Pumping the Periosteum: A Feasibility Study

Periosteal Distraction Osteogenesis in a Rat Model

Nikola Saulacic, DDS, PhD,[a] Gordana Vunjak-Novakovic, PhD,[b,c] Maiko Haga-Tsujimura, DDS, PhD,[d] Ken Nakahara, DDS, PhD,[e] Maude Coline Gerbaix, PhD,[f] and Serge Livio Ferrari, MD, PhD[f]

Purpose: Gradual elevation of periosteum from the bone surface is known to promote the adaptation of soft tissues and the formation of hard tissues. The aim of our study was to estimate the benefit of periosteal distraction osteogenesis (PDO) on de novo bone formation in a rat model.

Materials and Methods: After device placement, animals were allowed for a latency period of 7 days. Animals in the PDO group were subjected to distraction at a rate of 0.1 mm/d for 10 days. In the periosteal pumping (PP) group, the animals were subjected to distraction at a rate of 0.1 mm/d. The direction of distraction was alternated every 2 days. The animals were euthanized at 17, 31, and 45 days after surgery, and the samples were analyzed histologically and by microcomputed tomography.

Results: In both groups, the new bone was characterized as primary woven bone that was located at the leading edge of bone apposition. Bone volumes significantly increased throughout the observation period both in the PP group ($P = 0.018$) and in the PDO group ($P < 0.001$). The new bone was denser and more mature in the PP group than in the PDO group, and the difference was significant at the 31-day time point ($P = 0.024$). However, the volume of the new bone was higher in the PDO at the 45-day time point ($P < 0.001$).

Conclusions: We propose that the PP may be applied to enhance the osteogenic capacity of periosteum without plate elevation. Because this is only a proof-of-principle study, the alternated protocol of periosteal distraction warrants evaluation in the future studies.

Key Words: animal model, periosteum, bone, distraction osteogenesis

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Distraction osteogenesis (DO) is a method for endogenous formation of hard and soft tissue by a progressive elongation of the bone fragments created by osteotomy and is progressing in a mechanically controlled environment. Standard DO protocols involve the periods of latency, activation, and consolidation. The original protocol developed by Ilizarov[1,2] included an overcorrection at the end of distraction, followed by compression. The concept was based on the successful results obtained by compression osteosynthesis in the treatment of osteomyelitis, without the use of antibiotics. The benefit of compression at the end of activation was more recently confirmed in several studies.[3–5]

Alteration of distraction and compression was subsequently applied to enhance the quality and quantity of the regenerated bone. The active dynamization or "accordion maneuver" was successfully used in long bones to reduce the duration of treatment[6–8] or enhance the formation of bony union at the docking site.[9,10] The alternation protocol performed during[11–14] or after the mandibular DO[13,15] was superior compared with conventional DO protocol in terms of mineralization and thickness of the forming cortical bone. In contrast, an interruption of distraction by 2 days of compression showed no substantial difference in histological or radiological appearances in the mandible of rats.[16] Despite its apparent potential, the protocol of an accordion maneuver has still not been standardized.[17]

Periosteum is considered a crucial structure for successful bone regeneration during DO.[1,18–22] Actually, the principle of DO can be applied to maintain the osteogenic capacity of elevated periosteum.[23] Compared with conventional DO, the distraction gap in a periosteal distraction osteogenesis (PDO) is bordered by the periosteal (ie, cambial) layer and the original intact surface of the bone. The need for performing an osteotomy and its associated difficulties could thus be avoided. A series of experimental studies have demonstrated the formation of new bone induced by PDO to varying extents using different animal models, sites, surgical techniques, devices, and parameters of distraction.[24–29] More recently, it has been shown that the nature and kinetics of bone formation may be influenced by varying the rate and rhythm of distraction.[30] In comparison with an immediate periosteal elevation, however, the PDO resulted with contradictory results.[31–33] It is still poorly understood how would the alternated protocol of PDO affect bone formation from the original bone surface. The aim of this preliminary study was to assess the potential of a "pumping protocol" on de novo bone formation on the calvaria of rats.

MATERIALS AND METHODS

Animals

Twenty-four adult male Wistar rats weighing approximately 300 g were used for the study. The animals were acclimated for 14 days, housed in a room with a controlled climate (temperature, 22°C–24°C ± 2°C; humidity, 30%–60% ± 5%) and special sun-substitution ultraviolet light (photoperiod, 6–18 hours), without excessive or surprising noises. Three rats were housed individually in aired cages, fed with a standard rodent diet and water ad libitum. The protocol was approved by the Committee for Animal Research, State of Bern, Switzerland (approval number 95/12).

Surgery

The anesthesia and surgery were performed as previously described.[33,34] Briefly, after anesthesia induction by 8% isoflurane (Attane; Minrad Inc, Orchard Park, NY) and 600 mL/min of oxygen in the induction chamber, the rats were placed in prone position, and a nonrebreathable facemask (half-open system) was applied with a flow of 3% to 6% isoflurane in 200 mL/min of oxygen. Using aseptic technique (shaving of the operative area and disinfection with betadine), a local anesthesia with Xylocaine Spray 10% (AstraZeneca, Zug, Switzerland) was administrated in the operative filed. A midsagittal incision was made through the skin and the periosteum, and the flaps were carefully lifted from the forehead to expose the calvarial bone.

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From the [a]Department of Cranio-Maxillofacial Surgery, Inselspital, University Hospital Bern, University of Bern, Switzerland; Departments of [b]Biomedical Engineering, and [c]Medicine, Columbia University, New York, NY; [d]Department of Histology, and [e]Advanced Research Center, The Nippon Dental University School of Life Dentistry Niigata, Japan; and [f]Division of Bone Diseases, Department of Internal Medicine Specialties, Faculty of Medicine, Geneva University Hospital, Geneva, Switzerland.
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Reprints: Nikola Saulacic, DDS, PhD, Department of Cranio-Maxillofacial Surgery, Inselspital, University Hospital Bern, University of Bern, CH-3010, Bern, Switzerland. E-mail: nikola.saulacic@insel.ch.

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Distraction device with a perforated plate was placed on the calvarial bone (Fig. 1). The periosteum was closed over the distraction plate with interruptive sutures. The skin was sutured over the periosteum. The pain level during surgery was determined by the observation of vital parameters and movement after pain stimulation. The perioperative dose of buprenorphine subcutaneous 0.1 mg/kg (Tamgesic; ESSEX Chemie, Luzern, Switzerland) was continued for 4 days postoperative.

The animals were randomly divided into experimental groups using the software package www.randomization.com to PDO group (n = 12) or periosteal pumping (PP) group (n = 12). All animals were allowed a latency period of seven days.

The distraction devices were manipulated at a rate of 0.1 mm/d for a total of 10 days in all animals. The standardized distraction protocol applied in PDO group included 1 activation per day, for a total of augmentation achieved of 1 mm. The same amount of activation in PP group was alternated by turning the distraction screw back at 0.1 mm/d every second day. Consequently, the distraction plate after pumping protocol remained in contact with the underlying bone. The animals were controlled daily during the first 3 weeks and afterward every second day up to the euthanasia on the health symptomatology using the standardized scoore sheets. The rats were euthanized at the end-distraction (day 17), midconsolidation to mid-consolidation (day 31), and end-consolidation period (day 45) by an overdose of gaseous carbon dioxide. The specimens were excised and processed for the histological and microcomputed tomography (μ-CT) analysis.

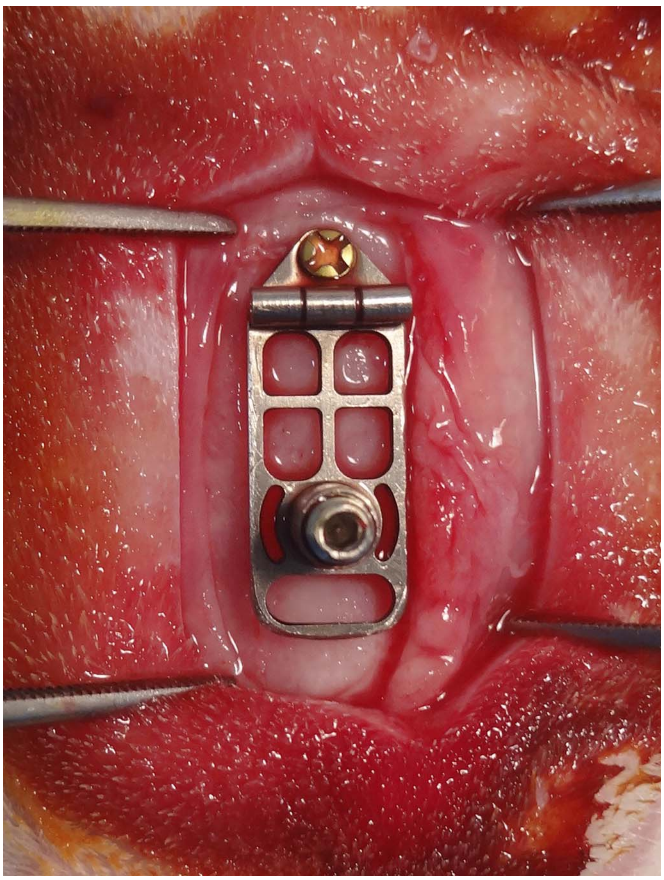

FIGURE 1. Intraoperative view of the area after flap elevation. The distraction device with a perforated plate was placed onto the calvarial bone.

Histological Analysis

The recovered samples were immediately immersed into 10% buffered formaldehyde/1% $CaCl_2$ for 48 hours. The specimens were processed for the production of undecalcified ground sections. The samples were rinsed in running tap water, dehydrated in ascending concentrations of ethanol (40%–100%) and xylol, and embedded in methylmethacrylate. From each sample, we prepared 10 tissue slices 1 mm apart from each other, using a low-speed diamond saw (Varicut VC-50; LECO, St. Joseph, MI). The embedded tissue blocks were cut along the axis of the distraction device into approximately 800-μm-thick sections. The sections were mounted onto acrylic glass slabs, grounded and polished to a final thickness of about 300 μm (Knuth-Rotor-3; Struers, Rodovre/Copenhagen, Denmark). The sections were stained with basic fuchsin and toluidine blue/McNeal and imaged using a Nikon DS-Ri1 digital camera connected to a Nikon Eclipse E800 microscope (Nikon Corporation, Tokyo, Japan).

μ-Ct Analysis

The distraction sites were subjected to radiography (25 kVP for 10 seconds) in 2 projections using a desktop Cone-Beam scanner (μCT 40; Scanco Medical AG, Brüttisellen, Switzerland). The X-ray source (E) was set at 70 kVp with 114 mA at high resolution (1000 projections/180°). An isotropic voxel size of 15 μm generated an image matrix of 2048 × 2048 pixels. Integration time was set on 3 seconds. The μ-CT slices (1000) were taken perpendicular to the saggital axis of the calvarium. Mineralized tissue was selected on the gray-scale images (0–1000) with the specific threshold set at 220, corresponding to the value of 530 mg HA/cm^3. Voxels above this value could be categorized as mineralized bone, background, or titanium. The new bone between the old bone surface and the distraction plate was selected manually. Bone volume (BV; mm^3) and bone mineral density (BMD; mg HA/mm^3) were determined by a blinded and calibrated examiner, using a computational program (Scanco Medical). The reconstructed 2-dimensional images were evaluated using 3D segmentation of volume of interest, gauss sigma at 0.8, and gauss support at 1.

Statistical Analysis

Differences between groups in BV and BMD were assessed using 1-way analysis of variance by univariate *t* test (Tukey test). Significance level of 0.05 was chosen in all tests. The statistical analysis was processed using SPSS for Windows (Release 19.0, standard version; IBM SPSS, Chicago, IL).

RESULTS

Clinical Observation

All animals recovered well and quickly from the surgery. One distraction device in the PP group (45 days) was exposed; despite the treatment using conservative measures, the device was lost. There were no major signs of infection or inflammation at the treated sites. Clinical examinations revealed an uneventful healing in all other animals with normal behavior, without impairment on their appereance, water and food intake.

Histological Analysis

The old calvarial bone was consisting of tabula externa and interna with intervening small marrow cavities. Formation of new bone in the distraction gap was observed in all animals. The type of new bone was always the primary woven bone at the leading edge of bone apposition. The bone height in the PDO group was generally greater than in the PP group. The thickness of this new bone was more asymmetrical in the PDO group and more equally distributed in the PP group. More dense bone was found as the bone matured over time.

17-Day Observation Period

The newly formed bone within the distraction gap consisted only of woven bone that contained some bone marrow cavities (Fig. 2). More bone formation was observed in higher part of distraction gap toward the plate perforations in the PDO group (Fig. 2A) than in the PP group (Fig. 2G). The gap between the new bone and the skin was filled with loose connective tissue that contained remnants of the coagulum. Signs of bone resorption and apposition were observed at sites where the tip of the screw touched the calvarial bone surface (Fig. 2I). A contiguous layer of new bone sporadically reinforced by parallel-fibered bone was observed on the top of the old bone outside the distraction plate (Figs. 2D, J). The surface contour of the newly formed bone was even and covered by a distinct periosteal layer.

31-Day Observation Period

The newly formed woven bone was reinforced by parallel-fibered bone in both groups (Fig. 3). In the PP group, new bone was seen penetrating through the perforation holes of the distraction plate (Fig. 3G). The new bone underneath the distraction plate contained large cavities with immature bone marrow (Figs. 3B, H). Osteoids were observed along the woven bone surface and lined bone marrow cavities. Outside the plate, the layer of new bone was comparatively thicker and contained small bone cavities (Figs. 3D, J). The newly formed bone was more mature toward the old calvarial bone and less mature toward the adjacent soft tissue. Fine orbicular structures indicating an intensive bone formation toward the skin was observed more in the PDO group than in the PP group.

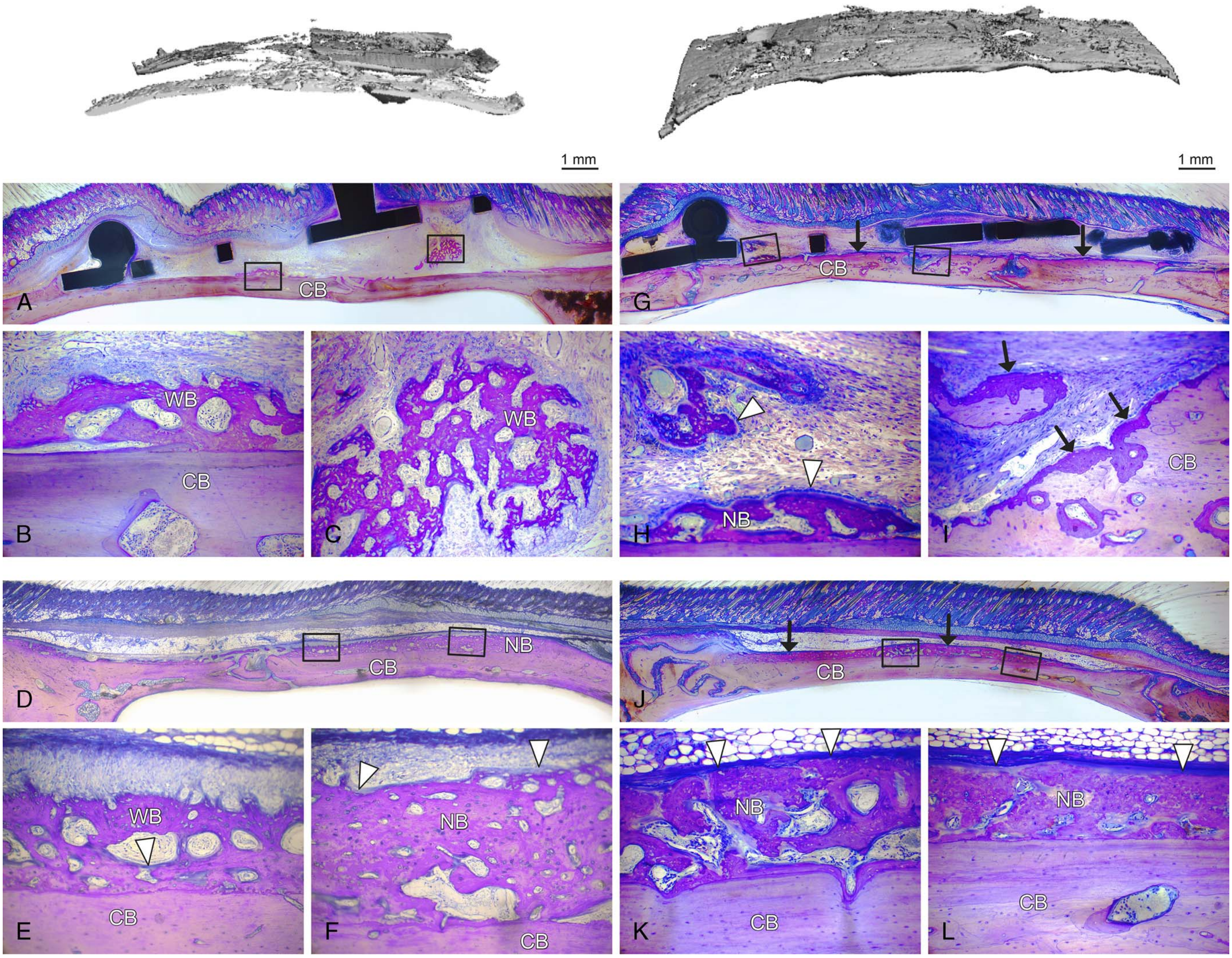

FIGURE 2. New bone formation at the 17-day time point. Microcomputed tomography images illustrate new bone in the PDO group (left) and PP group (right). Histological section of the midaxis of the distraction device in the PDO group (A) and next to the midaxis in the PP group (G) shows new bone formed on top of the old calvarial bone. Remnants of blood clots are evident within the loose connective tissue. High magnifications in (A) and (G) are outlined in (B, C) and (H, I), respectively. A fine trabecular network of the new woven bone is connected to the old bone (B) or seemingly isolated (C). H, Small areas of newly formed bone in the PP group are covered with the osteoid layer (arrowheads). I, Bone resorption and new bone formation (arrows) are observed next to the contact region between the distraction screw and the calvarial bone. A more contiguous layer of new bone is formed outside the distraction plate in the PDO group (D) and in PP group (J). Higher magnification of the boxed areas in (D) and (J) are enlarged in (E, F) and (K, L), respectively. An osteoid layer (arrowhead) indicative of ongoing bone formation is covering the surface of newly formed bone deposited on the calvarial bone. CB, calvarial bone; NB, new bone; WB, woven bone.

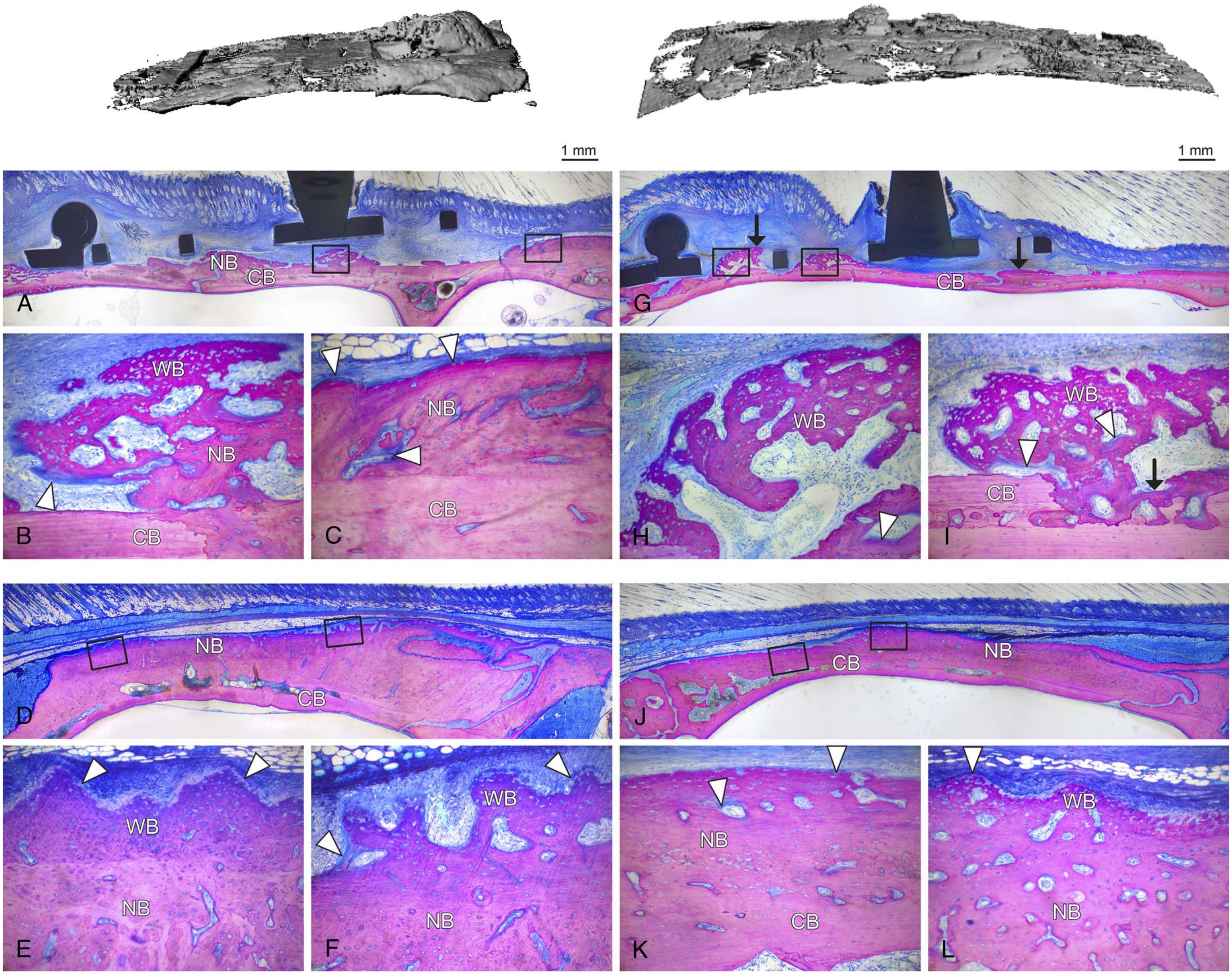

FIGURE 3. New bone formation at the 31-day time point. Microcomputed tomography images illustrate new bone in the PDO group (left) and PP group (right). Uneven formation of new bone is observed in the midaxis of the distraction device in the PDO group (A) and in the PP group (G). Higher magnification of the boxed areas in (A) and (G) show newly formed bone in (B, C) and (H, I), respectively. B, New woven bone is connected to the old bone lateral to the distraction screw. C, A contiguous layer of new bone outside the distraction gap is reinforced by parallel-fibered bone. New bone surface is covered with the osteoid layer (arrowheads). H, I, Woven bone with large cavities of immature bone marrow and osteoid linings (arrowheads) is extending into the plate perforations and rebuild previously resorbed calvarial bone (arrows). A thick layer of the new bone is deposited external to the distraction plate in the PDO group (D) and in PP group (J). High magnifications of images in (D) and (J) are shown in (E, F) and (K, L), respectively. A gradient of bone maturation between the mature bone formed toward the calvaria and trabecules of woven bone (arrowheads) toward the periosteum is evident more in the PDO group (E, F) than in the PP group (K, L). CB, calvarial bone; NB, new bone; WB, woven bone.

45-Day Observation Period

The features of bone formation in both groups (Fig. 4) were similar to those at the end of the 31-day observation period. New dense bone underneath the plate consisted mostly of parallel-fibered and lamellar bone with large cavities (Figs. 4B, H). The presence of bone marrow within new bone indicated bone maturation. Primary woven bone was still present at the leading edge of bone apposition, and more new bone was found on the sections outside the plate in both groups (Figs. 4D, J). The thickness of the new bone was greater in PDO group than in PP group, with orbicular bone structures facing the soft tissues (Figs. 4E, F). A uniform layer of dense new bone was found in the PP group. Several layers of new bone oriented parallel to each other were evident toward the periosteum (Figs. 4K, L). The surface contour of the newly formed bone was even, without signs of resorption.

μ-Ct Analysis

The BV values in both groups increased throughout the observation period (Table 1). Significant increase in BV in PDO group was observed at 45-day healing period compared with 17-day and 31-day healing periods ($P < 0.001$). Highest BV values at 45-day healing period were seen also in the PP group, being significantly different relatively to the 31-day healing period ($P = 0.018$). More new bone was found in the

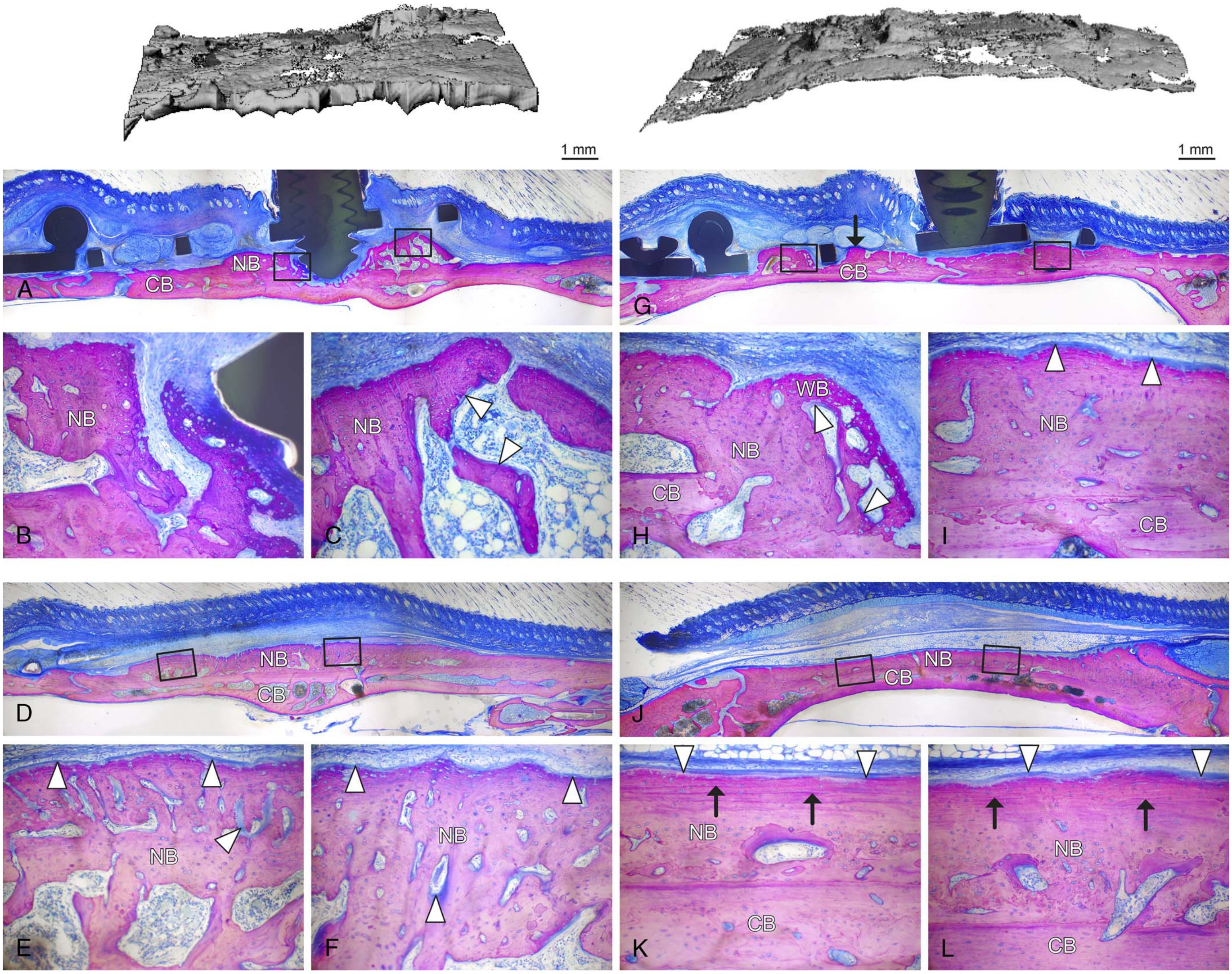

FIGURE 4. New bone formation at the 45-day time point. Microcomputed tomography images of the new bone in the PDO group (left) and PP group (right). Histological section in the midaxis of the distraction device shows newly formed bone on top of the old calvarial bone in the PDO group (A) and the PP group (G). Boxed areas in (A) and (G) are outlined in (B, C) and (H, I), respectively. Osteoid (arrowheads) lining large cavities with immature bone marrow (C, H) and covering the superior layer of new bone reinforced by parallel-fibered bone (I). A contiguous layer of the new bone seen laterally to the distraction plate is thicker in the PDO group (D) than in PP group (J). Higher magnification of the boxed area in (D) and (J) is enlarged in (E, F) and (K, L), respectively, showing new parallel-fibered and lamellar bone covered with the osteoid layer (arrowheads). E, F, Trabecules of woven bone are indicative of ongoing bone formation in the PDO group. K, L, Several layers of newly formed bone (arrows) oriented parallel to each other toward the osteoid layer are evident in the PP group. CB, calvarial bone; NB, new bone; WB, woven bone.

PDO than in the PP group at 45-day healing period ($P < 0.001$). The values of BMD in both groups remained relatively stable throughout the observation period (Table 1). Higher BMD values were observed in PP than in PDO group at the 17-day and 31-day, reaching the statistical significance at 31-day healing period ($P = 0.024$).

Discussion

The aim of the present study was to assess the possibility for inducing de novo bone formation by an alternated "pumping" protocol applied to calvarial periosteum in a rat model. The formation of new bone in the PP group increased throughout the observation period but remained lower than in the PDO group. Notably, this preliminary study shows that the original bone thickness may be doubled by pumping the periosteum without elevation of the distraction plate.

Periosteum is a natural scaffold and a source of cells and bioactive factors necessary for bone formation.[35] The role of the periosteum in bone biology has received renewed attention because of the data showing different regulation of periosteal and endosteal cells.[36] Periosteal activity is particularly important for the craniofacial region, because of the exclusively membranous apposition and the absence of cartilaginous growth.[37]

The staining detected the type of ossification at different states of bone maturity. The distinction between intramembranous and endochondral bone formation is more important for long bone distraction,[38] whereas gradual-distracted mandibles displayed direct intramembranous

TABLE 1. Volume and Density of New Bone for 2 Groups of Animals as Measured on the μ-CT

Healing Period	BV		BMD	
	PDO	PP	PDO	PP
17 d	12.81 ± 2.96	12.39 ± 4.64	875.30 ± 56.08	893.42 ± 24.85
31 d	13.07 ± 1.69*	11.06 ± 1.14	832.01 ± 47.69	932.02 ± 46.23*
45 d	30.11 ± 1.04†	17.52 ± 1.17	909.34 ± 33.35	897.66 ± 16.67

All values are expressed as mean ± SD.

*Statistically significant difference at $P < 0.05$.

†Statistically significant difference at $P < 0.001$.

ossification.[22] The signaling pathways involved in the periosteal-mediated regeneration of the craniofacial region may differ from those involved in long bone periosteal-mediated regeneration because the craniofacial periosteum is neural crest derived.[39]

Mechanical manipulation of the periosteum induced the bone formation in both groups at the site of its apposition from the existing bone surface. The newly formed bone resembled the original bone, corresponding to the previous findings at the extraoral sites.[25,28,30] The penetration of the newly formed bone in the PP group through the plate perforations was not unexpected. The overgrowth of the newly formed bone outside the distraction plate has been previously observed, and this overgrowth extended the amount of distraction performed and covered the distraction plate.[40,41]

Following a stimulus, the remaining cells divide and differentiate into osteoblasts to maintain a progenitor population.[42] Periosteal stem cells and osteoprogenitors particularly contribute to initial callus production through paracrine activation and recruitment of host cells.[43,44] Skeletal-lineage-specific stem or progenitor cells from lateral periosteum are responsible for massive tissue regeneration seen in DO.[22] The focal adhesion kinase-dependent, embryonic-like program reverts adult stem cells to a developmentally plastic state during DO, in contrast to the more restricted homeostatic program sufficient for the regeneration in fracture healing.

The contact between the periosteum and bone seems to be essential for its osteogenic capacity.[45,46] Actually, the special type of fibroblasts extend to the perforating fibers located inside the bone matrix.[47] These fibers provide resistance to pulling and transmit mechanical stress directly to the bone. Osteoblasts exposed to different stresses (ie, distraction vs compression) may activate distinct pathways and ultimately result in proliferation and differentiation profiles unique to the type of stress applied.[48]

To the best of our knowledge, the pumping protocol was for the first time applied in the periosteal distraction as described in this study. Increase in BV up to 45-day observation period indicates the potential of PP. One of the limitations of this study is the absence of the negative control group, without plate activation. This group was not included, as displacement of the distraction device was frequently observed in animals without activation (unpublished results). Further modifications are needed to increase the stability of the distraction device.

The same rate and duration of the distraction in the 2 groups were applied to evaluate the process of bone formation at a given time point. The apposition of the new bone in layers may have been induced by the alternated protocol of distraction. Of note, an ongoing woven bone formation in PDO group has continued once the activation ceased.[30] Besides the mode of periosteal manipulation, the main difference between PDO and PP groups was the size of the distraction gap. Histomorphometry would be advantageous to assess the area of osteoid and mineralized bones within the region of interest and extend the results of μ-CT evaluation. Enhanced bone formation in the PDO group compared with the PP group was confirmed by a significant increase in the BV at 45-day healing period. Lower distance between the periosteum and the original bone in the PP group appeared to advance bone mineralization. Higher values of BMD for the PP group than the PDO group were expected according to the histological findings, but the statistical significance was confirmed only at the 31-day healing period. It is possible that the BV values were underestimated on the μ-CT images by removal of the inherent halation artifacts. The use of more sophisticated procedures at the molecular level will be needed in future studies to detect subtle differences in bone formation.[49]

The overall impact of the applied PP protocol of distraction at the given time point was moderate and was dominated by the total amount of distraction plate elevation performed. Significant increase in bone formation in the PDO group compared with the PP group thus confirmed the relevance of the preserved space provided by the device. From the clinical perspective, the size of distraction gap is of primary importance. Nevertheless, the PP protocol leads to more progressive soft tissue adaptation than the PDO. This might be beneficial for intraoral applications, where the risk of wound dehiscence and device exposure should be carefully considered. At the next step, the results of the present study should be translated to a more clinically relevant large animal model.

CONCLUSIONS

Data collected in this study demonstrated that an alternated protocol of periosteal distraction promotes de novo bone formation form the original bone surface, without elevation of the distraction plate. Gradual elevation of periosteum from the original bone surface was beneficial in terms of new BV. Future studies should explore the optimal moment, amplitude, and period of relaxation throughout the pumping protocol of periosteal distraction.

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REFERENCES

1. Ilizarov GA. The tension-stress effect on the genesis and growth of tissues: part II. The influence of the rate and frequency of distraction. *Clin Orthop Relat Res*. 1989;263–285.
2. Ilizarov GA. Clinical application of the tension-stress effect for limb lengthening. *Clin Orthop Relat Res*. 1990;8–26.
3. Mori S, Akagi M, Kikuyama A, et al. Axial shortening during distraction osteogenesis leads to enhanced bone formation in a rabbit model through the HIF-1alpha/vascular endothelial growth factor system. *J Orthop Res*. 2006;24:653–663.
4. Kim UK, Chung IK, Lee KH, et al. Bone regeneration in mandibular distraction osteogenesis combined with compression stimulation. *J Oral Maxillofac Surg*. 2006;64:1498–1505.
5. Kim UK, Park SJ, Seong WJ, et al. Expression of TGF-beta1, osteonectin, and BMP-4 in mandibular distraction osteogenesis with compression stimulation: reverse transcriptase-polymerase chain reaction study and biomechanical test. *J Oral Maxillofac Surg*. 2010;68:2076–2084.
6. Hente R, Fuchtmeier B, Schlegel U, et al. The influence of cyclic compression and distraction on the healing of experimental tibial fractures. *J Orthop Res*. 2004;22:709–715.
7. Claes L, Augat P, Schorlemmer S, et al. Temporary distraction and compression of a diaphyseal osteotomy accelerates bone healing. *J Orthop Res*. 2008;26:772–777.
8. Grunwald L, Dobele S, Hontzsch D, et al. Callus massage after distraction osteogenesis using the concept of lengthening then dynamic plating. *Strategies Trauma Limb Reconstr*. 2015;10:129–135.
9. Tsuchiya H, Tomita K, Minematsu K, et al. Limb salvage using distraction osteogenesis. A classification of the technique. *J Bone Joint Surg Br*. 1997;79:403–411.

10. Iacobellis C, Berizzi A, Aldegheri R. Bone transport using the Ilizarov method: a review of complications in 100 consecutive cases. *Strategies Trauma Limb Reconstr*. 2010;5:17–22.
11. Mofid MM, Inoue N, Atabey A, et al. Callus stimulation in distraction osteogenesis. *Plast Reconstr Surg*. 2002;109:1621–1629.
12. Makhdom AM, Hamdy RC. The role of growth factors on acceleration of bone regeneration during distraction osteogenesis. *Tissue Eng Part B Rev*. 2013;19: 442–453.
13. Ozgul S, Akdeniz ZD, Celebiler O, et al. The effect of 2 different distraction-compression models on new bone generation. *J Oral Maxillofac Surg*. 2012;70: e490–e499.
14. Krishnan A, Pamecha C, Patwa JJ. Modified Ilizarov technique for infected non-union of the femur: the principle of distraction-compression osteogenesis. *J Orthop Surg (Hong Kong)*. 2006;14:265–272.
15. Lazar FC, Klesper B, Carls P, et al. Callusmassage. A new treatment modality for non-unions of the irradiated mandible. *Int J Oral Maxillofac Surg*. 2005;34: 202–207.
16. Greenwald JA, Luchs JS, Mehrara BJ, et al. "pumping the regenerate": an evaluation of oscillating distraction osteogenesis in the rodent mandible. *Ann Plast Surg*. 2000;44:516–521.
17. Alzahrani MM, Anam E, AlQahtani SM, et al. Strategies of enhancing bone regenerate formation in distraction osteogenesis. *Connect Tissue Res*. 2018;59: 1–11.
18. Kojimoto H, Yasui N, Goto T, et al. Bone lengthening in rabbits by callus distraction. The role of periosteum and endosteum. *J Bone Joint Surg Br*. 1988;70: 543–549.
19. Yasui N, Kojimoto H, Shimizu H, et al. The effect of distraction upon bone, muscle, and periosteum. *Orthop Clin North Am*. 1991;22:563–567.
20. Sun Z, Herring SW. The effect of periosteal injury and masticatory micromovement on the healing of a mandibular distraction osteogenesis site. *Arch Oral Biol*. 2009; 54:205–215.
21. Takeuchi S, Matsuo A, Chiba H. Beneficial role of periosteum in distraction osteogenesis of mandible: its preservation prevents the external bone resorption. *Tohoku J Exp Med*. 2010;220:67–75.
22. Ransom RC, Carter AC, Salhotra A, et al. Mechanoresponsive stem cells acquire neural crest fate in jaw regeneration. *Nature*. 2018;563:514–521.
23. Schmidt BL, Kung L, Jones C, et al. Induced osteogenesis by periosteal distraction. *J Oral Maxillofac Surg*. 2002;60:1170–1175.
24. Sencimen M, Aydintug YS, Ortakoglu K, et al. Histomorphometrical analysis of new bone obtained by distraction osteogenesis and osteogenesis by periosteal distraction in rabbits. *Int J Oral Maxillofac Surg*. 2007;36:235–242.
25. Oda T, Kinoshita K, Ueda M. Effects of cortical bone perforation on periosteal distraction: an experimental study in the rabbit mandible. *J Oral Maxillofac Surg*. 2009;67:1478–1485.
26. Sato K, Haruyama N, Shimizu Y, et al. Osteogenesis by gradually expanding the interface between bone surface and periosteum enhanced by bone marrow stem cell administration in rabbits. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2010;110:32–40.
27. Zakaria O, Kon K, Kasugai S. Evaluation of a biodegradable novel periosteal distractor. *J Biomed Mater Res B Appl Biomater*. 2012;100:882–889.
28. Yamauchi K, Takahashi T, Tanaka K, et al. Self-activated mesh device using shape memory alloy for periosteal expansion osteogenesis. *J Biomed Mater Res B Appl Biomater*. 2013;101:736–742.
29. Saulacic N, Schaller B, Iizuka T, et al. Analysis of new bone formation induced by periosteal distraction in a rat calvarium model. *Clin Implant Dent Relat Res*. 2013; 15:283–291.
30. Saulacic N, Nakahara K, Iizuka T, et al. Comparison of two protocols of periosteal distraction osteogenesis in a rabbit calvaria model. *J Biomed Mater Res B Appl Biomater*. 2016;104:1121–1131.
31. Claes L, Veeser A, Gockelmann M, et al. A novel method for lateral callus distraction and its importance for the mechano-biology of bone formation. *Bone*. 2010; 47:712–717.
32. Lethaus B, Tudor C, Bumiller L, et al. Guided bone regeneration: dynamic procedures versus static shielding in an animal model. *J Biomed Mater Res B Appl Biomater*. 2010;95:126–130.
33. Nakahara K, Haga-Tsujimura M, Sawada K, et al. Periosteal distraction osteogenesis versus immediate periosteal elevation in a rat model: histological and micro-CT analysis. *J Craniomaxillofac Surg*. 2017;45:620–627.
34. Nakahara K, Haga-Tsujimura M, Iizuka T, et al. Periosteum-induced bone formation by distraction osteogenesis: histologic and microcomputed tomography analysis. *Int J Oral Maxillofac Implants*. 2016;31:785–792.
35. Allen MR, Hock JM, Burr DB. Periosteum: biology, regulation, and response to osteoporosis therapies. *Bone*. 2004;35:1003–1012.
36. Colnot C. Skeletal cell fate decisions within periosteum and bone marrow during bone regeneration. *J Bone Miner Res*. 2009;24:274–282.
37. Lin Z, Fateh A, Salem DM, et al. Periosteum: biology and applications in craniofacial bone regeneration. *J Dent Res*. 2014;93:109–116.
38. Fink B, Pollnau C, Vogel M, et al. Histomorphometry of distraction osteogenesis during experimental tibial lengthening. *J Orthop Trauma*. 2003;17:113–118.
39. Leucht P, Kim JB, Amasha R, et al. Embryonic origin and Hox status determine progenitor cell fate during adult bone regeneration. *Development*. 2008;135: 2845–2854.
40. Saulacic N, Hug C, Bosshardt DD, et al. Relative contributions of osteogenic tissues to new bone formation in periosteal distraction osteogenesis: histological and histomorphometrical evaluation in a rat calvaria. *Clin Implant Dent Relat Res*. 2013;15:692–706.
41. Saulacic N, Schaller B, Bosshardt DD, et al. Periosteal distraction osteogenesis and barrier membrane application: an experimental study in the rat calvaria. *J Periodontol*. 2012;83:757–765.
42. McBride SH, Falls T, Knothe Tate ML. Modulation of stem cell shape and fate B: mechanical modulation of cell shape and gene expression. *Tissue Eng Part A*. 2008;14:1573–1580.
43. Chen L, Tredget EE, Wu PY, et al. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. *PLoS One*. 2008;3:e1886.
44. Zhang X, Xie C, Lin AS, et al. Periosteal progenitor cell fate in segmental cortical bone graft transplantations: implications for functional tissue engineering. *J Bone Miner Res*. 2005;20:2124–2137.
45. Canalis RF, Burstein FD. Osteogenesis in vascularized periosteum. Interactions with underlying bone. *Arch Otolaryngol*. 1985;111:511–516.
46. Kostopoulos L, Karring T. Role of periosteum in the formation of jaw bone. An experiment in the rat. *J Clin Periodontol*. 1995;22:247–254.
47. Hirashima S, Ohta K, Kanazawa T, et al. Anchoring structure of the calvarial periosteum revealed by focused ion beam/scanning electron microscope tomography. *Sci Rep*. 2015;5:17511.
48. Gabbay JS, Zuk PA, Tahernia A, et al. In vitro microdistraction of preosteoblasts: distraction promotes proliferation and oscillation promotes differentiation. *Tissue Eng*. 2006;12:3055–3065.
49. Shah HN, Jones RE, Borrelli MR, et al. Craniofacial and long bone development in the context of distraction osteogenesis. *Plast Reconstr Surg*. 2021;147:54e–65e.