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ORIGINAL ARTICLE

Induced osteogenesis using biodegradable and titanium periosteal distractors

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KEYWORDS

Hydroxyapatite;
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Abstract *Background:* New bone formation can result from periosteal distraction. This is achieved through progressive uplifting of the periosteum by increasing the interface between it and the bone surface.

Objective: This study investigated the impact of gradual periosteal distraction using biodegradable materials and titanium distraction devices.

Materials and methods: 20 rabbits were separated into 2 groups. Distraction devices were placed in all groups after reflecting the calvarial periosteum. The device was actuated following 7 days. Group 1 got titanium device and Hydroxyapatite HA with poly-L-lactide (PLLA) device was utilized in group 2. Five animals were sacrificed from each group following 4 and 6 weeks. Newly formed bone was histologically and radiographically assessed.

Results: The histological observations showed that both distraction devices successfully induced osteogenesis and effectively distracted the soft tissue following 4 and six weeks. The study showed scattered bone trabeculae, with adipose tissue and multiple dome-shaped bones. Micro-computed tomography showed newly formed bone that was far less radiopaque than the initial basal bone. The connective tissue appeared as a radiolucent area that decreased gradually toward the fixation point of the device. At 6 weeks, the percentage of new bone was significantly higher than at 4 weeks for both devices. The PLLA device showed more bone than did the titanium device at both 4 and 6 weeks, but no significant difference was observed.

Conclusions: Both distraction devices were effective in distracting the periosteum and inducing new vascularized bone. The PLLA device induced more bone than the titanium device. Thus, the distractor composition may influence the new bone.

1. Introduction

New bone formation, with or without cortical perforation, can result from periosteal distraction. This is achieved through progressive uplifting of the periosteum by increasing its contact with the bone surface (Schmidt et al., 2002). Schmidt et al. (2002) were among the first to conduct a periosteal

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distraction device study. A gradual process of periosteal lifting triggers expansion of the fissure between the initial bone surface and periosteum, which induces supraosseous neogenesis. Therefore, Schmidt et al. proposed further improvements to remove the effect of displacement on the distraction device. Subsequent studies on periosteal distraction have also indicated some practical device-related issues. The issues most reported include device instability, soft-tissue dehiscence, and displacement (Estrada et al., 2007; Saulacic et al., 2011).

The contact with bone appears to be essential in influencing the osteogenicity of the periosteum (Canalis and Burstein, 1985; Kostopoulos et al., 1994; Bosch and Vargervik, 1995; Kostopoulos & Karring, 1995). The process of periosteal distraction primarily entails separating the periosteum from the underlying bone, but different studies provide evidence of periosteal bone formation (Yamauchi et al., 2008; Zakaria et al., 2011).

However, the success of periosteal distraction greatly depends on the effectiveness of the improvements made to the device and the suitability of the underlying protocol that regulates the device activation (Canalis and Burstein, 1985). Previous studies on periosteal distraction have applied distraction rates ranging from 0.2 to 0.5 mm per day, distraction periods from 8 to 32 days, consolidation periods from 7 to 60 days, and latency periods from 1 to 10 days (Schmidt et al., 2002; Estrada et al., 2007; Yamauchi et al., 2008; Zakaria et al., 2011).

Many periosteal distraction studies have applied different devices and protocols. However, none have clarified the optimal activation conditions (Shikinami et al., 2005). The most studied biodegradable materials are poly-L-lactide and synthetic polymers for their feasibility in medical devices. Some of their benefits are their ability to undergo complete hydrolysis into their constituting components and their absorbability in the body (Shikinami et al., 2005). In 2007, the United States Food and Drug Administration approved a thin biodegradable mesh, made of unsintered hydroxyapatite (u-HA) and PLLA fine particles, for use in fracture repair and fragment fixation in specific parts, particularly the maxillofacial region.

Numerous successful clinical trials have used this biodegradable mesh in the repair and fixation of bone fragments (Ueki et al., 2006, 2011; Ito et al., 2008; Kawachi et al., 2008). The mesh is made with u-HA fine particles, which are naturally bioactive, bioresorbable, and essential in osteoconductivity. They are responsible for the rapid dissolution of the materials compared to others (Shikinami et al., 2005). For the current study, a simple device primarily using biodegradable mesh was developed for periosteal distraction.

The purpose of this study was to examine the result of gradual periosteal distraction using devices made of titanium and biodegradable materials.

2. Materials and methods

A total of 20 Japanese male rabbits, aged 6 weeks and averaging 2.5–3 kg, were selected for use in this study. The animal distribution and grouping are presented in Table 1. The trial protocol was approved by the Committee of Animal Experiments at Tokyo Medical and Dental University, Tokyo, Japan (EA 0150212A).

Table 1 The experimental design and device properties used in group 1 and 2.

Groups	Group 1	Group 2
Distractor material	Titanium distractor	PLLA distractor
4 weeks	5	5
6 weeks	5	5
Device dimension	$20 \times 10 \times 0.3$ mm^3	$20 \times 10 \times 0.5$ mm^3
Fixation screw	L = 3 mm and D = 1 mm	L = 3 mm and D = 1 mm
Elevation screw	L = 5 mm and D = 2 mm	L = 5 mm and D = 2 mm

2.1. Device description

Two distraction devices were used: the first made of titanium mesh and the second made of bioactive, bioresorbable u-HA combined with PLLA (TAKIRON, Japan). The components and device dimensions are presented in Table 1 and Fig. 1.

2.2. Surgical procedures

The study animals were anesthetized before the operation, using an intramuscular injection of thiopental sodium (25 mg/kg Rabonal) and ketamine (50 mg/kg Ketalar). An additional 1.8 mL of a locally available anesthetic containing 2% xylocaine/epinephrine was also injected before commencing the surgery.

Aseptic conditions were applied for all operations. For the animals in both intervention groups, the forehead was shaved, then disinfected with a 1% iodine tincture solution. A u-shaped incision was made in the skin and then subperiosteally on the calvaria bone. The periosteal flap and skin were then opened to reveal the underlying surface of the bone. Cortical perforations of the occipital bone were made using a no. 4 round bur under saline irrigation.

Using two fixation screws, the device was fixed on one end of the bone surface so that it rested on the perforated area. The periosteum was closed to cover the whole device. The skin flaps were sutured using no. 3–0 silk.

2.3. Device activation

One week after surgery, an incision of 2 mm was made in the soft tissue approximately over the screw holes of both devices. The device was raised by threading the elevation screw. A distraction at the 0.5-mm rate was then applied twice daily for 5 days. During the entire observation period, all rabbits under the study received water and ordinary feed to the quantity required.

Five animals were sacrificed using a lethal dose of thiopental sodium at 4 and 6 weeks after the consolidation period in both groups 1 and 2. The cranial bone was removed and placed in a neutral 10% solution of formalin for 14 days.

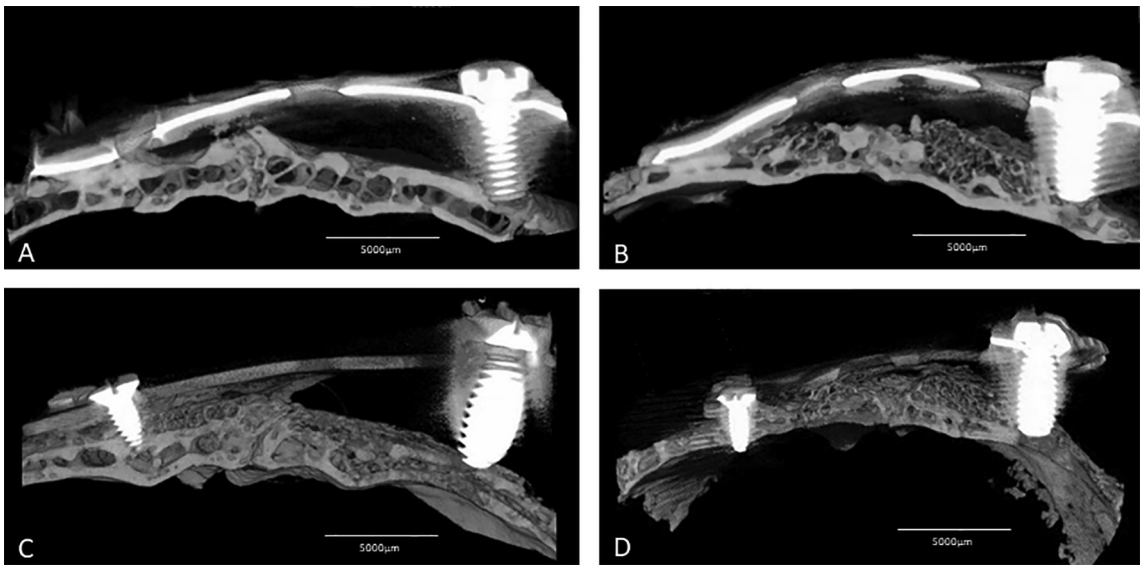

Fig. 1 Micro-CT transverse section showing newly formed bone under both devices in group 1 after 4 (A), 6 weeks (B) and group 2 after 4 weeks (C) and 6 weeks (D). Scale bar is 5000 µm.

Table 2 Showing The total amount of the newly formed bone area per Distracted area in Group 1 and 2 at 4 and 6 weeks. (The measuring unit is mm^2).

T BA/DA	Group 1 (Ti)	Group 2 (PLLA)	*t*-test	P value
4 weeks	231.8 ± 33.3	266.3 ± 34.6	t = 1.4369	P = 0.2008
6 weeks	329.9 ± 40.5	361 ± 34.7	t = 1.1663	P = 0.2878
T-test	t = 3.7420	t = 3.8651		
P value	P = 0.0096*	P = 0.0083*		

* Significant difference where P value is less than 0.05.

2.4. Micro-computed tomography

Soon after the fixation period, all the specimens were imaged using a micro-computed tomography (CT) device (SMX-90CT, Japan) capable of high resolution using continuous increments of 60 µm. Bone images were then extracted by processing the grayscale images obtained, using a median filter that removed noise and set an unchangeable threshold for the mineralized bone extraction phase. Images were calibrated using Phantom Compass and scrutinized using 3D analysis software (TRI/3D-BON, Ratoc, Japan). A 10 micro-CT serial longitudinal images were then obtained for each specimen (1 image per millimeter). The calculation of the amount of bone formed per distracted area (TBA/DA) for all samples used ImageJ software (1.43 Hz; Table 2).

SPSS software (SPSS, Chicago, IL, USA) was used to perform statistical analysis. Descriptive statistics were also used to describe means and standard deviations. The *t*-test was used for comparative analysis of the newly formed bone in both groups at 4 and 6 weeks. The level of significance was set at 95%.

2.5. Histologic processing

Ascending grades of ethanol were prepared to dehydrate the calvarial bone after fixation. Once fully dehydrated, the bone was fixed in polyester resin (Rigolac-70F and Rigolac-2004).

Histological sections were obtained with the distraction devices in place using an Exakt machine (Mesmer, Germany) and ground to a thickness of around 60 µm. A solution of 0.1% toluidine blue was then used to stain the sections to allow histological observation using a light microscope.

3. Results

The animals showed uncomplicated recovery. No device exposure or infection was detected in any animal during the period of experimentation.

3.1. Micro-CT findings

In weeks 4 and 6, micro-CT-extracted 3D images revealed a small amount of new bone, characteristically less radiopaque than the original basal bone. The connective tissue was represented as a radiolucent area with considerable thickness towards the elevation screw that decreased gradually toward the fixation point (Fig. 1).

Quantitative data indicated a relatively high percentage of new bone volume in week 6 compared to week 4 for both devices (Table 2). Some statistical differences were evident between 4 and 6 weeks for both devices. The PLLA device showed more bone volume than the titanium device both at

weeks 4 and 6. Despite this, no significant differences were observed at either point.

3.2. Histological findings

The histological sections for group 1 (titanium device) at week 4 revealed evidence of multiple dome-shaped bones surrounded by a thin layer of trabeculae and further scattered trabeculae within abundant adipose tissue. A connective tissue layer covering the new bone was also observed (Fig. 2A). Similar histological patterns were obtained after 6 weeks, but bone trabeculae were observably thicker and made up of less adipose tissue (Fig. 2B).

For group 2 (PLLA device), bone trabeculae at the fixation screw appeared denser, but with notably decreased adipose tissue. Bone trabeculae in the middle area of the device appeared less compact but with more connective tissue (Fig. 2C and D). In addition, trabeculae showed a potential to extend beyond the device perforation toward the distracted periosteum, and various blood vessels could be seen in the overlying periosteum (Fig. 3).

4. Discussion

This study evaluated periosteal distraction using titanium and biodegradable devices, histologically and radiographically using micro-CT. The distractor device was simple in design, creating a wedge-like space between the cortical bone and periosteum. This was necessary to prevent the invasion of the surrounding tissues and allow space for bone formation.

Skull periosteum was chosen for distraction in this study. Initially, distraction devices were implanted under the periosteum of the mandible. It was a challenging operation and risked dislodgment of the device due to chewing (Kostopoulos & Karring, 1995; Schmidt et al., 2002).

Stevens et al. (2005) distracted the tibial periosteum in rabbits for obtaining new bone, reporting satisfactory results showing defect filling. Due to the limited space, this procedure does not regenerate sufficient bone tissue for treating expansive bone defects.

This has led researchers to initiate the study of perosteal distraction osteogenesis using the cranium (Kessler et al., 2007; Zakaria et al., 2011, 2012; Dziewiecki et al., 2016; Saulacic et al., 2016). The skull is suitable because it is flatter and thus makes placing the device easier. Further, the skull's periosteum is more robust. This makes the calvarial periosteum better for conducting periostal distraction osteogenesis research.

Researchers must protect the integrity of the periosteum when performing animal experiments since it is a barrier that protects against soft tissues invasion. Maintaining its integrity during the distraction process is also important, along with preventing its dehiscence by placing distraction screws far from the periosteum incision.

Distraction devices were first constructed from titanium alloy and stainless steel, then biodegradable materials were used to fabricate scaffolds. The types of materials used include PLLA/HA, hydrogel, polyglycolic acid, beta-tricalcium phosphate, and poly-DL-lactide (Stevens et al., 2005; Yamauchi et al., 2010; Zakaria et al., 2011; Dziewiecki et al., 2016). Dziewiecki et al. (2016) found that the choice of biodegradable or metal material does not affect the formation of new bone. However, resorbable composites are more appropriate for developing tissue-engineered scaffolds (Wubneh et al., 2018).

The two processes of osteogenesis and angiogenesis are intricately interrelated. The existence of new bone tissue largely depends on the regeneration ability of the new vessels (Mercado-Pagán et al., 2015). In our study, numerous blood vessels were observed in the periosteal tissue with the new bone in both distraction devices.

Undeniable competition is evident between soft-tissue cells and osteoblasts in the space created during periosteal distraction. The soft-tissue cells from the periosteum can invade the maintained space due to their rapid proliferation compared to osteoblasts (Rompen et al., 1999).

The absence of bone marrow cells could trigger fatty tissue occurrence. The absence of stimulation could hinder the

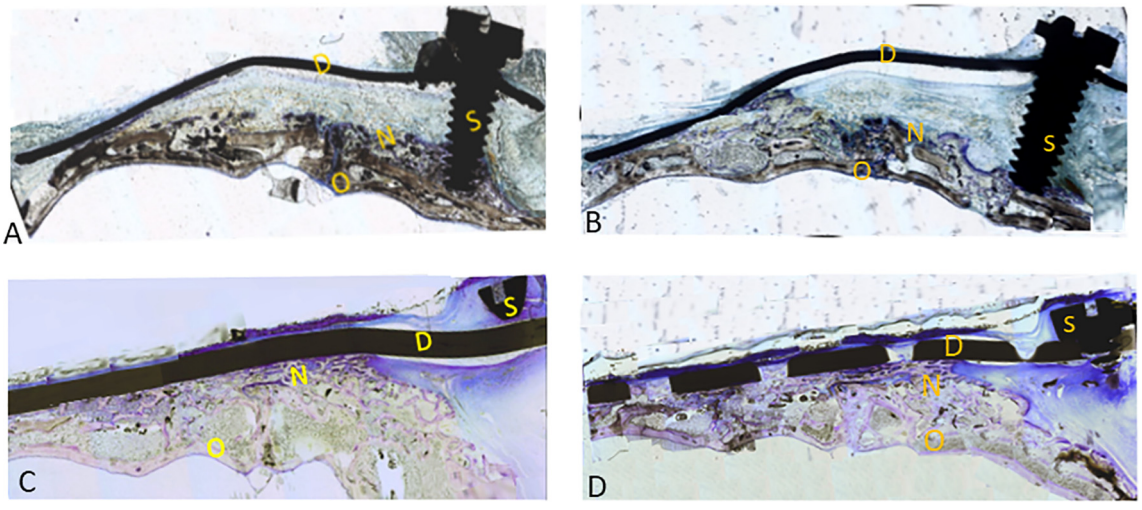

Fig. 2 Histological images showing group 1 after 4 (A) and 6 weeks (B) and Group 2 after 4 (C) and 6 weeks (D) showing the newly formed bone (N) above the original bone (O), Elevation screw (S) and distractor (D). Toluidine blue staining.

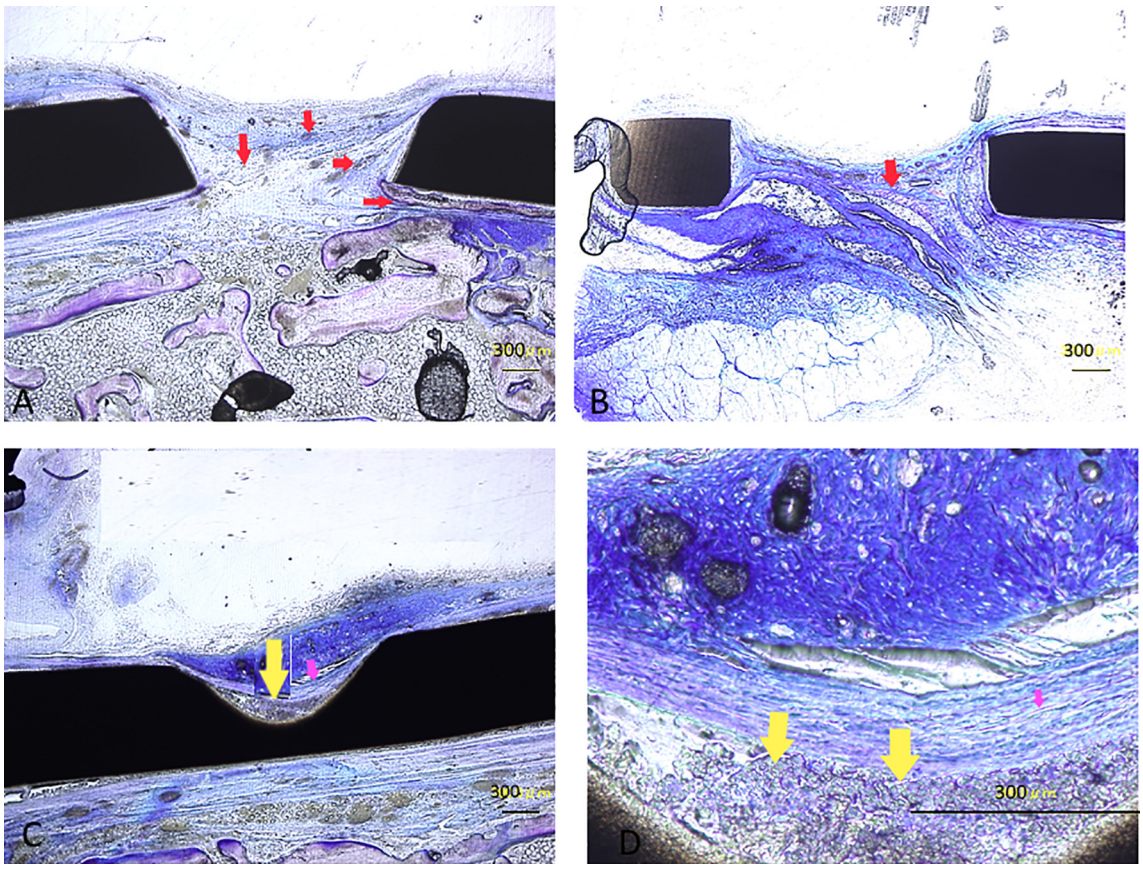

Fig. 3 Histological images with higher magnification for group 2 showing bone trabeculae (Red arrows) extending through perforation of the distractor reaching the overlying periosteum (A and B). New bone trabeculae (Yellow arrows) are present over the device and under the distracted periosteum (Pink arrows) (C and its higher magnification in D).

maturation of the new bone (Altug et al., 2011). The perforation of cortical bone is intended to enhance the movement of bone marrow cells to the sites of distraction. However, the calvarial bone received insignificant mechanical stimulation, and this showed a negative effect on the quality of new bone. Once the periosteum is elevated, its osteogenicity is controversial. For some researchers, it loses osteogenicity, while others have found it retains its osteogenic capacity, conditional on the nature of contact with the bone (Kostopoulos et al., 1994; Weng et al., 2000).

The process of periosteal distraction entails the separation of periosteum from its bone (Simon et al., 1994). However, previous studies on periosteal distraction have reported the formation of new bone close to the periosteum (Yamauchi et al., 2008, 2010; Zakaria et al., 2011). This has been attributed to the application of distractor composed of osteoconductive material.

In the current study, the histological findings showed more bone volume induced by the biodegradable distraction device (group 2) than the titanium device (group 1). This could be due to the osteoconductive property of the HA microparticles in the device. Thus, the composition of the distractor may have influenced the resulting bone.

The process of periosteal distraction typically entails subjecting periosteal tissues to substantial tension. This can affect the stimulation of cambium cell layer proliferation, which subsequently triggers the formation of new bone (Kanno et al., 2005). Some subperiosteal callus was formed during distraction osteogenesis. This is partly due to the appropriate levels of stimulation of periosteal mesenchymal stem cells and their subsequent differentiation into osteoblasts (Delloye et al., 1990; Hikiji et al., 2000; Takeuchi et al., 2010). In vitro studies have shown evidence of upregulation of Runx2 and osteogenic factor expression when mechanical strain is applied to human periosteal cells (Kanno et al., 2005).

However, tension may not be the only reason for the little new bone observed in this study. The presence of periosteal bone in both the experimental and control groups suggests the input of stimulation resulting from direct contact with the bone formed and located below the mesh. More bone cells come from the inner parts of the periosteum (Takeuchi et al., 2010).

In previous studies, the distraction rate ranged from 0.2 to 0.5 mm per day. The current study included rates 1 mm per day. The total newly formed bone area after 4 weeks was 231.8 ± 33.3 mm^2 for group 1 and 266.3 ± 34.6 mm^2 for group 2. After 6 weeks, group 1 showed 329.9 ± 40.5 mm^2, and group 2 showed 361 ± 34.7 mm^2. Previous reports have recommended 0.4 mm per day as the proper periosteal distraction rate in a rodent model (Saulacic et al., 2011). Although applying a different animal model, this is close to the rate in the current study.

Vertical bone augmentation and soft-tissue expansion are achievable using osteogenic distractors. However, it is

practically inconvenient for patient use. The device applied in the current study is compact and acceptable for intraoral application. By reducing the distraction rate, the amount of connective tissue in the periosteal distraction site can be minimized. This agrees with the propositions of Ilizarov's principle, which recommends slowing the rate of distraction to induce bone (Ilizarov et al., 1989).

5. Conclusion

Both distractors were successful in elevating the periosteum and inducing bone formation. The PLLA device showed more bone than did the titanium device, so the distractor material may impact the new bone.

Ethical statement

All experimental procedures were conducted in accordance with the highest standards, under the approval of the Committee of Animal Experiments at Tokyo Medical and Dental University, Tokyo, Japan (EA 0150212A).

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CRediT authorship contribution statement

Marwa Madi: Conceptualization, Methodology, Writing - review & editing, Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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