department of Regenerative Oral Surgery, Nagasaki University, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan.

[e] Professor and Chair, Department of Orthodontics and Dentofacial Orthopedics, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan.

Corresponding author: Dr Hitoshi Hotokezaka, Associate Professor, Department of Orthodontics and Dentofacial Orthopedics, Graduate School of Biomedical Sciences, Nagasaki University, 1-7-1 Sakamoto, Nagasaki 852-8588, Japan
(e-mail: hotoke@nagasaki-u.ac.jp)

Accepted: December 2022. Submitted: August 2022.

reported that the success rate of secondary OAS insertion significantly decreased.

In the case of dental implants, when the bone mass is small it is often augmented by autologous, allogeneic, heterologous, or alloplastic graft material.[4–6] Bioactive ceramics have also been used as possible alternatives for bone regeneration.[7] These biomimetic materials can be bone-inducible materials in some cases.[8] One of the most clinically used materials in this category is beta-tricalcium phosphate (β-TCP). β-TCP is biocompatible, with a calcium/phosphorus ratio similar to that of bone components, and it promotes bone growth.[9,10] Additionally, it has a more rapid decomposition rate compared to other bone substitutes.[11]

Approaches used for dental implants provide a rationale for using biomimetic materials, such as β-TCP, in the loosening site as a fixative tool for reinserted OAS in the initially drilled location. However, few studies have examined the reinsertion and fixation of OASs after failure of the first insertion. More fundamentally, there is no animal model of OAS loosening. Hence, this study aimed to develop an OAS-loosening model using rat tibia and to investigate whether filling the loosened bone hole with β-TCP could fix the inserted OAS.

MATERIALS AND METHODS

This study was approved by the Animal Welfare Committee of Nagasaki University (1803301444-4). Eleven-week-old male Wistar rats (SLC Shizuoka, Japan [body weight, 250–300 g]) were used in this study. The rats were housed in a colony room and fed a standard pellet diet and water ad libitum.

All operations were performed under general anesthesia with an intraperitoneal injection of 0.375 mg/kg medetomidine (Zenoaq, Fukushima, Japan), 2 mg/kg midazolam (Sandoz, Tokyo, Japan), and 2.5 mg/kg butorphanol tartrate (Meiji Seika Pharma Co Ltd., Tokyo, Japan).

Time Course of Self-Healing of the Bone Defect Hole

A bone hole, 3 mm in depth and 1.6 mm in diameter, was made in the rat tibia, and self-healing was observed for 8 weeks ($n = 4$). A bone hole was formed 1 mm below the knee joint perpendicular to the tibial surface using a fissure bur (MS Steel Bur HP Fissure 016, Morita, Tokyo, Japan). The healing status of the bone defect hole was evaluated serially using in vivo micro–computed tomography (CT) (R_mCT, Rigaku, Tokyo, Japan). To analyze the bone morphometry, a volume of interest (VOI, $1600 \times 1600 \times 400$ μm) was set in the CT image (Figure 1A). Bone mineral density (BMD), bone mineral content (BMC), and bone volume (BV) of the cortical tibia were measured using three-dimensional (3D) image analysis software (TRI/3D-BON, Ratoc System Engineering, Tokyo, Japan).

Development of OAS-Loosening Model

The anchor-loosening model was evaluated under three different OAS hole diameters: 1.6 mm ($n = 20$), 2.1 mm ($n = 20$), and 2.5 mm ($n = 20$). A hole with a depth of 3 mm was drilled in the rat tibia. The OAS, 4.5 mm in length and 1.2 mm in diameter (ACE, Brockton, Mass), was placed in the drilled hole using a hand screw, and the OAS was stabilized in the center of the bone hole using a 0.008-inch–diameter twisted stainless-steel wire (Figure 1B). The surgical site was closed and was maintained for a healing period of 2 or 4 weeks (Figure 1C). After the healing period, the twisted wire for retention was removed, and a 0.06-mm–diameter small hole was made on the long axis of the tibia 15 mm distal to the OAS. A 100-g nickel-titanium coil spring (Tomy, Fukushima, Japan) was ligated between the small hole and the OAS to apply orthodontic force (Figure 1D). Subsequently, the diameter of the OAS hole that prevented the OAS from falling out but loosened the inserted OAS was determined. As described in the "Results" section, a 1.6-mm–diameter bone hole was appropriate for the OAS-loosening model and was used for the following experiment.

Effect of β-TCP on the Fixation of Reinserted OAS

To test whether filling the created 1.6-mm–diameter bone hole with β-TCP would cause the OAS to resist the orthodontic force the rats were randomly divided into two groups: the β-TCP (HOYA Technosurgical Co, Tokyo, Japan) filling ($n = 18$) and control ($n = 20$) groups. No material was filled in the control group. Subsequently, the screw was inserted into the bone hole, and the skin was sutured. After 2 ($n = 10$ in each group) or 4 ($n = 8$ or 10 in β-TCP and control groups, respectively) weeks (2-week or 4-week healing group), the wound was reopened, and orthodontic force was applied according to the procedures described previously. One week after the orthodontic force was applied, linear distance, mesial tipping angle, and bone morphometry were measured using in vivo micro-CT (Figure 1E). 3D micro-CT images from R_mCT were superimposed and analyzed using 3D image analysis software. Movements of the OAS and bone morphometry after applying the orthodontic force were compared between the β-TCP and control groups (Figure 1F). To compare the bone morphometry parameters, a volume of interest (VOI, $2000 \times 2000 \times 3000$ μm) was set in the CT image (Figure 1G).

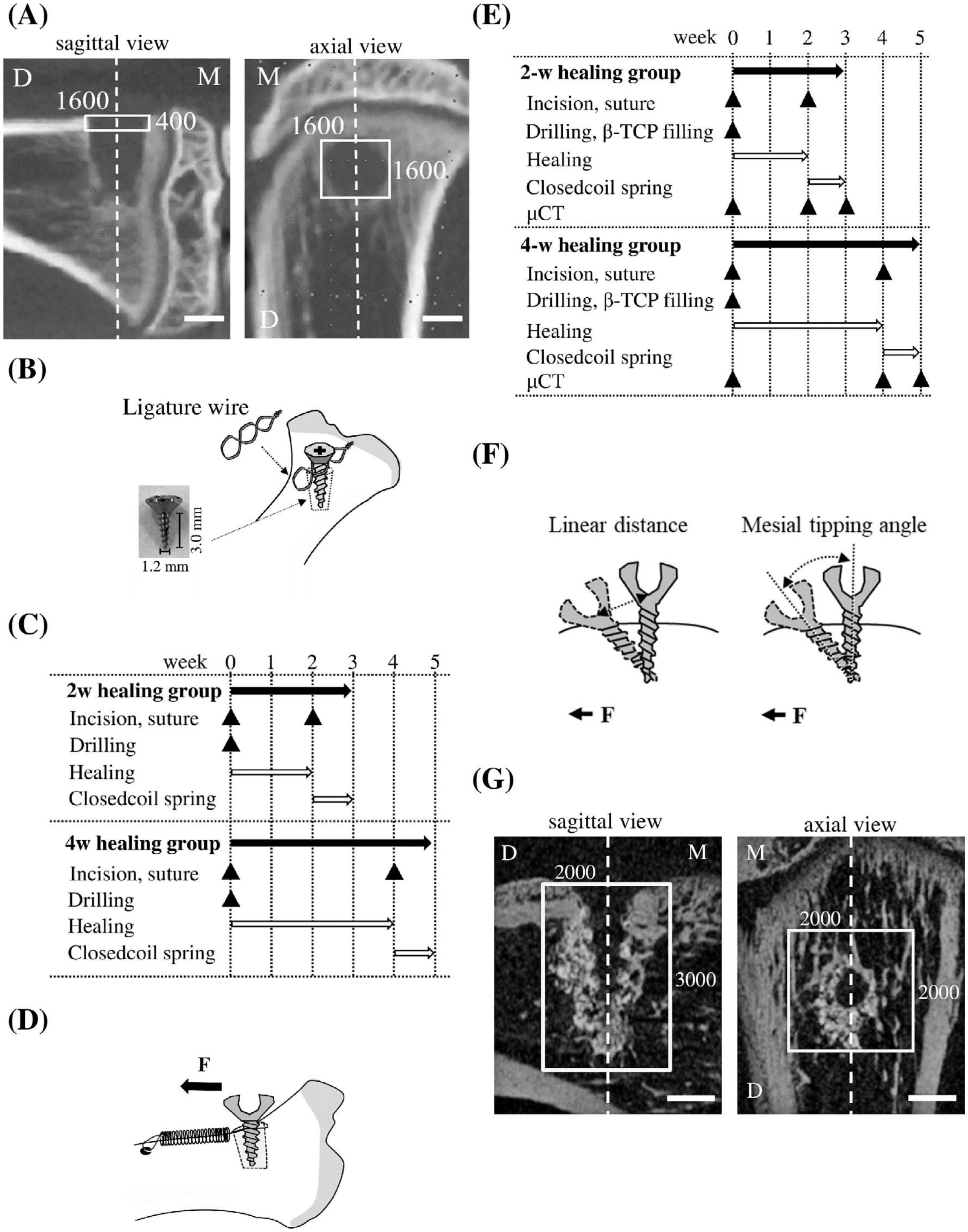

Figure 1. (A) Bone morphometry and the volume of interest. Sagittal and axial views of a rat tibia in which the bone hole was drilled. Boxes indicate the volume of interest (1600 × 1600 × 400 μm). D indicates distal; M, mesial. Scale bar: 1 mm. (B) A screw used in this study. Illustrations of the rat tibia, bone hole, screw, and twisted ligature wire. (C) Time schedule of the experiment to determine the bone hole diameter. (D) Illustrations of the closed coil spring ligated between the small hole and the orthodontic anchor screw (OAS) to apply orthodontic force. (E) Time schedule of the experiment of beta-tricalcium phosphate (β-TCP) filling. (F) Measurements of OAS movement: Linear distance, mesial tipping angle. (G) Bone morphometry and the volume of interest. Sagittal and axial views of a rat tibia in which the bone hole was drilled. Boxes indicate the volume of interest (2000 × 2000 × 3000 μm). D indicates distal; M, mesial. Scale bar: 1 mm.

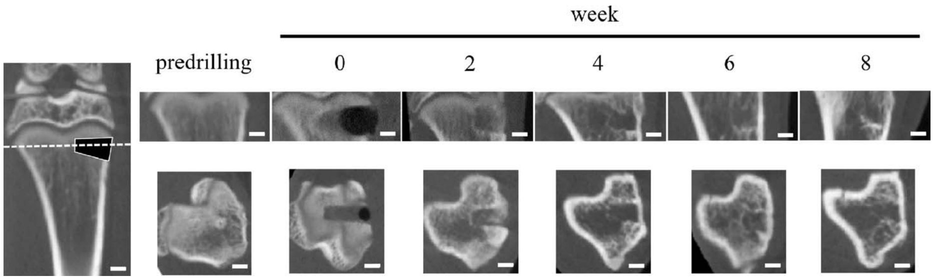

Figure 2. Observation of self-healing process of the bone hole by in vivo micro–computed tomography. Scale bar: 1 mm.

After completion of the experiment, the rats were euthanatized with an overdose of carbon dioxide, and the knee joint of the tibia was excised. Subsequently, the finer bone structures were visualized by high-resolution ex vivo micro-CT and histology.

In Vivo Micro-CT

In vivo micro-CT was performed under anesthesia. The parameters of image acquisition were as follows: voltage = 90 V; current = 100 μA; scanning time = 2 minutes; and resolution = 20 μm/pixel.

High-Resolution Ex Vivo Micro-CT

High-resolution ex vivo micro-CT images using SkyScan 1272 scanner (Bruker, Kontich, Belgium) were taken under the following parameters of resolution: 9 μm, 360° rotation, 0.6° rotation steps, and 0.1-mm copper filter (90 KV, 276 μA). The status of bone induction around the OAS was quantified using CT software, version 1.14.4 (Bruker).

Histological Analysis

The excised tibia was fixed in a formalin solution (FUJIFILM Wako, Osaka, Japan) for 48 hours. Subsequently, the tibia was decalcified with 17% ethylenediamine tetraacetic acid (Merck Millipore, Darmstadt, Germany) at 20°C for 4 weeks. After decalcification, the tibia was dehydrated and embedded in paraffin. Continuous slicing (6-μm thickness) was performed, and hematoxylin and eosin staining was performed.

Statistical Analyses

The same researcher (KN) performed all measurements three times, and the mean value was used.

Statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan). A paired *t*-test was used to test the differences between the two groups. One-way analysis of variance was used to compare the mean changes in bone morphometry. All data are presented as mean ± standard deviation.

RESULTS

Time Course of Self-Healing of the Bone Defect Hole

No healing of cortical bone was observed 2 weeks after the bone hole was made. After 4 weeks, regeneration of thin cortical bone was observed. After 6 weeks, regeneration of cortical bone with the same thickness as the nearby cortical bone was observed (Figure 2; Table 1).

Development of OAS-Loosening Model

All OASs placed in the bone hole with the 2.1- and 2.5-mm diameters fell out during application of orthodontic force. In contrast, only two out of the 10 OASs placed in the bone hole with 1.6-mm diameter fell out after a 2-week healing period, and 1 out of the 10 OASs fell out after a 4-week healing period when orthodontic force was applied. Therefore, the bone hole diameter of 1.6 mm was used as the OAS-loosening model.

Effect of β-TCP Filling on Fixation of Reinserted OAS

In the β-TCP filling group, only one out of 10 OASs fell off during application of orthodontic force in the 2-week healing group, and no OASs fell off in the 4-week healing group. On the contrary, in the control group, three out of 10 of OASs fell off in the 2-week healing

Table 1. Self-Healing of the Bone Defect Hole (Cortical Bone)[a]

	BMD	BMC	BV
Predrilling	720.13 ± 41.41	4.01 ± 0.74	7.72 ± 1.42
0 wk	0*	0*	0*
2 wk	518.73 ± 11.54*	1.23 ± 0.86*	5.83 ± 1.85
4 wk	577.45 ± 38.25*	2.55 ± 1.73	6.25 ± 0.60
6 wk	623.08 ± 97.93	3.13 ± 1.18	6.74 ± 0.92
8 wk	717.00 ± 51.53	3.78 ± 0.80	6.82 ± 1.53

[a] BMD indicates bone mineral density (mg/cm^3); BMC, bone mineral content ($mg \times 10^{-2}$); and BV, bone volume ($cm^3 \times 10^{-4}$). Values are presented as means ± standard deviation.

* $P < .01$ compared with predrilling.

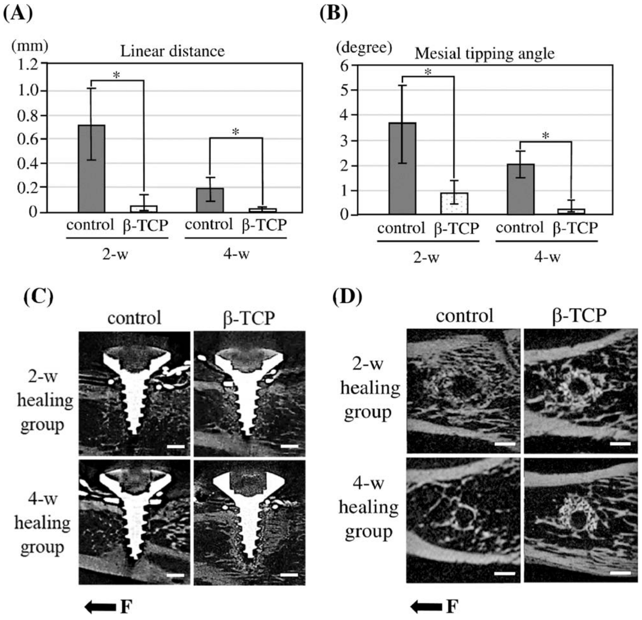

Figure 3. (A, B) Measurements of orthodontic anchor screw movement. (A) The linear distance in the 2-week and 4-week healing groups. (B) The medial tipping angle in the 2-week and 4-week healing groups. * $P < .01$. (C, D) Ex vivo high-resolution micro–computed tomography images. (C) Sagittal view; (D) axial view. Scale bar: 1 mm.

group, and two out of 10 OASs fell off in the 4-week healing group during application of orthodontic force.

The linear distance and mesial tipping angle movements of the OAS in the β-TCP group were remarkably smaller than those in the control group for both the 2- and 4-week healing groups (Figure 3A,B). All measurements of OAS movements were significantly smaller in the 4-week than in the 2-week healing group.

As shown in Table 2, BMD, BMC, and BV of the cortical bone were significantly higher in the β-TCP filling group than in the control group, both in the 2-week and 4-week healing groups. For trabecular bone, BMD was significantly higher in the β-TCP filling group than in the control group in the 2-week and 4-week healing groups. BV and BV/TV were significantly higher in the β-TCP filling group than in the control in the 4-week healing groups.

Ex vivo micro-CT images demonstrated that in the control group, a low-density radiopaque image around the OAS was observed in the 2-week healing group, and the density was increased in the 4-week healing group. In contrast, in the β-TCP group, many radiopaque β-TCP granules were observed around the OAS in the 2-week healing group. Additionally, thick new bone-like radiopaque images were observed around the OAS in the 4-week healing group (Figure 3C,D).

Histological analyses showed that in the control group, a large amount of fibrous tissue was observed around the OAS in the 2-week healing group. Then, in the 4-week healing group, the amount of fibrous tissue was reduced, and a partially localized new bone-like tissue was observed. On the other hand, in the β-TCP filling group, residual β-TCP granules and a thin layer of regenerated new bone were observed around the granules in the 2-week healing group. Then, in the 4-week healing group, a new bone-like tissue apparently increased around the OAS (Figure 4).

Table 2. Bone Morphometry After Orthodontic Force Applied to the 2-Week and 4-Week Healing Groups[a]

	BMD	BMC	BV	BV/TV	BMC/TV
2-wk healing group					
Cortical bone					
Control	863.18 ± 46.23	302.25 ± 10.21	31.38 ± 6.68		
β-TCP	1030.18 ± 44.36	498.50 ± 99.58	43.15 ± 6.80		
P-value	.001	.008	.046		
Trabecular bone					
Control	636.50 ± 47.96	0.22 ± 0.22	0.03 ± 0.03	6.25 ± 3.50	45.75 ± 25.49
β-TCP	890.73 ± 18.48	1.08 ± 1.03	0.10 ± 0.09	6.93 ± 3.43	73.25 ± 43.62
P-value	.001	.153	.172	.792	.318
4-wk healing group					
Cortical bone					
Control	974.85 ± 49.30	304.75 ± 75.98	42.55 ± 1.80		
β-TCP	1164.03 ± 49.20	538.50 ± 40.34	52.28 ± 1.68		
P-value	.002	.002	.001		
Trabecular bone					
Control	760.70 ± 29.16	1.71 ± 1.09	0.19 ± 0.12	12.33 ± 6.28	109.78 ± 56.15
β-TCP	1056.48 ± 128.92	2.73 ± 0.49	0.43 ± 0.04	28.25 ± 4.41	180.93 ± 40.58
P-value	.001	.14	.011	.006	.086

[a] BMD indicates bone mineral density (mg/cm^3); BMC, bone mineral content (mg $\times 10^{-2}$); BV, bone volume (cm$^3 \times 10^{-4}$); BV/TV, bone volume/tissue volume (%); and BMC/TV, bone mineral content/tissue volume (mg/cm^3). Values are presented as means ± standard deviation. *P*-values, paired *t*-test.

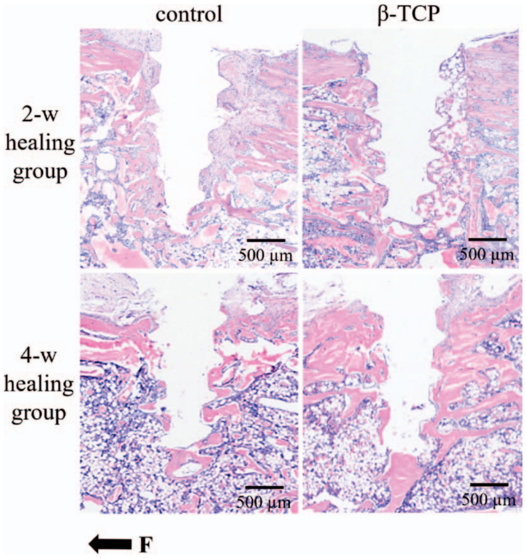

Figure 4. Histological micrographs of hematoxylin and eosin staining around the inserted orthodontic anchor screw.

DISCUSSION

In this study, a rat model of OAS loosening was established and demonstrated that filling the bone defect with β-TCP stabilized the loosening OAS when an orthodontic force was applied. This is the first report to establish an OAS-loosening model. The following conditions are required for an OAS-loosening model: the bone hole must be sufficiently large to loosen an OAS but must prevent the OAS from falling out when orthodontic force is applied. Three different sizes of bone holes were evaluated, with diameters of 1.6, 2.1, and 2.5 mm, and OASs were placed with a diameter of 1.2 mm. After either a 2- or 4-week healing period, orthodontic force was applied to the OASs. Subsequently, all of the OASs fell out in the bone holes with 2.1- and 2.5-mm diameters, whereas only 10–20% of the OASs fell out in the bone hole with a 1.6-mm diameter. These results indicated that a bone hole diameter of 1.6 mm is not too large for the OAS to fall out but still loosens the inserted OAS. Therefore, this condition was appropriate for an OAS-loosening model.

Investigating self-healing of the bone defect using micro-CT showed that cortical bone was not detected within 2 weeks, but thin cortical bone regenerated in 4 weeks. Complete recovery of cortical bone was observed in 6 weeks (Figure 2). Several previous studies[12,13] on OAS demonstrated that the bone around the microcrack is repaired with new bone after 4 weeks. Similarly, self-tapping titanium cortical bone screws, commonly used in orthopedics, require one month to repair microscopic damage.[14] These reports support the findings of the current study regarding the time course of cortical bone regeneration. Additionally, cortical bone regeneration was less as measured 2 and 4 weeks after drilling a bone hole than as measured at 6 weeks, suggesting that reinserted OASs may be more loosened after 2- and 4-week healing periods, compared to 6 weeks and thereafter. Consequently, to study the effect of β-TCP on OAS loosening and bone regeneration, 2 and 4 weeks were used as the healing periods in this study.

The stability of the OAS was previously tested in a variety of ways.[15,16] However, there is no report that bone-filling materials were applied for fixation of reinserted OASs. Among those bone-filling materials, β-TCP was selected as the fixation tool for OAS reinsertion because β-TCP is absorbed by osteoclasts and is quickly replaced by new bone. Rabbit femurs filled with β-TCP granules demonstrated[17] in an increase in new bone formation from 4 to 12 weeks after filling. The histological and micro-CT analyses of this study showed that β-TCP resorption and new bone formation were observed in 4 weeks.

The linear distance and mesial tipping angle of OAS movement by the orthodontic force were remarkably smaller in the β-TCP filling group compared to the control group. The values of BMD in the trabecular and cortical bone were significantly higher in the β-TCP group than in the control group. Accordingly, new bone formation by β-TCP can fix a loosening OAS against orthodontic force.

Reinsertion of OASs in orthodontic treatment has been performed at the site where the OAS was first inserted or at another appropriate site. However, it is often difficult to determine another site because of anatomical limitations. Additionally, the site at which the OAS is inserted requires good bone quality and quantity. The present study showed that the bone was reinforced by filling with β-TCP and that filling of β-TCP with a 4-week healing period will stabilize the OAS and allow it to resist orthodontic forces. β-TCP filling may contribute and provide a new solution to increase the success rate of OAS reinsertion at the same site when an OAS is loosening or falling out.

CONCLUSIONS

- An OAS-loosening model was established in rat tibia and demonstrated that β-TCP filling with a healing period of 4 weeks can significantly reinforce the stability of OASs through the promotion of bone formation.
- β-TCP filling can be used as a novel tool for fixation of loosening OASs against orthodontic force.

ACKNOWLEDGMENTS

We appreciate Dr Shinsuke Ohba for his constructive ideas for the experimental design and Dr Takeshi Moriishi for his comments on the histological results. This work was supported by research grants from Japanese Grant-in-Aid for Scientific Research (grant 19K10407 to MH, grant 21K17187 to IK, and grant 20K10229 to HH).

REFERENCES

1. Papadopoulos MA, Tarawneh F. The use of miniscrew implants for temporary skeletal anchorage in orthodontics: a comprehensive review. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2007;103:e6–e15.
2. Uesugi S, Kokai S, Kanno Z, Ono T. Stability of secondarily inserted orthodontic miniscrews after failure of the primary insertion for maxillary anchorage: maxillary buccal area vs midpalatal suture area. *Am J Orthod Dentofacial Orthop*. 2018;153:54–60.
3. Chen YH, Chang HH, Chen YJ, Lee D, Chiang HH, Yao CC. Root contact during insertion of miniscrews for orthodontic anchorage increases the failure rate: an animal study. *Clin Oral Implants Res*. 2008;19:99–106.
4. da Silva HF, Goulart DR, Sverzut AT, Olate S, de Moraes M. Comparison of two anorganic bovine bone in maxillary sinus lift: a split-mouth study with clinical, radiographical, and histomorphometrical analysis. *Int J Implant Dent*. 2020;6:17.
5. Scarano A. Maxillary sinus augmentation with decellularized bovine compact particles: a radiological, clinical, and histologic report of 4 cases. *Biomed Res Int*. 2017;2594670.
6. Ting M, Afshar P, Adhami A, Braid SM, Suzuki JB. Maxillary sinus augmentation using chairside bone marrow aspirate concentrates for implant site development: a systematic review of histomorphometric studies. *Int J Implant Dent*. 2018;4:25.
7. Hernigou P, Dubory A, Pariat J, et al. Beta-tricalcium phosphate for orthopedic reconstructions as an alternative to autogenous bone graft. *Morphologie*. 2017;101:173–179.
8. Rh Owen G, Dard M, Larjava H. Hydroxyapatite/beta-tricalcium phosphate biphasic ceramics as regenerative material for the repair of complex bone defects. *J Biomed Mater Res B Appl Biomater*. 2018;106:2493–2512.
9. Di Raimondo R, Sanz-Esporrín J, Plá R, et al. Alveolar crest contour changes after guided bone regeneration using different biomaterials: an experimental in vivo investigation. *Clin Oral Investig*. 2020;24:2351–2361.
10. Cömert Kılıç S, Güngörmüş M, Parlak SN. Histologic and histomorphometric assessment of sinus-floor augmentation with beta-tricalcium phosphate alone or in combination with pure-platelet-rich plasma or platelet-rich fibrin: a randomized clinical trial. *Clin Implant Dent Relat Res*. 2017;19: 959–967.
11. Trombelli L, Franceschetti G, Stacchi C, et al. Minimally invasive transcrestal sinus floor elevation with deproteinized bovine bone or β-tricalcium phosphate: a multicenter, double-blind, randomized, controlled clinical trial. *J Clin Periodontol*. 2014;41:311–319.
12. Wu J, Bai YX, Wang BK. Biomechanical and histomorphometric characterizations of osseointegration during miniscrew healing in rabbit tibiae. *Angle Orthod*. 2009;79:558–563.
13. Zhang L, Zhao Z, Li Y, Wu J, Zheng L, Tang T. Osseointegration of orthodontic micro-screws after immediate and early loading. *Angle Orthod*. 2010;80:354–360.
14. Wang L, Ye T, Deng L, et al. Repair of microdamage in osteonal cortical bone adjacent to bone screw. *PLoS One*. 2014;9:e89343.
15. Al-Thomali Y, Basha S, Mohamed RN, et al. Effect of surface treatment on the mechanical stability of orthodontic miniscrews: a systematic review with meta-analysis. *Angle Orthod*. 2022;92:127–136.
16. Yamagata K, Oga Y, Kwon S, Maeda-Iino A, Ishikawa T, Miyawaki S. A novel auxiliary device enhances miniscrew stability under immediate heavy loading simulating orthopedic treatment. *Angle Orthod*. 2022;doi:10.2319/022222-163.1.
17. Gao P, Zhang H, Liu Y, et al. Beta-tricalcium phosphate granules improve osteogenesis in vitro and establish innovative osteo-regenerators for bone tissue engineering in vivo. *Sci Rep*. 2016;6:23367.