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Clinical Device-Related Article

Evaluation of a biodegradable novel periosteal distractor

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Abstract: We developed a new device composed of a thin biodegradable mesh (poly-L-lactide/hydroxyapatite composite) for distracting periosteum. The purpose of this study is to evaluate the effect of using this device as a periosteal distractor.
Materials and Methods: Eight Japanese male rabbits were divided into two groups according to time of sacrifice. The calvarial periosteum was elevated and one side of a biodegradable mesh was fixed to the bone surface with two titanium screws. Seven days after the surgery, an elevating screw was inserted into the other side of the mesh. Then, the calvarial periosteum was elevated at maximum 0.5 mm every 12 h for 5 days. The device was designed to distract the periosteum at different rates along its entire surface. At 4 and 6 weeks of the consolidation, the animals were sacrificed and newly formed bone was histologically and radiographically evaluated.
Results: The new device simplified periosteal distraction and reduced its invasiveness. Moreover, it successfully induced new bone formation from two sources; the periosteum and the underlying basal bone. Histomorphometric analysis of the distracted space showed that there is a relation between the rate of distraction and the amount of newly formed bone. We suggest that the optimal speed range for periosteal distraction in rabbit calvarial model could be less than 0.33 mm/day.
Conclusions: The new device is slim, biodegradable and the procedure is simple. Thus, periosteal distraction with this device is potential for vertical and horizontal ridge augmentation in oral cavity. © 2011 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 100B: 882–889, 2012.

Key Words: periosteal distraction, bone regeneration, biodegradable material, dental implants

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INTRODUCTION

Oral rehabilitation with endosseous dental implants is effective and predictable; however, osseous defect is a frequent obstacle for this treatment. To achieve satisfactory functional and aesthetic requirements, ridge augmentation is required before implant installation.[1] There are several augmentation techniques: bone graft, guided bone regeneration, and alveolar distraction.[2] However, these augmentation options have some disadvantages. Although autogenous bone graft is still a gold standard for bone augmentation, donor side morbidity, and limitation of harvestable bone are problems in this technique.[1] Although autogenous bone substitutes are used; however, they are not effective enough compared with autogenous bone because of their material and chemical characteristics.[2] Although augmenting the bone with guided bone regeneration is effective, this technique is usually limited to undersized and regularly shaped defects and membrane exposure is a common problem.[3] Distraction osteogenesis is applicable to challenging case in which large augmentation is required. This technique is advantageous especially in vertical bone augmentation; however, it needs osteotomy and two surgeries.[4]

The osteogenic potential of the periosteum is well established.[5] Progressively uplifting the periosteum and expanding the interface between the bone surface and periosteum has resulted in the formation of new bone either with cortical perforation[6] or without.[1] Although Schmidt et al. were the first to demonstrate a device for periosteal distraction, they suggested the further modification of the periosteal distraction device to eliminate the dislodging action of the distracted tissues on the distracting end.[1] Estrada et al. reported device instability, displacement and perforation of soft tissue. In these previous studies, the devices were applied in the rabbit forehead or in the dog oral cavity and loss of the devices commonly occurred.[7] Casap et al. also reported difficulty of device placement thus the selection of the experimental animal was restrained by adequate size and facial anatomy.[8] Their device was similar to the one designed by Schmidt et al.[1] It is a metallic u-shaped device of 15 mm height with a distraction rod passing through it

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and the distraction plate was only 6 mm length.[9] This device was fixed to the body of the mandible and extended to the outside, which adversely affected the animal feeding habit. Although this technique seems to be promising, improvement of periosteal distraction device is strongly required. First, the device should be compact for less interfering of oral functions. Second, it should not harm mucosa or periosteum and, finally, it should be easily activated.

Studies of periosteal distraction with different devices and with different protocols have been reported; however, optimal activation conditions have not yet been clarified.[8] In previous periosteal distraction studies, the distraction rates varied from 0.2–0.5 mm/day, distraction period ranged from 8–32 days and latency period ranged from 5–10 days, whereas consolidation period ranged from 7 to 56 days.[1,2,6–10]

Poly-L-lactide is a synthetic polymer that has been extensively studied as a biodegradable material for medical devices. This material has the advantage of complete hydrolysis into its components and finally absorbed totally inside the body.[11] A slim biodegradable mesh, which consists of poly-L-lactate and hydroxyapatite (u-HA) fine particles, has been approved by FDA in 2007 for the repair of fracture and fixation of bone fragments in maxillofacial region. Some clinical studies reported using this composite in fractures of maxillofacial and other regions.[12–16] u-HA fine particles in this mesh are bioactive and totally bioresorbable inorganic component being responsible for osteoconductivity and faster dissolution of this composite when compared with that without u-HA particles.[11] We developed a simple device using this biodegradable mesh for periosteal distraction. The purpose of this study is to evaluate the effect of gradually distracting the periosteum using this biodegradable device.

MATERIALS AND METHODS

Japanese male white rabbits, weighing from 2.5 to 3 kg, were used and were divided into two groups, four rabbits in each group, according to the time of sacrifice. The experimental protocol was approved by the Committee of Animal Experiments in Tokyo Medical and Dental University.

Device description

The device consisted of composite materials of bioactive, bioresorbable unsintered HA (u-HA; Ca/P = 1.69 (mol.ratio), carbonate ion = 3.8 mol %, having fine particles (average size 3–5 μm) of 40% weight combined with poly-L-lactide (PLLA), which has been reinforced using a unique compression forging process. The u-HA fine particles are uniformly distributed throughout the composite materials. (Super FIXSORB MX, TAKIRON, Osaka, Japan) The distraction device has three components [Figure 1(A,B)].

i. Rectangular shape bioresorbable mesh ($20 \times 10 \times 0.5$ mm^3), in which three holes were prepared: two holes for fixation screws and 1 serrated hole for distraction screw [Figure 1(A)].
ii. Two titanium fixation mini screws (3 mm in length, 1 mm in diameter) [Figure 1(B)].
iii. Titanium elevating screw (5 mm in length, 2 mm in diameter) [Figure 1(B)].

Surgical procedures

Animals were anesthetized preoperatively with an intramuscular injection of ketamine (50 mg/kg Ketalar, Sankyo, Tokyo, Japan) and thiopental sodium (25 mg/kg Rabonal, Tanabe, Tokyo, Japan). In addition, 1.8 mL of a local anesthetic (2% xylocaine/epinephrine 1:80,000, Dentsply Sankin, Tokyo, Japan) was injected into the surgical sites before the start of surgery.

All operations were performed under aseptic condition. The forehead of the animal was shaved and disinfected with tincture of 1% iodine solution. A U-shaped skin and subperiosteal incision was done over the calvarial bone. The skin and periosteal flap were carefully raised to expose the bone surface and, then, the periosteum was retracted away from the operative site. Under irrigation with saline, 2 or 3 perforations were made with a round bur of number 4 to expose the modularly cavity in the external cortical plate of the occipital bone. The mesh was first placed over the perforated area and then fixed to the bone surface from one end by means of the two mini screws [Figure 2(A)]. The periosteum was then positioned back in its place and stabilized by suturing. [Figure 2(B)]. Finally, the skin flaps were sutured with 3–0 silk [Figure 2(C)].

Device activation

After 1 week a soft tissue incision of 2 mm length was done over the screw place of the mesh. The elevating screw was threaded through the mesh to raise it. The rotating screw perforated the covering soft tissue and was resting on the external cortical layer of the calvarial bone. Rotating the screw 180° cause the titanium mesh to be elevated by 0.5 mm. A rate of 0.5 mm distraction was applied twice a day for 5 days [Figure 2(D)].

During the observation period, all rabbits were given water and a standard rabbit feed *ad libitum*. Rabbits were sacrificed after 4 and 6 weeks consolidation period with a lethal dose of thiopental sodium. The entire cranial bone was removed and fixed for 14 days in neutral 10% formalin.

Microcomputed tomography (micro-CT) analysis

After fixation, specimens were scanned using a high resolution micro CT imaging system (SMX-90CT, Shimadzu, Kyoto, Japan) continuously in increments of 60 μm. The bone images were extracted by processing the gray scale images using a median filter to remove noise and a fixed threshold to extract the mineralized bone phase. Following phantom calibration of the images, scanned images were analyzed with three-dimensional 3D image analysis software (TRI/3D-BON; Rotac system engineering, Tokyo Japan). 10 Micro CT serial longitudinal images were obtained for each specimen (1 image/mm). The distracted area in each image was divided equally into three segments by the imaginary lines L1, L2, L3, and L4 [Figure 3(A)].

In each segment the area occupied by new bone (BA) and the total distracted area (DA) were measured using image analysis software (Image j, 1.43 Hz, NIH, Bethesda, MD). Images were automatically corrected for brightness and contrast, then were converted into 8-bit gray scale before measurement.[17]

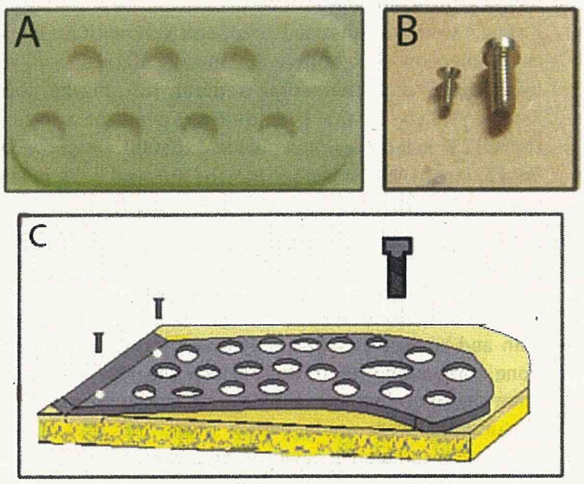

FIGURE 1. Image of PLLA/HA distraction device (A).Image of Distraction and fixation screws (B). Illustration showing the device and Position of fixation and elevation screws (C). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The percentage of the newly formed bone per distracted volume (BV/DV) in each segment was calculated. A table of (BV/DV) means for all segments in the eight animals representing the two groups was formed including the mean and SD of each segment. (Table I)

Heights attained by the periosteum at the end of activation at L2 and L3 axis were calculated by rules of right angled triangle [Figure 3(A)] and consequently elevation rates at these points were calculated (Table II).

Statistical analysis was performed with SPSS statistical package. Descriptive statistics included mean and standard deviation as well as one way analysis of variance test to compare the ratio of newly formed bone volume per distracted volume in the three segments between the two groups. The level of significance was set to 95%.

Histological processing

Following fixation calvarial bone was dehydrated in ascending grades of ethanol, and then embedded in polyester resin (Rigolac- 70F, Rigolac-2004, Nisshin EM Co., Tokyo, Japan). The distraction devices were kept in place then sections were cut (Exakt, Mesmer, Ost Einbeck, Germany) and ground to a thickness of about 100 µm. The sections were finally stained with 0.1% toluidine blue. Histological observation was performed under a light microscope.

RESULTS

In all animals normal dietary habit was resumed immediately after cessation of general anesthesia effect. No infectious symptom was detected during the entire period of the experiment. In two animals slightly more inflammation was observed compared with the other animals after the first surgery; however, it diminished before activation of the distraction. All devices remained rigidly fixed to the calvarium during the experiment. They were totally concealed under the soft tissue during activation and until time of sacrifice.

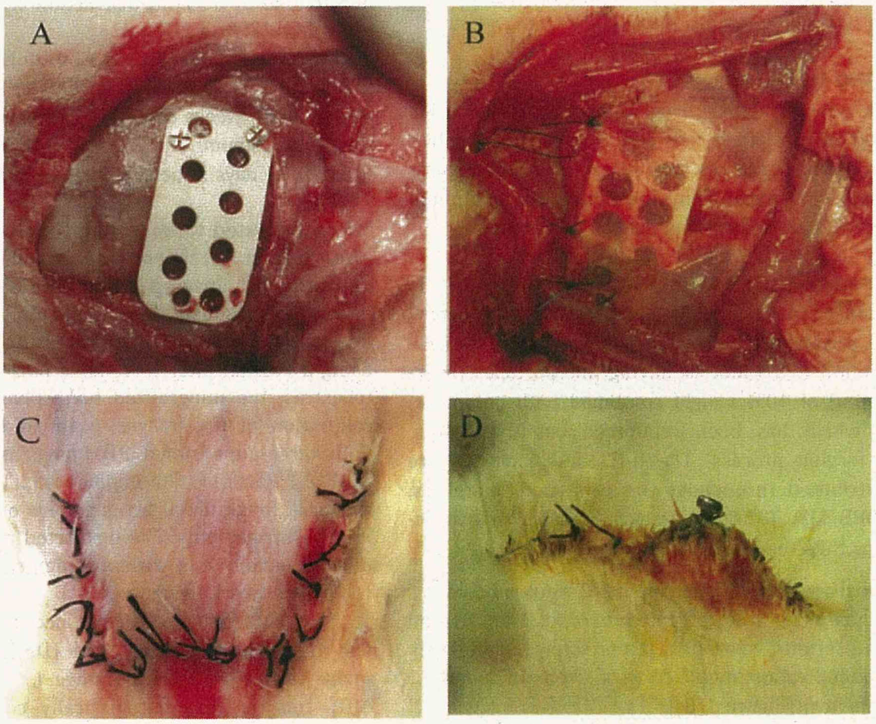

FIGURE 2. Image of Periosteal distractor fixed on calvarium bone (A). Distractor covered by periosteum (B). Flab closure (C). Device activation (D). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

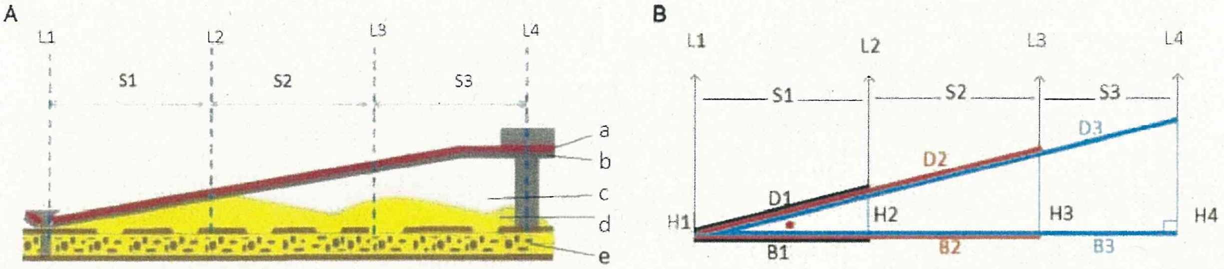

FIGURE 3. Illustration showing a transverse section showing the 3 distracted segments,S1, distracted segment close to the fixed end.S2,middle distracted segment .S3, distracted segment close to the movable end. L1, L2, L3, L4 are imaginary line passing between the S1, S2, and S3 segments. a, periosteum; b, device. c connective tissue. d, newly formed bone. e ,original bone. (A) Geometrical Illustration of the device and distracted site after full activation (B). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

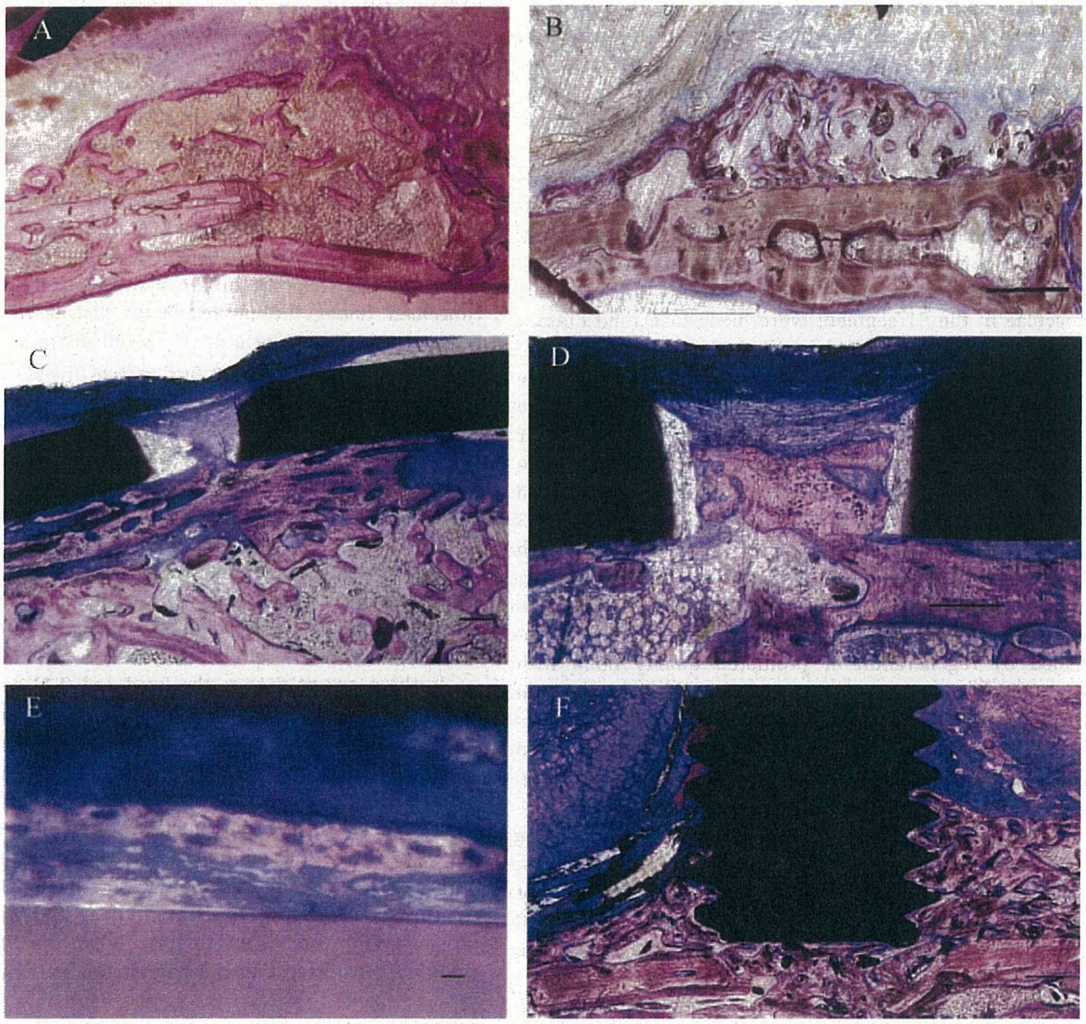

FIGURE 4. Representative histological images stained with toluidine blue for group 1 and group 2 Scale = 300μ in A,B,C,D,F 10 μ in E. Newly formed bone in S2 segment in group1. (A) Newly formed bone in S2 segment in group2. (B) Newly formed bone in S1 segment in group 2 showing more dense trabeculae than those in S2 segment. (C) Extended bone trabeculae through device perforations and contact with periosteum. (D) New bone trabeculae over the device and under the periosteum. (E) New bone trabeculae in S3 segment creeping over the apical 2 mm of the distraction screw (F). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE I. Showing Percent of Volume of Newly Formed Bone per Distracted Volume in Each Segment in Group 1 and 2

	Group 1			Group 2		
Distracted Segment	S1	S2	S3	S1	S2	S3
R1	69.37	20.79	27.37	100	45.39	9.21
R2	84.28	19.6	15.27	94.21	73.76	18.89
R3	98.33	29.32	17.25	100	70.82	18.2
R4	96.84	47.81	6.51	85.89	82.79	22.89
Mean	87.205	29.38	16.6	95.025	68.19	17.2975
SD	13.45662	13.02696	8.56334	6.673672	16.03061	5.774492

R, rabbit; S, distracted segment.

Distraction screws were easily adjusted and remained attached to the device until the end of the experiment.

At 4 weeks of the consolidation period in transverse histological sections multiple dome shape bone outlined by thin bone trabeculae and scattered bone trabeculae within abundant adipose tissue were evident. A layer of connective tissue was covering the newly formed bone layer [Figure 4(A)]. At 6 weeks of the consolidation period transverse sections demonstrated the similar histological patterns; however, bone trabeculae were thicker and adipose tissue was less abundant [Figure 4(B)].

Newly formed bone trabeculae showed different patterns among the distraction segments at 4 and 6 weeks of the consolidation period. Bone trabeculae in S1 Segment at both time points showed more compact appearance with marked decrease in adipose tissue [Figure 4(C)]. In addition, trabeculae in the S1 segment were likely to extend outside the limits of the distraction segment through the holes of the plate and attach to proliferating periosteum [Figure 4(D)]. Bone trabeculae in S2 segment showed more loose appearance with thicker overlying connective tissue layer [Figure 4(A,B)]. In S1 and S2 segments at 6 weeks of consolidation period small bone trabeculae was observed between the periosteum and the mesh [Figure 4(E)]. S3 showed almost similar histological pattern to S2 with thicker connective tissue layer although minute bone trabeculae was not observed between the periosteum and the device. At both 4 and 6 weeks of consolidation S3 was distinguished by regeneration of promoted quality bone with scant adipose tissue over the serrations of the apical 2 mm of the distraction screw [Figure 4(F)]. Periosteum was characterized by numerous blood vessels and tended to extend down into the distraction area through the plate holes [Figure 4(D)].

At 4 and 6 weeks of consolidation micro-CT 3D image showed that the new bone is less radiopaque than the original basal bone. Connective tissue appeared as radiolucent area of considerable thickness in S3; however, it tapered off from S3 to S1 and disappeared in the S1 segment [Figure 5(A,B,C)].

Quantitative data showed that S1 segment at both time points contain the highest new bone volume per distracted segment volume compared with S2 and S3 segments. The percentage of new bone volume at 6 weeks was higher than the one at 4 weeks (Table I). There were statistical differences between S1 and S2 segments and between S1and S3 segments at 4 weeks. At 6 weeks there were significant differences between all segments [Figure 6(A,B)].

DISCUSSION

In this study, we demonstrated that our new device induced osteogenesis and distracted soft tissue successfully in a 6 weeks rabbit calvarial model. The results also showed that there is a relation between the rate of periosteal distraction and quality of formed bone. In addition, the device sensibly simplified the periosteal distraction procedure and reduced the invasiveness.

The success of our new device in inducing new bone and distracting the periosteum in the rabbit model was attributed to several key design features. First, the whole device except the part of the activating screw is entirely concealed under the periosteum and soft tissues, which reduced the dislodging effect to the distracted tissues. Second, the design of the new device kept the integrity of the periosteum because the device perforated the periosteum only at the site of the activation screw. These points are advantageous compared with the previous devices, which perforated the periosteum at more than one site. Indeed, periosteal integrity following its elevation is the principle factor for success of periosteal distraction.

Interestingly, the sloping design of the device enabled to apply different distraction speeds simultaneously depending on the distance from the fixation screws, all of which were 1 mm per day and less.

In the segment near to the fixed end (S1) newly formed bone underneath the device is characterized by relatively thick trabecular bone and less adipose tissue. Moreover, growth of periosteal bone over the mesh was usually confined in this segment. This may suggest that the use of slower speed during periosteal distraction is prudent to promote newly formed bone. It is likely that the slower periosteal distraction speed decreased the opportunity for soft tissue to occupy the created space and increased the possibility for osteoblasts to form bone. Furthermore, this study demonstrated that the periosteum over the mesh had

TABLE II. Showing Elevation Rates in S1, S2, and S3 Segments

Segment	S1	S2	S3
Elevation rate range (mm/day)	0–0.33	0.33–0.66	0.66–1

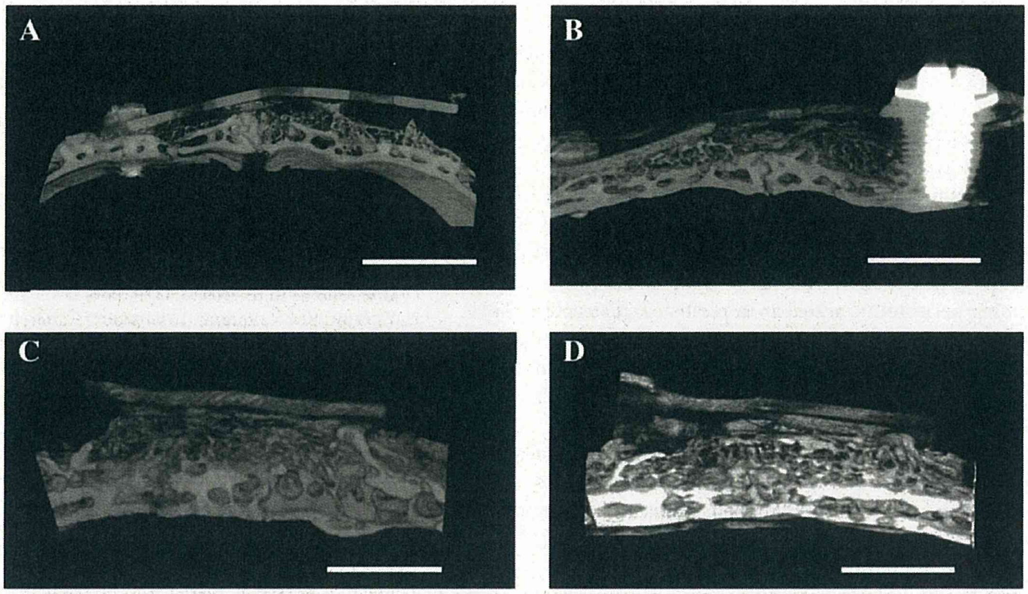

FIGURE 5. Micro CT images showing Transverse view of newly formed bone under the device in group1 (A) and in group2 (B). Micro CT images showing Crossectional view of newly formed bone under the device in group2 in S1 segment (C) and S2 segment (D). Scale bar = 5000 μ in A,B,C,D.

capacity to form minute bone when it received appropriate level of tension. However, the periosteum could not produce bone under the condition of excess tension level, which existed at the place away from the fixation screws in this study.

In this study at the area of the highest distraction speed, the segment 3, the less bone and more connective tissue were observed; however, the threads of the titanium distraction screw were covered with large amount of good quality of bone. Bone trabeculae crept on the titanium surface and covered the thread in which the distraction screw worked as a scaffold for bone regeneration. [Figure 4(f)]

In this study the histological finding demonstrated that newly formed bone originated mainly from the basal bone. It is very likely that progenitor cells of blood vessels and osteoblasts were provided from the basal bone, especially through the perforated bone holes. These cells proliferated and differentiated in the space, which was gradually produced by the elevating mesh. Nutrition to the regenerated area was also mainly provided from the basal bone. Furthermore, the distracted periosteum through the mesh holes was also an additional nourishment source for the new bone.

The speed of the regenerative space expansion by periosteum elevation should be optimum, thus osteoprogenitor cell supply, proliferation and differentiation of these cells and nutritional supply are able to catch up. The periosteal distraction is consistent to Elizarov's principle of tissue distraction in which the slow distraction speed for tissues is recommended.

Sencimen et al. reported dominance of adipose tissue under the periosteum in the periosteal distraction.[9] This study clearly demonstrated that the quality of the newly formed bone depended on the distraction speed. Slowest speed produced bone of thicker trabeculae and less connective tissue and radiopacity closer to original bone. This newly formed bone can be sustained and matured if it receives appropriate level of mechanical stress. Periosteal

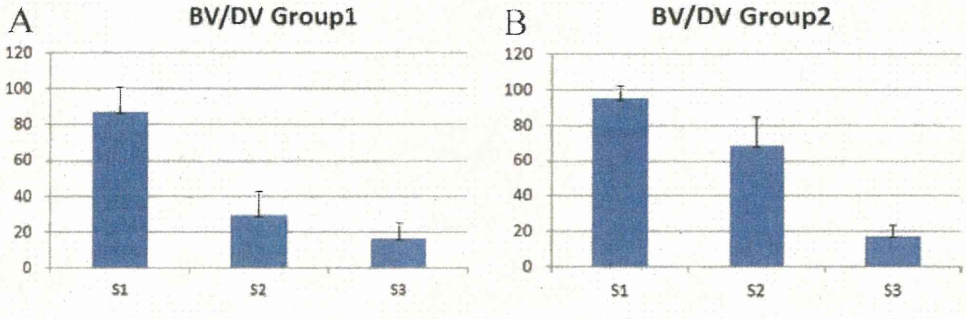

FIGURE 6. A, B: Graphic representation of the mean and standard deviation of the percent of bone volume /distracted volume (BV/DV) among segments in the 2 groups. $P < 0.01$ Scheffe test. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

distraction followed by dental implant installation could be beneficial because the implants transmit mechanical stress to the newly formed bone. The important finding in this study was that the optimum speed range of periosteal distraction for bone augmentation was 330 μm per day and less, which decreased soft tissue formation. In bone distraction speed from 0.5 mm to 1.0 mm or sometimes more per day is clinically applied, which is based on previous animal and clinical studies.[10,18,19] In osteogenic distraction, cell and nutrition supplies originate from both ends of bone and from the surrounding periosteum, whereas in periosteal distraction those supplies are derived only from basal bone and overlying periosteum. Therefore, it seems to be reasonable that in osteogenic distraction normal distraction speed is more than that of periosteal distraction suggested in this study.

In addition to autologous bone graft and distraction osteogenesis, several bone augmentation modalities have been developed and clinically applied: applications of osteogenic cells[20,21] or signal molecules, such as BMP-2[22,23] or PDGF[24,25] combining with scaffold materials. Although these modalities are clinically effective and promising, vertical bone augmentation in oral cavity is still challenging except distraction osteogenesis because of difficulties in covering grafted bone or material with soft tissue.

Distraction osteogenesis makes vertical augmentation together with soft tissue expansion possible; however, bone distraction in the oral cavity is technically sensitive because of difficulties in segmenting bone and setting a bone distraction device in narrow space. In addition, a patient is uncomfortable when bone distraction device exists in the oral cavity.

Future intraoral application of this biodegradable device as a periosteal distraction could be less sensitive technically especially when it is set free from fixation screws. Moreover, as the accessible elevation screws can be easily removed before complete bone mineralization a second surgery will not be necessary.

Lethaus et al. have recently reported a new device for periosteal distraction demonstrating effectiveness of periosteal distraction in vertical bone augmentation.[26,27] This study further demonstrated effectiveness of periosteal distraction in vertical augmentation with a new biodegradable device. Conclusively, although improvement of a device is still required, periosteal distraction would be potentially effective in vertical bone augmentation in dental field.

ACKNOWLEDGMENTS

The authors like to thank Mr. Toshimitsu Yamamoto and all staff members in the Department of Dental Anatomy, Tokyo Medical and Dental University for their kind assistance.

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Induced Osteogenesis Using a New Periosteal Distractor

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Purpose: We developed a new device mainly composed of a titanium mesh to distract the periosteum. The purpose of this study is to evaluate induced osteogenesis by periosteal distraction with the new device.

Materials and Methods: We divided 12 Japanese male rabbits into 3 groups, with 4 rabbits in each. In all groups the calvarial periosteum was reflected, and 1 side of the titanium mesh was fixed to the bone surface with 2 micro-screws. In groups 1 and 2, an elevation screw was inserted into a serrated hole on the other side of the plate 7 days after surgery. Then the device was activated at a rate of 0.5 mm every 12 hours for 5 days. At 4 weeks of the consolidation period, group 1 was killed, followed by group 2 at 6 weeks. Group 3 (control) received no screws, and hence no activation was performed. In group 3, 2 animals were killed 4 weeks after titanium mesh insertion, followed by the other 2 animals at 6 weeks. The device was designed to simultaneously distract the periosteum at different rates along its inclined surface. Newly formed bone was histologically and radiographically evaluated.

Results: The new device effectively induced osteogenesis and successfully distracted the soft tissue after 6 weeks in a rabbit model.

Conclusions: The new device is slim, and the procedure is straightforward. Thus, periosteal distraction with this device can potentially be used for vertical and horizontal ridge augmentation in the oral cavity. In addition, the results suggest that connective tissue growth in the distraction site might be controlled by reducing the speed of periosteal distraction.

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Oral rehabilitation with endosseous dental implants is effective and predictable; however, implant placement, without augmentation procedures, may lead to an esthetically compromised situation.[1] The presence of an osseous defect is a frequent obstacle for this treatment. To achieve satisfactory functional and esthetic requirements, ridge augmentation is often required before implant installation.[1]

Several reconstruction procedures have been proposed to augment the alveolar ridge, including bone grafting, guided bone regeneration, and alveolar distraction.[2] However, the outcome of some of these augmentation options varies. Although autogenous bone graft is still a gold standard for bone augmentation, donor-side morbidity and limitation of harvestable bone are problems of this technique.[1] Instead of autogenous bone, bone substitutes are used; however, they are not effective enough compared with autogenous bone because of their material and chemical characteristics.[2] Though effective, the guided bone regeneration technique is usually limited to undersized and regularly shaped defects; moreover, membrane exposure is a common problem.[3]

Distraction osteogenesis is used in more extensive alveolar augmentation, especially vertical type; however, limitations of this technique have been reported. They have ranged from aggressive procedures[4] and frequent lingual inclination of the distracted segment[5,6] to some relapse of the initial bone gain before implant placement.[7]

Schmidt et al[1] were the first authors to apply a device for periosteal distraction. By gradual periosteal lifting, this technique enlarges the interface between the original bone surface and the periosteum, inducing supraosseous neogenesis. They recommended ad-

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FIGURE 1. Titanium device.

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ditional improvement of the periosteal distraction device to eliminate the displacing effect of the distracted tissues on the device.[1] Subsequent periosteal distraction studies also reported device problems. Some studies reported device instability, displacement, and soft tissue dehiscence.[8,9] Casap et al[10] also reported difficulty in device placement; thus the selection of the experimental animal was restrained by adequate size and facial anatomy.

The periosteal tissue was proved highly osteogenic.[11] Contact with bone seems to be essential for its osteogenicity.[12-14] Periosteal distraction procedures involve separation of the periosteum from its underlying bone; however, some periosteal distraction studies showed periosteal bone formation.[15,16]

Though promising, periosteal distraction greatly requires enhancements to the device and the protocol regulating the device activation.[12] In previous periosteal distraction studies, distraction rates varied from 0.2 to 0.5 mm/d, the distraction period ranged from 8 to 32 days, and the latency period ranged from 1 to 10 days, whereas the consolidation period ranged from 7 to 60 days.[1,2,8,10,15-19]

We developed a compact titanium device for periosteal distraction that is activated in an inclined position. The purpose of this study is to evaluate the induced osteogenesis as a result of using this device.

Materials and Methods

We divided 12 Japanese male rabbits aged 6 weeks and weighing from 2.5 to 3 kg into 3 groups, with 4 rabbits in each. Two groups were experimental, and one group served as a control. The experimental protocol was approved by the Committee of Animal Experiments at Tokyo Medical and Dental University, Tokyo, Japan.

Device Description

The distraction device has 3 components (Figs 1-3): (*1*) titanium mesh (20 mm × 10 mm × 0.3 mm), in which 3 holes were prepared—2 holes for fixation screws and 1 serrated hole for distraction screw (Fig 1); (*2*) 2 titanium fixation mini-screws (3 mm in length and 1 mm in diameter) (Fig 2); and (*3*) a titanium elevation screw (5 mm in length and 2 mm in diameter) (Fig 2).

Surgical Procedures

Animals were anesthetized preoperatively with an intramuscular injection of ketamine (50-mg/kg Ketalar; Sankyo, Tokyo, Japan) and thiopental sodium (25-mg/kg Rabonal; Tanabe, Tokyo, Japan). In addition, 1.8 mL of a local anesthetic (2% Xylocaine/epinephrine 1:80,000; Dentsply Sankin, Tokyo, Japan) was injected into the surgical sites before the start of surgery.

All operations were performed under aseptic condition. In all groups the forehead of the animal was shaved and disinfected with tincture of 1% iodine solution. A U-shaped skin and subperiosteal

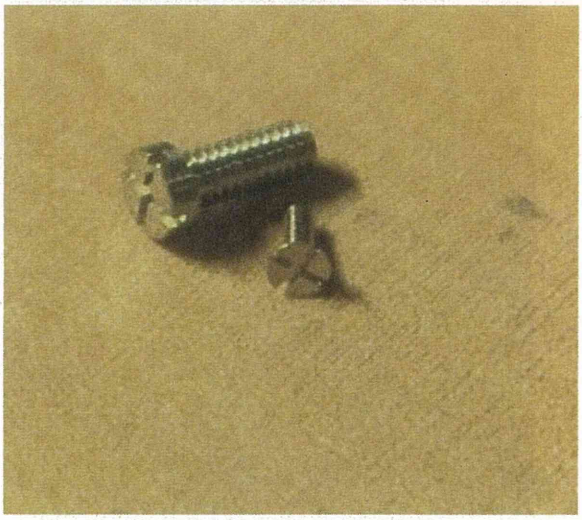

FIGURE 2. Distraction and fixation screws.

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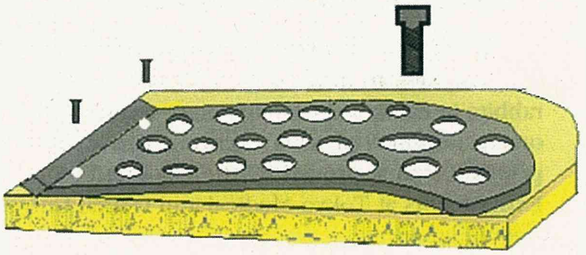

FIGURE 3. Device and position of fixation and elevation screws.

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incision was performed over the calvarial bone. The skin and periosteal flap were carefully raised to expose the bone surface, and then the periosteum was retracted away from the operative site. Under irrigation with saline solution, 2 or 3 perforations were made with a No. 4 round bur to expose the modular cavity in the external cortical layer of the occipital bone. This exposure acted as an active source for osteoblast progenitor cells in the site during distraction. The mesh was first placed over the perforated area and then fixed to the bone surface from one end by means of 2 micro-screws (Fig 4). The periosteum was sutured back in place, covering the whole mesh (Fig 5). Finally, the skin flaps were sutured with No. 3-0 silk (Ethicon, Somerville, NJ) (Fig 6).

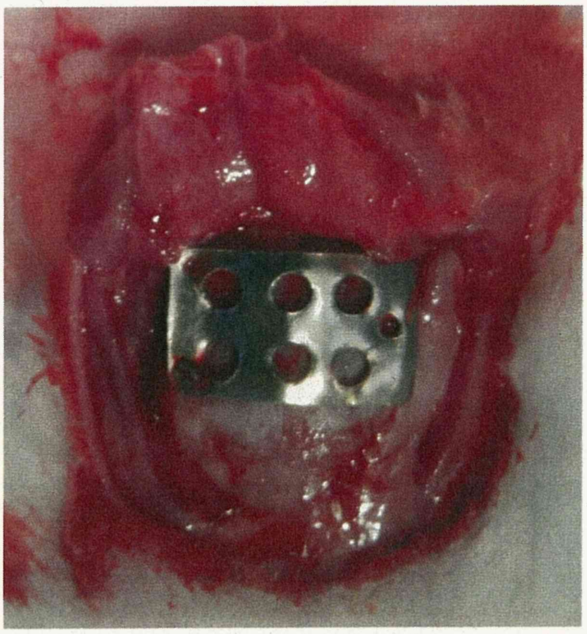

FIGURE 4. Periosteal distractor fixed on calvaria bone.

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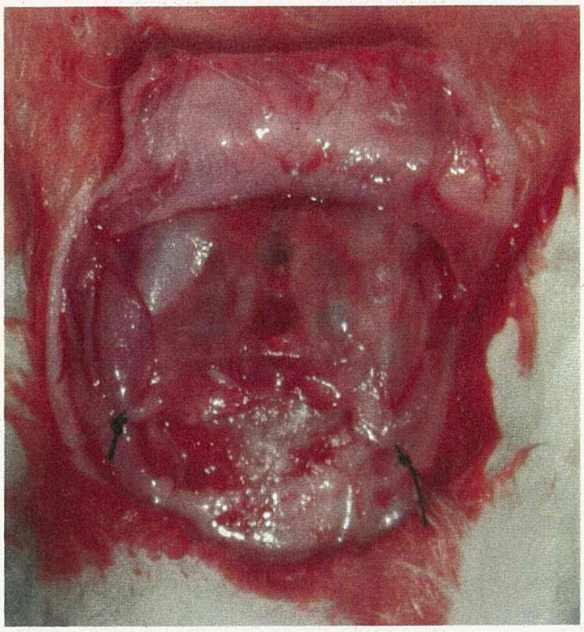

FIGURE 5. Distractor covered by periosteum.

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Device Activation

In groups 1 and 2, at 1 week after device placement, a soft tissue incision of 2 mm in length was made over the screw hole in the mesh. The elevation screw was threaded through the mesh to raise it. The

FIGURE 6. Flap closure.

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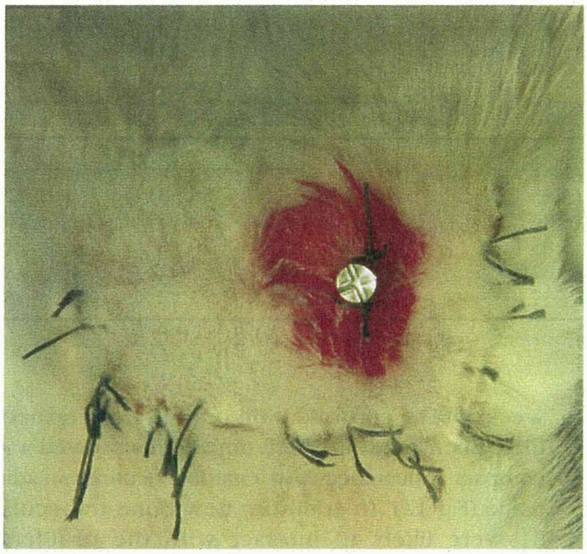

FIGURE 7. Elevation screw insertion.

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rotating screw perforated the covering soft tissue and was resting on the external cortical layer of the calvarial bone (Fig 7). Rotating the screw 180° caused the titanium mesh to be elevated by 0.5 mm. A rate of 0.5 mm of distraction was applied twice a day for 5 days. Group 3 (control group) animals received no elevation screw, and, hence, devices were not activated.

During the observation period, all rabbits were given water and standard rabbit feed ad libitum. Group 1 and 2 animals were killed after 4 and 6 weeks of the consolidation period, respectively, with a lethal dose of thiopental sodium. In group 3, 2 animals were killed 4 weeks after titanium mesh insertion whereas the other 2 were killed after 6 weeks. The entire cranial bone was removed and fixed for 14 days in neutral 10% formalin.

Micro–Computed Tomography Analysis

After fixation, specimens were scanned with a high-resolution micro-computed tomography (CT) imaging system (SMX-90CT; Shimadzu, Kyoto, Japan) continuously in increments of 60 μm. The bone images were extracted by processing the grayscale images with a median filter to remove noise[20] and a fixed threshold to extract the mineralized bone phase. After phantom calibration of the images, scanned images were analyzed with 3-dimensional image analysis software (TRI/3D-BON; Ratoc System Engineering, Tokyo, Japan). We obtained 10 micro-CT serial longitudinal images for each specimen (1 image per millimeter). The distracted area in each image was divided equally into 3 segments by the imaginary lines L1, L2, L3, and L4 (Fig 8).

In each segment the area occupied by new bone and the total distracted area were measured by image analysis software (ImageJ, 1.43 Hz; National Institutes of Health, Bethesda, MD) (Supplementary Fig 1). Images were automatically corrected for brightness and contrast and then were converted into 8-bit grayscale before measurement.[16]

The percentage of the newly formed bone per distracted volume (BV/DV) in each segment was calculated. Table 1 shows the BV/DV means for all segments in the 8 animals representing the 2 experimental groups, including the mean and standard deviation of each segment. Heights attained by the periosteum at the end of activation at the L2 and L3 axis were calculated by rules of a right-angled triangle (Fig 9), and consequently, elevation rates at these points were calculated (Table 2). In the control group specimens were histologically inspected to observe new bone formation over or under the device.

Statistical analysis was performed with the SPSS statistical package (SPSS, Chicago, IL). Descriptive statistics included mean and standard deviation, as well as the 1-way analysis of variance test, to compare the BV/DV ratio in the 3 segments between the 2 groups. The level of significance was set at 95%.

Histologic Processing

After fixation, calvarial bone was dehydrated in ascending grades of ethanol and then embedded in polyester resin (Rigolac-70F and Rigolac-2004; Nisshin EM, Tokyo, Japan). The distraction devices were kept in place, and then sections were cut (Exakt; Mesmer, Ost Einbeck, Germany) and ground to a thickness of about 100 μm. The sections were finally stained with

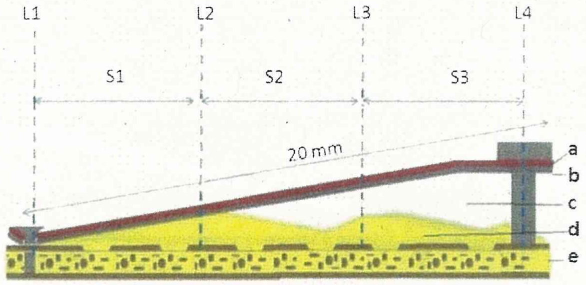

FIGURE 8. Transverse section showing 3 distracted segments: S1, distracted segment close to fixed end; S2, middle distracted segment; and S3, distracted segment close to movable end. L1, L2, L3, and L4 are imaginary lines passing between S1, S2, and S3. (a, periosteum; b, device; c, connective tissue; d, newly formed bone; e, original bone.)

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Table 1. BV/DV VALUES IN PERCENT

	Group 1			Group 2		
	S1	S2	S3	S1	S2	S3
Rabbit 1	58.9	17.8	23.81	88	40.39	8.28
Rabbit 2	72.48	17.05	13.43	83.84	66.38	17.18
Rabbit 3	85.54	25.8	15.35	90	64.44	16.744
Rabbit 4	85.21	42.55	5.85	87.29	76.16	21.28
Mean	75.53	25.8	14.61	87.28	61.84	15.87
SD	12.64	5.45	7.37	2.56	7.37	5.45

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0.1% toluidine blue. Histologic observation was performed under a light microscope.

Results

In all animals normal dietary habits were resumed immediately after cessation of the general anesthesia effect. No infections were detected during the entire period of the experiment. All devices remained rigidly fixed to the calvaria during the experiment. They were totally covered by the soft tissue during activation and until time of sacrifice. Distraction screws were easily adjusted and remained attached to the device until the end of the experiment.

At 4 weeks of the consolidation period, on transverse histologic sections, multiple dome-shaped bones outlined by thin bone trabeculae and scattered bone trabeculae within abundant adipose tissue were evident. A layer of connective tissue was covering the newly formed bone layer (Fig 10). At 6 weeks of the consolidation period, transverse sections showed similar histologic patterns; however, bone trabeculae were thicker and adipose tissue was less abundant (Fig 11).

Newly formed bone trabeculae showed different patterns among the distraction segments at 4 and 6 weeks of the consolidation period. Bone trabeculae in segment 1 (S1) at both time points showed a more compact appearance with a marked decrease in adipose tissue (Fig 12). In addition, new bone trabeculae in S1 were likely to interact with the proliferating periosteum through the holes of the titanium mesh (Fig 13). Bone trabeculae in segment 2 (S2) showed a more loose appearance with a thicker overlying connective tissue layer (Fig 10). At 6 weeks of the consolidation period, small bone trabeculae were observed between the periosteum and the titanium mesh near the anchored end in S1 (Fig 14). Segment 3 (S3) showed an almost similar histologic pattern to S2 with a thicker connective tissue layer. At both 4 and 6 weeks of consolidation, S3 was distinguished by regeneration of promoted quality bone with scant adipose tissue over the serrations of the apical 2 mm of the distraction screw (Fig 15).

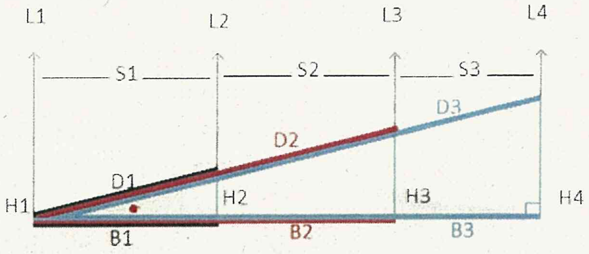

FIGURE 9. Geometric illustration of device and distracted site after full activation. It represents a right angled triangle in which: Red dot is the angle of elevation, L1 and L2 divide the triangle hypotenuse and adjacent sides into 3 equal parts D1, D2 and D3 & B1, B2 and B3. H4(5mm), H3(3.33mm), H2(1.66mm) are the maximum heights of S3, S2, S1 respectively while H1 is minimum height of S1 (0mm).

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In group 3 (control) microscopic observation showed the titanium mesh resting on the cortical bone with a small gap in between (Fig 16). A small amount of new bone and connective tissue was observed to fill the gap in all control specimens, whereas diminutive bone was detected over the mesh in 1 specimen of an animal killed after 6 weeks of device insertion (Fig 17).

At 4 and 6 weeks of consolidation, micro-CT 3-dimensional images showed that the new bone was less radiopaque than the original basal bone. Connective tissue appeared as a radiolucent area of considerable thickness in S3 (Figs 18, 19 [arrows]); however, it tapered off from S3 to S1 and almost disappeared in S1 (Figs 18-21).

Quantitative data showed that S1 at both time points contains the highest new bone volume per

Table 2. ELEVATION RATES IN S1, S2, AND S3

	S1	S2	S3
Elevation rate range (mm/d)	0-0.33	0.33-0.66	0.66-1

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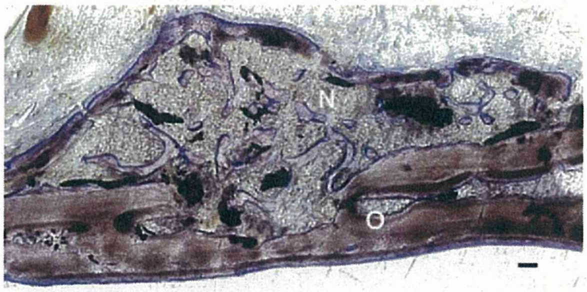

FIGURE 10. Newly formed bone in S2 in group 1. (O, original bone; N, new bone.) (Toluidine blue staining; *scale bar,* 300 μm.)

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distracted segment volume compared with S2 and S3. The percentage of new bone volume at 6 weeks was higher than that at 4 weeks (Table 1). There were statistical differences between S1 and S2 and between S1 and S3 at 4 weeks. At 6 weeks, there were significant differences between S1 and S3 and between S2 and S3 (Figs 22, 23).

Discussion

The results of this study showed that the new device has effectively induced osteogenesis and successfully distracted the periosteum and overlying soft tissue after 6 weeks in a rabbit calvarial model.

Advantages have been acquired because of the design of the new device. Primarily, it maintained the integrity of the periosteum without interruption. We reported no device displacement, because it was completely hidden under the soft tissues away from the dislodging action of the distracted tissues. Margins of the device were modified to follow the normal contour of the soft tissue when it is activated; hence, no soft tissue dehiscence was reported. Activating the device in an inclined position allowed the application of different distraction speeds to the periosteum simultaneously. The length of the device (20 mm) and its large surface area (200 mm^2) permitted the observation of different bone regeneration patterns in response to different distraction rates.

Sencimen et al[18] and Altuğ et al[19] used the mandible of the rabbit as a model to perform periosteal distraction. They reported an abundance of adipose tissue within the newly formed bone. This was attributed to the distractor design that required incising the periosteum at 3 sites during its application. The device used in our study remarkably preserved the continuity and integrity of the periosteum.

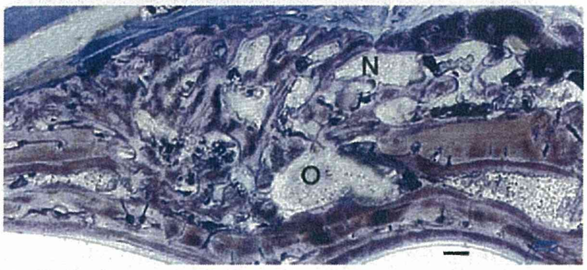

FIGURE 11. Newly formed bone in S2 in group 2. (O, original bone; N, new bone.) (Toluidine blue staining; *scale bar,* 300 μm.)

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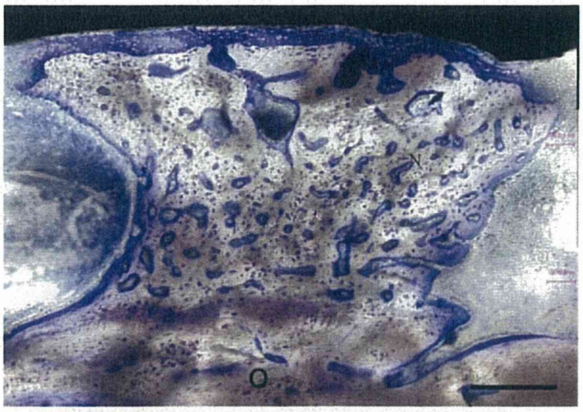

FIGURE 12. Newly formed bone in S1 in group 2 showing more dense trabeculae than in S2. (O, original bone; N, new bone.) (Toluidine blue staining; *scale bar,* 300 μm.)

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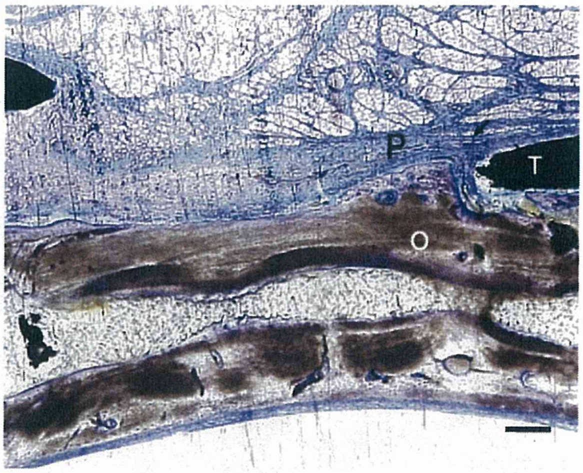

FIGURE 13. Extended bone trabeculae through device perforations and contact with periosteum. (O, original bone; P, periosteum; T, titanium device.) (Toluidine blue staining; *scale bar,* 300 μm.)

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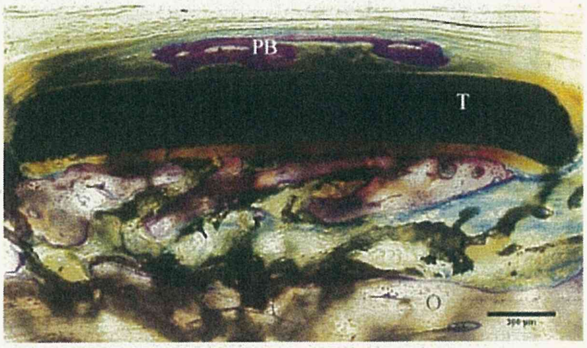

FIGURE 14. Periosteal bone formed over device at S1 in experimental group. (O, original bone; PB, periosteal bone; T, titanium device.) (Toluidine blue staining; *scale bar*, 300 μm.)

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New bone formed in a supra-osteal manner after raising the periosteum is mainly due to the process of natural bone resorption leading to bone marrow access.[21] Cortical perforation may further ease that access.[16] During periosteal distraction, a competition arises between soft tissue cells derived from periosteum and osteoblast cells originating from cancellous bone in the gradually created space. The former cells have the ability to invade the maintained space and multiply faster than the latter.[21]

In S1 the distance between the mesh and bone surface is compared least with the other segments (Figs 20, 21 [arrows]). This affords a comparatively restricted inlet for soft tissue cells and, consequently, less chance to invade the S1 space. Simultaneously, this gives a better chance for osteoblasts to multiply and occupy the space. This may explain the lesser interference of connective tissue and the high ratio of BV/DV at S1 compared with the other segments (Table 1).

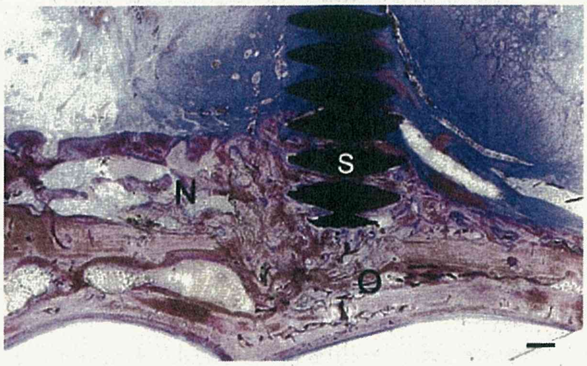

FIGURE 15. New bone trabeculae in S3 creeping over serrations of elevation screw. (O, original bone; N, new bone; S, screw.) (Toluidine blue staining; *scale bar*, 300 μm.)

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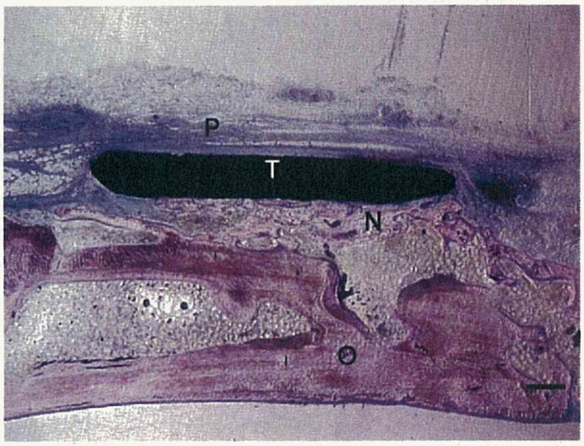

FIGURE 16. New bone filling the minute gap between the original bone surface and the titanium device (control group). (O, original bone; N, new bone; P, periosteum; T, titanium device.) (Toluidine blue staining; *scale bar*, 300 μm.).

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In S3 the distraction rate showed the highest value among the other segments (0.7-1 mm/d) and the lowest ratio of BV/DV. However, in that segment a large amount of bone was observed covering the apical 2 mm of the elevation screw. This can be attributed to the presence of the titanium screw, which acted as a scaffold for bone regeneration, provided that the underlying cortical bone in this

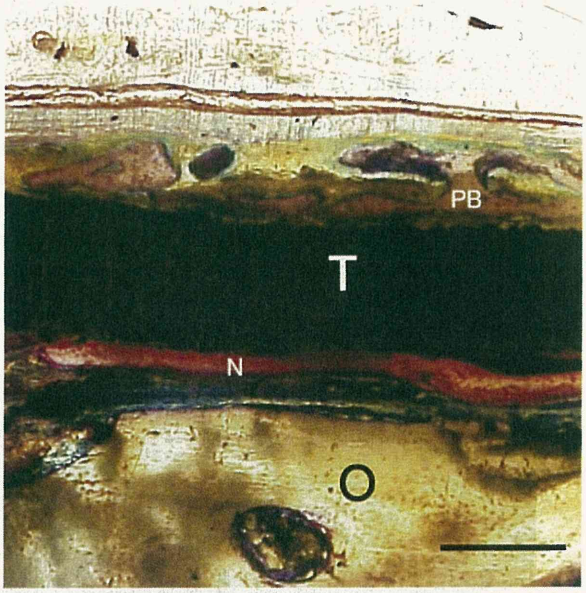

FIGURE 17. Periosteal bone formed over device close to anchored end at 6 weeks in control group. (O, original bone; N, new bone; PB, periosteal bone; T, titanium device.) (Toluidine blue staining; *scale bar*, 300 μm.)

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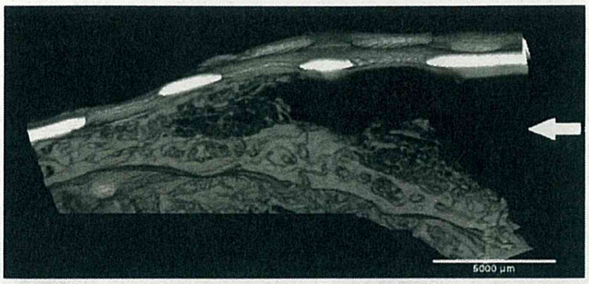

FIGURE 18. Micro-CT image showing transverse view of newly formed bone under device in group 1. *Scale bar,* 5,000 μm.

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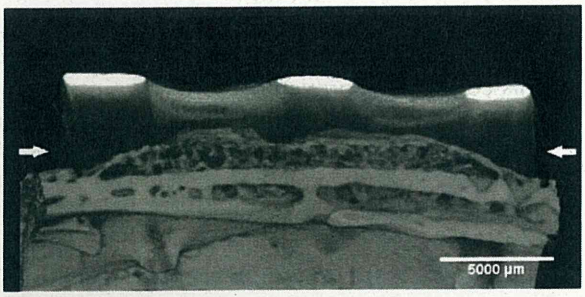

FIGURE 20. Micro-CT image showing cross-sectional view of newly formed bone under device in group 2 in S1. *Scale bar,* 5,000 μm.

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experiment was able to withstand the pressure of the advancing screw (Fig 15).

Altuğ et al[19] claim that lack of bone marrow cells may play a role in the occurrence of fatty tissue; in addition, lack of stimulatory forces may affect the maturity of the newly formed bone. In our study cortical bone perforation was performed to ease access of the bone marrow cells into the distracted site; however, the calvarial bone receives little stimulation, and this may affect the quality of new bone.

On elevation of the periosteum, its osteogenicity starts to be controversial. Some studies claim that it loses its osteogenicity,[22] whereas other studies have indicated that its osteogenic capacity is maintained conditional on contact with bone.[14] Periosteal distraction procedures include separation of periosteum from its underlying bone; however, 3 periosteal distraction studies reported new bone formation near the periosteum.[15,16,23] This was ascribed to the use of an osteoconductive biomaterial as a periosteal distractor. It induced an osteogenic response and promoted new bone formation. In our previous study we performed the same experiment using a device composed of a composite of biodegradable material and micro-hydroxyapatite osteoconductive particles.[16] The amount of new bone formed over and under the device was notably higher than that obtained in this experiment. The distractor composition might possibly have affected the induced bone.

The periosteal distraction process involves exerting tension on periosteal tissues. Altering tension of the periosteum may give rise to a stimulatory effect on the cambium cell layer to proliferate and form bone.[24] In distraction osteogenesis, early subperiosteal callus was formed in the gap after osteotomy. This is because the periosteal mesenchymal stem cells received an appropriate level of stimulation and were differentiated into osteoblasts.[25,26] In vitro studies proved that Runx2 and osteogenic factor expression were up-regulated in human periosteal cells upon application of mechanical strain.[27]

However, tension may not solely explain the minute periosteal bone observed in this study because of the presence of other factors. The presence of periosteal bone in the control and experimental groups suggests that it may result from stimulation due to contact with newly formed bone underneath the titanium mesh. The inner layer of periosteum is also a source of bone cells.[28]

Probably, the low distraction speed gave less chance for connective tissue to interfere and more

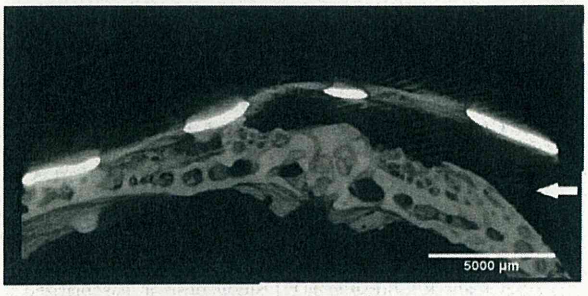

FIGURE 19. Micro-CT image showing transverse view of newly formed bone under device in group 2. *Scale bar,* 5,000 μm.

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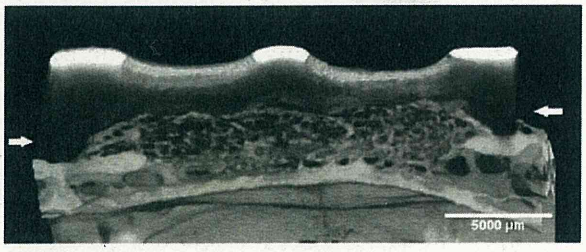

FIGURE 21. Micro-CT image showing cross-sectional view of newly formed bone under device in group 2 in S2. *Scale bar,* 5,000 μm.

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chances for newly formed bone to interact with proliferating periosteum. During osteogenic distraction, this contact was proven beneficial.[28]

It is reasonable that the optimum osteogenic distraction speed (0.5 to 1 mm/d or more[4,5]) is faster than that suggested in our study. This can be explained because cell and nutrition supplies come from both ends of the bone and the surrounding periosteum in osteogenic distraction; whereas, those supplies originate only from basal bone and periosteum in periosteal distraction.

In previous periosteal distraction studies, the distraction rates varied from 0.2 to 0.5 mm/d.[1,2,8,10,15-19,23] In this study all rates of less than 1 mm/d were included; however, a rate of 0.33 mm/d or less showed the least connective tissue interference. The maximum vertical bone formation, given the distraction rate of 0.33 mm/d in this rabbit model, was 1.49 mm, provided that this new bone height constitutes 90% of the maximum height attained by the device in that segment.

A recent study suggests 0.4 mm/d as an appropriate periosteal distraction rate in a rat calvarial model,[9] which is very close to our value, though applied in a different animal model.

Vertical augmentation of the alveolar bone accompanied by soft tissue expansion is feasible with an osteogenic distractor; however, it is technically sensitive and inconvenient to the patient.[6] Conversely, the device used in this study is compact; thus, it could be more acceptable in the patient's oral cavity.

Lowering the distraction rate seems to be 1 step toward diminution of the connective tissue in the periosteal distraction site. This is in agreement with Ilizarov's principle of tissue distraction that recommended the slow distraction speed of tissues.[29]

However, we recommend that further research should be performed to verify the suggested optimal rate for periosteal distraction using a symmetrically moving device.

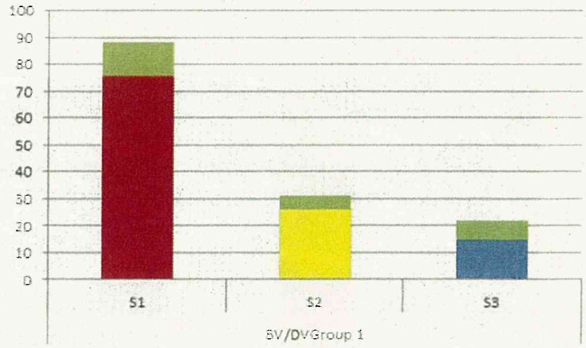

FIGURE 22. Mean and standard deviation of percent of BV/DV among segments in group 1. $P < .01$, Scheffé test.

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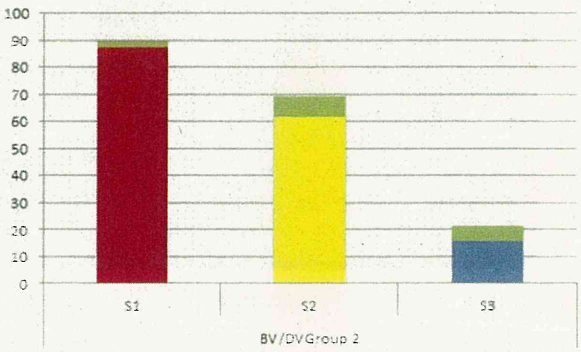

FIGURE 23. Mean and standard deviation of percent of BV/DV among segments in group 2. $P < .01$, Scheffé test.

Zakaria, Madi, and Kasugai. Induced Osteogenesis by Periosteal Distractor. J Oral Maxillofac Surg 2012.

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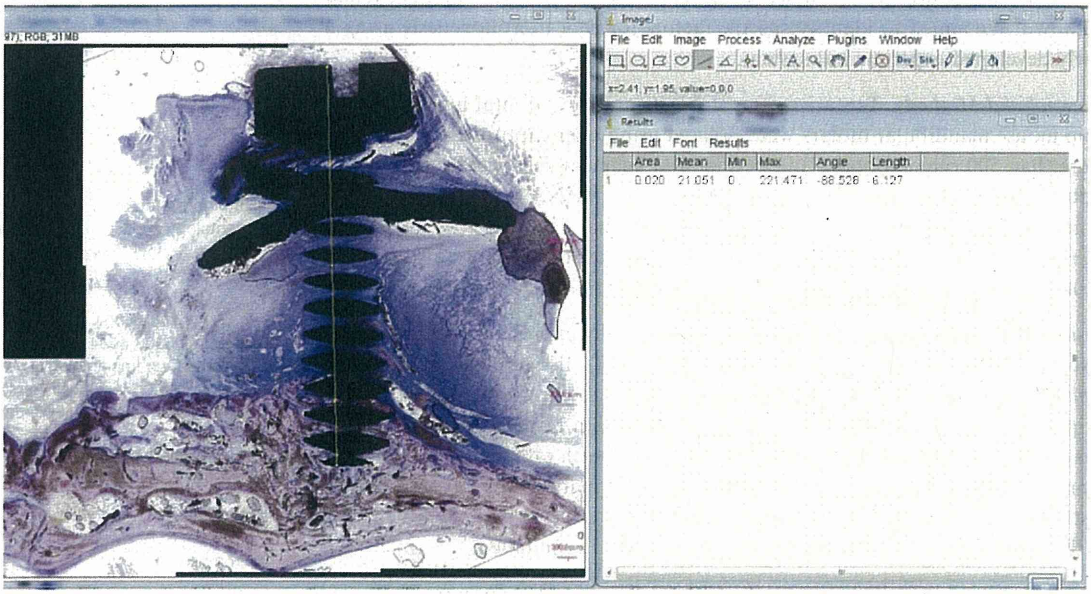
APPENDIX SUPPLEMENTARY DATA. Titanium elevation screw length measured using ImageJ software.



x=2.41, y=1.95, value=0,0,0

	Area	Mean	Min	Max	Angle	Length
1	0.020	21.051	0	221.471	-88.528	6.127

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Restricted expression of chromatin remodeling associated factor *Chd3* during tooth root development

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Date Y, Yokoyama Y, Kondo H, Kuroda S, Ohya K, Ota MS, Iseki S, Kasugai S. Restricted expression of chromatin remodeling associated factor Chd3 *during tooth root development. J Periodont Res 2012; 47: 180–187. © 2011 John Wiley & Sons A/S*

Background and Objective: The tooth root is one of the critical parts to maintain tooth function; however, the molecular mechanisms of root development remain unknown. We aimed to identify specific factors for root morphogenesis using a newly developed experimental system.

Material and Methods: Tentative cementoblasts and periodontal ligament cells from mouse mandibular molars were isolated using laser capture microdissection. More than 500 cementoblasts and periodontal ligament cells were separately captured. After RNA extraction and amplification, mRNA expression in isolated cementoblasts was compared with that of periodontal ligament cells by cDNA microarray analysis. Then, putative cementoblast-specific genes were subjected to *in situ* hybridization analysis to confirm the results in mouse mandible.

Results: Approximately 2000 genes were differentially expressed between these tissues. Among those genes, *zinc finger helicase (ZFH)*, also termed *chromodomain-helicase-DNA-binding protein 3 (Chd3)*, was one of the highly expressed transcripts in tentative cementoblasts. *In situ* hybridization revealed that *ZFH/Chd3* was strongly expressed in Hertwig's epithelial root sheath rather than in cementum. Moreover, its expression disappeared when root formation was advanced in the first molar. In contrast, *Chd3* was continuously expressed in dental epithelial cells of the cervical loop, in which root extension is never terminated.

Conclusion: These results suggest that *ZFH/Chd3* might play an important role in tooth root development and subsequent cementogenesis.

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Key words: chromodomain-helicase-DNA-binding protein 3; Hertwig's epithelial root sheath; microarray; tooth root

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The tooth root, which is developed by the reciprocal interaction between dental epithelium and mesenchyme, is one of the essential parts to maintain tooth function. However, the molecular basis for the signaling during root development and critical factors for root morphogenesis remain unknown. At the initiation of root formation, as previously reported, dental follicle cells penetrate the ruptured Hertwig's epithelial root sheath and subsequently differentiate into cementoblasts, or Hertwig's epithelial root sheath cells undergo an epithelial to mesenchymal transformation and become functional cementoblasts (1,2). It had been generally believed that cementoblasts and