Beneficial Role of Periosteum in Distraction Osteogenesis of Mandible: Its Preservation Prevents the External Bone Resorption

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Distraction osteogenesis (DO) is a method of new bone generation through the gradual extension of two segments of existing bone. DO is applied for maxillofacial surgeries to manage defects in mandibular continuity. Vertical DO with an oral device is often employed to augment the alveolar bone height for better implant anchorage for esthetic purposes or functional prosthetic requirements. To determine how the periosteum affects the vertical DO in mandibular reconstruction, we extracted the teeth and resected the alveolar parts of the mandible on both sides of dogs, along with removal of the surrounding periosteum on the right, but not left side. Three months later, box-shaped bone segments (vectors) were prepared from the resected alveolar part, and the segments were vertically elongated using a distraction device on both sides at 0.9 mm/day for one week. The extent of bone formation after distraction was determined with micro-focused computed tomography and by measuring incorporation of tetracycline and calcine with confocal laser scanning microscopy. During the initial two months after distraction, new bone formation was observed more prominently in the left side than in the right side of mandible with the periosteum. However, this difference was less clear during the bone-remodeling period. One notable change was the reduced height of the alveolar part of the right-side mandible, a sign of external bone resorption, observed in two out of three dogs at 6-month post-consolidation. These findings suggest that preservation of periosteum prevents the external bone resorption during the vertical DO of mandible.

Keywords: vertical distraction osteogenesis/periosteum/vector/micro-focused computed tomography/confocal laser scanning microscopy

Materials and methods

Animal model

Eleven 1- to 2-year-old beagles (8 female and 3 male), weighing around 10 kg, were used for this study. Use and care of animals in this study conformed to Association for Assessment and Accreditation of Laboratory Animal Care standards and was approved by the Tokyo Medical University Subcommittee on Research Animal Care.

Surgery

After sedation with ketamine hydrochloride (5 mg/kg), general
anesthesia was induced with intravenous pentobarbiturate (0.5 mg/kg). In the first operation, we extracted 4 premolar teeth, and performed marginal resection of the mandible over a height of 5 mm simulating tumor resection. The surrounding periosteum was completely excised over a width of 2 mm using a knife (Fig. 1A, arrow) only on the right side, and was preserved on the left side after resection of the mandible (Fig. 1A). The excised range was over 1.5 cm of the osteotomy line each mesially and distally, and through the lower border of the mandible bucco-lingually. This procedure was performed by a single qualified oral and maxillofacial surgeon (A.M.).

After 3 months (Fig. 1B), under general anesthesia vectors were produced 5 mm in height, 30 mm in length, and completely osteotomized bucco-lingually (7 to 8 mm in width) on the resected area under both conditions, and intra-oral distraction devices, TRACK 1.0 mm-System (Gebrüder Martin GmbH & Co. KG, Tuttlingen, Germany), were fixed using screws. At first, the vector was kept in the original position for 1 week (latency period), then 0.9 mm of vertical distraction was performed per day, and the bone height was increased by a total of 5.4 mm (Fig. 1C). The distraction devices were kept at the final position for 2 months (consolidation period), then removed. Observation was carried out for 6 months after completion of consolidation. The mandible including the whole distracted part was prepared for micro-CT scanning, Elescan (NS-ELEX Co. Ltd., Kita-kyushu, Japan). First, we scanned the whole image at low magnification under the following conditions: slice thickness 131 µm, magnification 1.72, pixel size 82 × 82 µm, and image matrix size 512 × 512 pixels. We created 3D reconstruction images by volume rendering method for morphological observations using the computer software program TRI/3D-BON (Ratok System Engineering Co., Ltd., Tokyo, Japan).

Bone morphometrical study
Using the above images, we selected two areas of volume of interest (VOI) of 2 × 2 × 1 mm in the trabecular bone for morphometrical analysis, which is independently set in the mesial and distal area of the distraction gap (Fig. 3). These areas were examined by micro-CT analysis under the following conditions: 20 µm of slice thickness, 5.56 × magnification, 25 × 25 µm pixel size, and 50 number of slices. Bone volume (BV) was calculated using tetrahedrons corresponding to the enclosed volume of the triangulated surface. Total tissue volume (TV) was the entire volume of the analysis. The bone volume index was determined as follows: 100 × BV/TV.

CLSM images
We then prepared the specimens to make the non-decalcified
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Fig. 2. Labeling schedule.
- Tetracyclin (TC) injection.
- Calcein (Cal) injection.

The numbers inside parentheses indicate the frequency of injections.
- A. Latency period.
- B. Distraction period.
- C. Consolidation period.

TC was injected at the first half and Cal at the latter half. Both agents were injected weekly.

Fig. 3. Setting of volume of interests (VOIs) for the bone morphometries.

We selected the two areas of VOI of 2 × 2 × 1 mm in the trabecular bone, which is independently set on the mesial and distal area of the distraction gap.

Fig. 4. Comparison of CLSM images between the original and the extracted condition.
- A. The original image. In the original CLSM images, the TC labeling area was colored red, and the Cal labeling area was green.
- B. The extracted image. To eliminate the background, we made CLSM extracted images drawn in the same color using computer software.
The specimens were dehydrated in graded alcohol for 3 weeks, saturated with styrene monomer for 10 days, embedded in polyester resin consisting of a 7:3 mixture of Rigorac 2004 and 70F (Riken, Tokyo, Japan), and polymerized. The block was cut into 1-mm continuous slice sections with a band saw, and each was ground down further to approximately 100 \( \mu \)m using Exakt Micro Grinding System (Exakt Apparatebau, Norderstedt, Germany). We scanned the ground sections at \( \times 20 \) magnification using CLSM (Zeiss LSM 510, Carl Zeiss AG, Oberkochen, Germany), and 20 images were combined to make one whole slice image. In the CLSM images, the TC labeling area representing the first half of the observation period was colored red fluorescence, and the Cal labeling area representing the latter half of the observation period was colored green fluorescence (Fig. 4A).

To eliminate the background fluorescence and to distinguish the native bone from the original image, we made CLSM extracted images using the computer software program Adobe Photoshop (Adobe Systems Inc., San Jose, CA, USA, Fig. 4B).

**Results**

**3D micro-CT reconstruction images**

At 1-month post-distraction, newly generated bony granulation tissues were observed and filled rapidly in the distraction gap of the periosteum-preserved side of mandible (i.e., left), but not of the periosteum-removal side (i.e., right) in two dogs examined (Fig. 5 second column). At 3-month post-consolidation, however, this difference became less evident and the distraction gaps in both sides were filled with bony granulation tissues (third column). At 6-month post-consolidation, new bone formation was more demonstrated and the height of distraction areas reached to the original level in the left side of mandible in all three dogs (fourth column bottom). In contrast, such tissue regeneration features in the right side of mandible were demonstrated in only one dog (fourth column top). The remaining two dogs exhibited reduction of height in the alveolar part of mandible, a sign of external bone resorption (fourth column middle). These findings suggest that preservation of periosteum prevents external bone resorption.

**CLSM images**

The CLSM images were taken at the trans-section of the central part of resected and basement bones. After completion of distraction (Fig. 6A), micropillar-like trabecular bones were formed longitudinally toward the distraction gap from the basement bone of both right and left sides (green arrows). In contrast, new bone formation outside of the resected bone fragments (vectors) was more evident in the left side of mandible compared with the right side (purple arrows; Fig. 7A blue triangles versus black arrows; Fig. 7B yellow triangles).

At 1- to 2-month post-distraction (Figs. 6B and 6C), new trabecular bones in the distraction gap extended from both the basement bone and the vector and connected to each other in both left and right sides of mandible (red arrows). Fig. 6C shows slightly less calcein-incorporated areas in the right side than the left side of mandible, suggesting periosteum promotes remodeling in the latter half period. The trabecular bones were apparently thicker, but irregular in shape (Fig. 7D).

At 1-month post-consolidation (Fig. 6D), the trabecular bone structure in the distraction gap and the bone remodeling were clearly demonstrated in both right and left sides of mandible (see also Fig. 8A). Notably, resorption of the native bone on the crestal area of the vector was observed in the right, but not left, side (blue arrows).
At 6-month post-consolidation, one case displayed the well corporation of TC and Cal (E). However, in the remaining two cases Cal labeling was reduced in the right side of mandible (F). These findings are consistent with the 3D micro-CT (Fig. 5) suggesting a protective role of periosteum from external bone resorption.
Fig. 7. High magnification images of CLSM during the new bone formation period.
A-C. The distraction gap at the completion of distraction. A. Outer border of the vector in the periosteum-preserved side. The blue triangles indicate actively formed new bone along with the outer border of the vector. B. Outer border of the vector in the periosteum-removal side. The yellow triangles indicate the outer border of the vector which has little new bone formed. NB. Native bone. NEW. New bone. C. The distraction gap in the periosteum-removal side. The green triangles indicate the top of the new trabecular bone which has not yet connected to the vector side. D. The distraction gap at 1-month post-distraction. The trabecular bone had become thicker, but the structure was still irregular.

Fig. 8. High magnification images of CLSM during the bone remodeling period.
A. The distraction gap at 1-month post-consolidation. The trabecular bone structure was clearly seen. B. Comparison of the vector between the periosteum-removal and -preserved sides at 1-month post-consolidation. Osteons at the top of the alveolar crest were sharply cut by bone resorption from outside in the periosteum-removal side (The same findings as Fig. 6D).
Higher magnification images revealed that bone remodeling in the osteons of native bone was developed in both right and left sides of vectors (Fig. 8B green arrows). However, the labeled osteons in the native bone were sharply cut by subsequent bone resorption from outside in the periosteum-removal side (yellow arrow), and new bone formed in the inner layer of the vector had already appeared on the outside (blue arrow).

At 6-month post-consolidation (Figs. 6E and 6F), both right and left side of mandible in one dog displayed the well incorporation of both tetracycline and calcein in the distracted gap and the vector (Fig. 6E). In the remaining two dogs, however, such incorporated areas were reduced in the right side of mandible compared with the left side (Fig. 6F). These findings are thus consistent with the reconstruction images of 3D micro-CT (see Fig. 5) and suggest a protective role of periosteum from external bone resorption during the vertical distraction osteogenesis in mandibular reconstruction.

**Bone morphometrical study**

Average trabecular BV index was 28.4% and 17.8% in the left and right side of mandible, respectively, before distraction (controls, n = 2). Trabecular BV indexes of the mesial and distal parts of the distraction gaps were 23.8% and 21.9% in the left side and 13.1% and 20.4% in the right side of mandible, respectively, at 6-month post-consolidation (n = 3). Fig. 9 shows the changes in BV index of the cancellous bone during mandibular distraction. BV index of the mesial part of distraction gap was increased during the consolidation period, but gradually decreased to approximate that of the cancellous bone before surgery. On the distal part, BV index of the distraction gap was very low, and it was restored after the consolidation period. BV index of the distraction gap was restored to approximate that of the native bone at 6-month post-consolidation on both mesial and distal parts, but it was delayed in the periosteum-removal side compared with preserved side (Fig. 9).

**Discussion**

Since Ilizarov (1989) began using DO in limb lengthening, it has been a procedure developed to reconstruct and lengthen the long bones. Since McCarthy et al. (1992) clinically applied this technique to correct craniofacial deformities, it has also been applied to repair segmental bone defects in the craniofacial bone after tumor resection and trauma. This technique has the advantage of initiating new bone growth without bone transplantation and promoting the growth of soft tissues (Nosaka et al. 2000). Costantino et al. (1990) first applied the horizontal distraction to the mandible in a dog model, which was followed by animal studies (Block et al. 1996) and clinical application (Chin and Toth 1996) of vertical distraction. Several clinical reports point out that long-term reduction of the alveolar height is one of the main problems of this procedure, in particular post traumatic atrophic alveolar ridge (Kanno et al. 2006). In addition, there are other serious problems when DO is performed after resection of a malignant tumor. The surrounding soft tissues including the periosteum are usually completely removed in mandible resection to prevent the recurrence of the tumor, and the soft tissue of the resected part is often reconstructed with free flaps or irradiated, resulting in scars or ischemia (Nocini et al. 2004; Shao et al. 2006).

Although Ilizarov (1989) confirmed that the critical factor of new bone formation was the preservation of the bone marrow for limb lengthening, the effect of the periosteum was not clear in mandibular distraction. The most interesting finding of our study was that at 6-month post-consolidation the height of the distraction area was almost preserved in the left side, but reduced in two out of three cases in the right (periosteum-removal) side of the mandible. What causes this difference and what role does the periosteum play in this phenomenon? Basically, there are two main sources of blood supply to the normal mandible: the inferior alveolar artery and the vessels perforating the periosteum (Miao et al. 1997). According to Elshahat et al. (2004) and Gosain et al. (2004), the periosteum is not as essential as the bone marrow. To the contrary, Costantino et
al. (1990) stressed the importance of the periosteum for mandibular lengthening. Zimmermann et al. (2005) wrote that in distraction of rat and rabbit long bones and sheep mandible, new bone formation was predominantly seen in the sub-periosteal region in every case. Cope and Samchukov (2000) reported that in osteogenesis in alveolar distraction, either from the bone marrow or the periosteum, the modeling period continued almost for the initial 2 months, and after that consolidation period, secondary remodeling began. We observed the same findings on our micro-CT and CLSM images in the periosteum-preserved side. However, in the periosteum-removal side, although remodeling of osteons inside the cortical bone was maintained, no bone formation but only resorption from the outside progressed after the consolidation period. Moreover, there was little Cal labeling at the distraction gap and the vector in two out of three cases exhibited reduction of height in the alveolar part of mandible in the periosteum-removal side at 6-month post-consolidation. This finding indicated that when periosteum was removed bone remodeling was sparse during the latter half period. On the contrary, in a case in which the alveolar height was maintained, both TC and Cal were labeled in both right and left sides in all periods. Morphometrical study confirmed the bone structures quantitatively (Parfitt et al. 1987). Three-dimensional observation is possible without destruction of the specimen using micro CT (Odgaard 1997). Some studies have adopted two-dimensional morphometrical parameters for DO (Zimmermann et al. 2005; Amir et al. 2006), but it has not been confirmed three-dimensionally. Our morphometrical observations that remodeling is delayed without periosteum are in line with the CLSM findings that bone remodeling was sparse during the latter half period. To summarize our results, only inner remodeling (remodeling in the distraction gap or in the osteon) was continuing in the periosteum-removal side, whereas inner and outer remodeling (remodeling on the outer bone surface along the periosteum) was continuing normally in the periosteum-preserved side. In addition, active inner remodeling maintained the bone height even without periosteum, the condition of which is, however, unpredictable.

If the total amount of bone formation is higher than that of resorption during the bone remodeling period, external bone resorption might be protected even without periosteum. Several methods are considered to compensate the external bone resorption to obtain prospective vertical DO. Firstly, excessive elongation (overcorrection) is an actual solution as many reports point out (Saulacic et al. 2005; Kanno et al. 2006; Ettl et al. 2009). Secondly, the distraction rate is an important condition, and it has been indicated that frequent distraction was more effective for good bone formation (Zimmerman et al. 2005; Amir et al. 2006). Thirdly, combination of growth factors may be useful. Hu et al. (2003) suggested that angiogenic factors like VEGF and bFGF may be effective as therapeutic agents. Weight bearing may also protect against resorption, because functional pressure promotes the bone remodeling (Chiba 1976). Many reports point out that implant placement protects against bone resorption of the alveolar crest on the grafted (Chiapasco et al. 2000) or distracted (Nosaka et al. 2000) mandible. According to Radomisli et al. (2001), weight bearing alerts the expression of collagen I and II, BMP, and osteocalcin to affect early healing in DO.

Some limitations exist in our study. Firstly, bone turnover is different according to species, and the results of the present study are limited to dogs. We selected the dog in this study because its bone turnover is similar to that of humans (Dannucci et al. 1987). Secondly, using young animals is also a limitation, because most malignant tumors occur in elderly people. Therefore, we should perform the same study in older animals in the future. Thirdly, quantitative evaluation was performed in this study but statistical evaluation was not performed because of the small number of cases.

In conclusion, the preservation of periosteum prevents the external bone resorption and maintains the augmented bone height in vertical DO of mandible.

Acknowledgments

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