Relative Contributions of Osteogenic Tissues to New Bone Formation in Periosteal Distraction Osteogenesis: Histological and Histomorphometrical Evaluation in a Rat Calvaria

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ABSTRACT

Background: The relative contributions of different, potential factors to new bone formation in periosteal distraction osteogenesis are unknown.

Purpose: The aim of the present study was to assess the influence of original bone and periosteum on bone formation during periosteal distraction osteogenesis in a rat calvarial model by means of histology and histomorphometry.

Methods: A total of 48 rats were used for the experiment. The contribution of the periosteum was assessed by either intact or incised periosteum or an occlusive versus a perforated distraction plate. The cortical bone was either left intact or perforated. Animals were divided in eight experimental groups considering the three possible treatment modalities. All animals were subjected to a 7-day latency period, a 10-day distraction period and a 7-day consolidation period. The newly formed bone was analyzed histologically and histomorphometrically.

Results: New, mainly woven bone was found in all groups. Differences in the maximum height of new bone were observed and depended on location. Under the distraction plate, statistically significant differences in maximum bone height were found between the group with perforations in both cortical bone and distraction plate and the group without such perforations.

Conclusions: If the marrow cavities were not opened, the contribution to new bone formation was dominant from the periosteum. If the bone perforations opened the marrow cavities, a significant contribution to new bone formation originated from the native bone.

KEY WORDS: animal study, calvarial bone, distraction osteogenesis, periosteum, rat

INTRODUCTION

The distraction osteogenesis (DO) technique is a progressive elongation of the bone fragment within the space created by osteotomy that results with the formation of hard and soft tissues.[1,2] The technique was subsequently recommended as a predictable, efficient treatment of vertically deficient alveolar ridges.[3–5] Still, the unfavorable anatomical conditions might present

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limitations for a DO procedure.[6,7] It has been even suggested that for vertical ridge augmentation, alveolar DO may be more prone to minor complications than the inlay procedure.[8]

Periosteal distraction osteogenesis (PDO) is a unique method for engineering endogenous hard tissue, which avoids bone harvesting and resolves the problem of tissue coverage by promoting a progressive process of soft tissue adaptation. It is thus simpler to perform and less invasive than other bone augmentation techniques. Several experimental studies have demonstrated the possibilities of new bone formation induced by PDO.[9–15] Various results were obtained using different distraction devices with different treatment parameters. In comparison between DO and PDO on the lateral mandible in rabbits, Sencimen and colleagues[16] found significant differences in the extent of newly formed bone and interstitial fatty tissue. The authors pointed to three vertical incisions that negatively influenced the osteogenic activity of the periosteum and suggested decortication of the underlying bone to stimulate the maturation of woven bone.

In a recent comparison of PDO in the mandible of rabbits with and without decortications at the 8-week consolidation period, Oda and colleagues[17] observed newly formed bone underneath the distraction mesh in both groups. The regenerated tissue was, however, more calcified in the decortication group. Aside from one study where the original bone was perforated,[11] it was left intact in other studies.[9,10,12–16]

Perforations of the cortical bone in the rat calvaria significantly enhanced the incorporation of bone grafts[18] and stimulated the bone generation in the experimental model of guided bone regeneration.[19] Nevertheless, the periosteum of adult animals that has been immediately elevated did not seem to contribute to the supraosteal bone formation.[20,21] In contrast to the bone grafting techniques, the periosteum in PDO is gradually elevated from the original bone surface. Apart from the influence of the osteogenic cells from the bone marrow,[17] the bone formation from the periosteum in PDO has not been evaluated in histological and histomorphometrical studies.

When the periosteum is injured, callus formation in DO is markedly disturbed and insufficient from the onset of the distraction process.[22,23] The influence of the periosteum to new bone formation in PDO was evaluated by immediate and intermittent forces. Significantly more bone marrow and osteoid was found through gradual distraction than through immediate elevation of the periosteum at the medial aspect of sheep tibia,[24] but no major differences were found between the groups on the calvaria of minipigs.[25]

The purpose of the present study was to investigate the mechanism of bone formation by PDO in a calvarium model of rats. The specific aim of the study was the histomorphometrical evaluation of the relative contribution of two osteogenic tissues on new bone formation in PDO.

MATERIALS AND METHODS

Animals and Surgery

PDO was performed on the calvarial bone of 48 male, adult Wistar rats approximately 400 g in weight. The rats were housed in compliance with the Swiss guidelines for animal experimentation. The rats were held in individually aired cages, in a room with an adjusted climate (temperature 22–24°C ± 2°C, humidity 30–60% ± 5%), and special sun substitution ultraviolet light (photoperiod 6–18 hours), without excessive or surprising noises. The protocol was approved by the Committee for Animal Research, State of Bern, Switzerland (Approval no. 96/09).

Surgery was performed under conditions of sevoflurane – induced general anesthesia (Sevoflurane 8%; Oxygen 600 ml/min) by nonrebreather mask. Before surgical procedure, a local anesthesia was performed with Mepivacaine (10 mg/ml) and Adrenaline (1:200,000). Buprenorphin s.c. (0.1 mg/kg) is applied for the pain control. Using an aseptic technique (shaving of the operative area and disinfection with betadine), a midsagittal incision through the skin and the periosteum was made. The skin was reflected and the periosteal flap carefully elevated from the midline laterally (Figure 1a).

To access the influence of bone marrow to bone formation, the original bone surface was perforated lateral to the saggital suture (Figure 1b). Participation of the apical periosteum was excluded by using an occlusive distraction plate (Figure 1c) or allowed by perforation of the distraction plate (Figure 1d). The lateral periosteum was left intact or incised along the lateral edge of the distraction plate from the hinge distally.

The animals were divided in eight groups with six rats each, established to assess the combined effects of

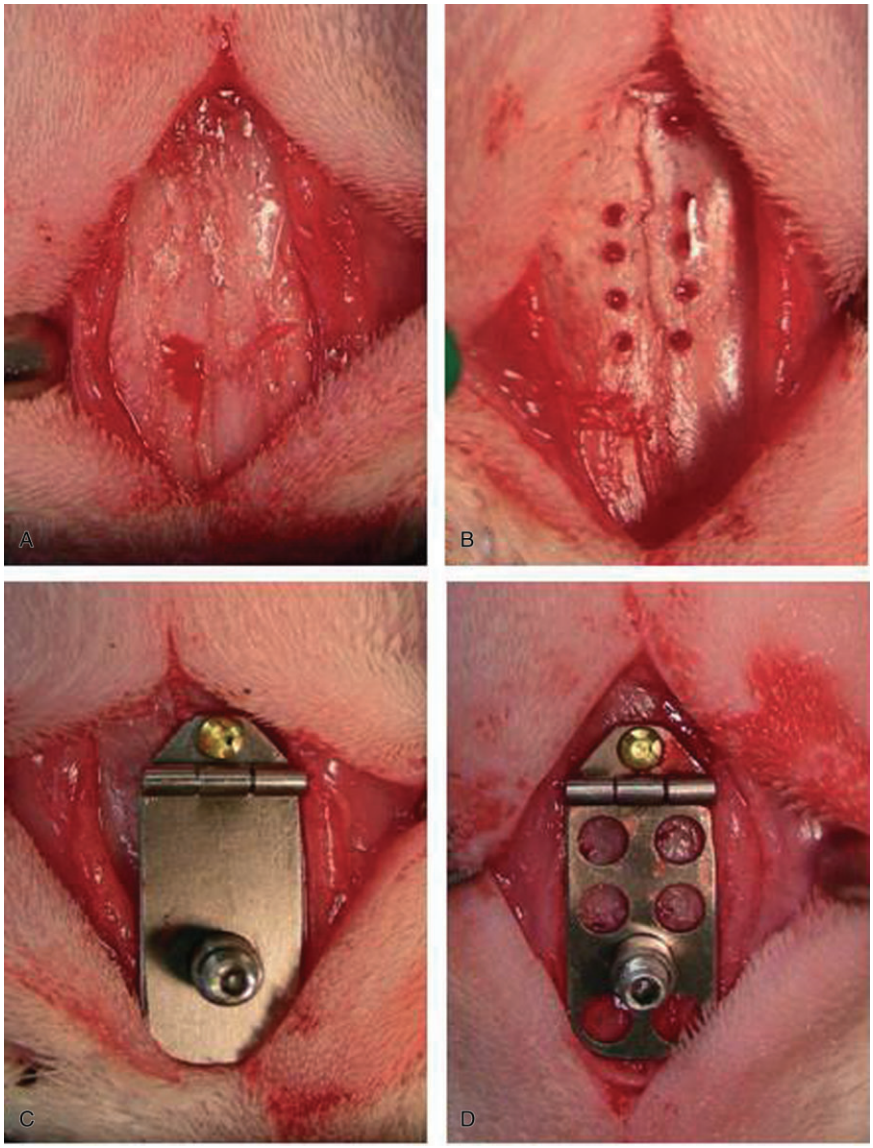

Figure 1 Intraoperative view of the area following (a) flap elevation and (b) perforation of calvarial bone. Placement of distraction device with (c) occlusive and (d) perforated distraction plate.

native bone and periosteum (Table 1). In group I, the entire surface of the bone base was kept intact, lateral periosteum incised and occlusive distraction plate used. In group II, the bone base was perforated, lateral periosteum left intact and distraction plate with perforations used to permit activity of osteogenic tissues. In group III, the bone base was perforated, whereas the lateral periosteum was incised and participation of the apical periosteal excluded. In group IV, the bone base was kept intact and the participation of the apical periosteal excluded; the lateral periosteum served as the source of osteogenic activity. In group V, the bone base was left intact and the lateral periosteum incised; the apical periosteal served as the source of osteogenic

TABLE 1 Treatment Protocol for Eight Groups with Six Rats Each Submitted to Periosteal Distraction

Group	Bone Base	Lateral Walls of Periosteum	Apical Covering of Periosteum
I	–	–	–
II	+	+	+
III	+	–	–
IV	–	+	–
V	–	–	+
VI	–	+	+
VII	+	–	+
VIII	+	+	–

Bone base: + = perforated, – = intact; Lateral walls of periosteum: + = intact, – = incised; Apical covering of periosteum: + = involved, – = excluded.

activity. In group VI, the bone base was left intact; the lateral and the apical periosteum served as the sources of osteogenic activity. In group VII, the bone base and the apical periosteum served as the sources of osteogenic activity; the lateral periosteum was incised. In group VIII, the participation of the apical periosteum was excluded; the bone base and the lateral periosteum served as the sources of osteogenic activity. According to this experimental design, the contribution of each layer alone and in combination with one another was assessed.

All animals were subjected to the same distraction protocol: a latency period of 7 days and distraction at 0.2 mm per 24 hours for 10 days. After 7 days of consolidation period, the animals were killed by placement in an empty Plexiglas box and administration of an overdose of gaseous carbon dioxide. The specimens were excised and processed for the histological analysis.

Histological and Histomorphometric Analysis

The calvarium of rats was block-resected using an oscillating autopsy saw. The recovered segments were immediately immersed in a solution of 4% buffered formaldehyde combined with 1% $CaCl_2$ for at least 48 hours at ambient temperature. The specimens were processed for the production of undecalcified ground sections as described previously.[26] Briefly, the samples were rinsed in running tap water, dehydrated in ascending concentrations of ethanol and embedded in methylmethacrylate. Ten tissue slices, 1 mm apart from each other, were prepared from each sample using a slow-speed diamond saw (Varicut® VC-50, Leco, Munich, Germany). The embedded tissue blocks were cut along the axis of the distraction device into approximately 400-µm-thick ground section. After mounting the sections onto acrylic glass slabs, they were ground and polished to a final thickness of about 100 µm (Knuth-Rotor-3, Struers, Rodovre/Copenhagen, Denmark) and surface stained with basic fuchsin and toluidine blue/McNeal. Digital photography was performed using a ProgRes® C5 digital camera (Jenoptik Laser, Optik, Systeme GmbH, Jena, Germany) connected to a Zeiss Axioplan microscope (Carl Zeiss, Göttingen, Germany).

All available ground sections were used for histomorphometric measurements of the maximum bone thickness at various regions. Measurements were made underneath and peripheral to the distraction plate.

Statistics

Statistical evaluation was performed using SPSS for Windows Version 15.0 (SPSS Inc., Chicago, IL, USA). The difference between the groups with regard to mean maximal bone height were analyzed using a multivariate *t*-test (Turkey's test). The mean values between the groups were compared simultaneously at the considered location. In statistical tests, a significance level of .05 was chosen.

RESULTS

Qualitative Histological Analysis

Three rats were excluded from the study, because of infection in one animal (group I) and exposure and loss of the distraction device during the consolidation period in three other animals (groups III, VI, VIII). Healing was uneventful in the remaining 44 rats, which showed a normal behavior throughout the experimental period. There were histological signs of minute exposure of the distraction devices in 5 rats (groups I, 2 × III, VI, VIII), without obvious signs of inflammation. The old bone consisting of a tabula externa and a tabula interna with intervening small marrow cavities was recognizable histologically. The bone marrow was absent, if the calvarium was thinner than 0.5 mm.

Within each group, except for group IV, there were no major differences observed between animals. Inspection of all ground sections revealed that it was necessary to make a distinction between three sagital regions (Figure 2): (R1) in the midaxis under the distraction plate (R2) at the periphery of but still under the distraction plate and (R3) outside the distraction plate. Each region was represented by two ground sections. The data for regions R2 and R3 from both sides were pooled for a given region. The regions R1 and R2 were further divided into 3 subregions: (a) lower part of the distraction gap (<1 mm of distraction); (b) higher part of distraction gap (1–2 mm of distraction); and (c) outside, distally from the distraction plate. The data for subregion c for regions R1 and R2 were pooled. The excluded animals were counted as drop-outs.

Newly formed bone formation in the distraction gap was observed in all animals. However, the thickness of this new bone varied between subregions and between some of the groups (see morphometric analysis). The basic morphologic pattern of new bone formation was comparable between groups and subregions.

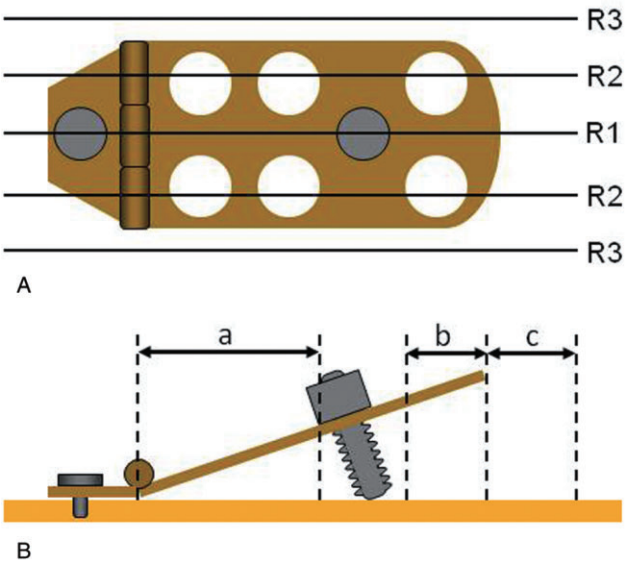

Figure 2 Schematic drawings of the distraction device and regions (a) and subregions (b) for the histological and histomorphometrical analysis. Region R1 is through the midaxis of the distraction plate, region R2 is at the lateral border but still under the distraction plate and region R3 outside the distraction device. Regions R1 and R2 are divided in subregions in the lower part of the distraction gap (a), higher part of distraction gap (b) and outside the distraction plate (c).

The type of new bone was always woven bone. While this new woven bone was more mature towards the old calvarial bone, it was less mature towards the adjacent soft tissue and ongoing bone formation was a common finding at this site. Both bone resorption and bone apposition were usually observed at sites were the tip of the screw touched the bone surface.

Group I (Bone-Intact, Periosteum-Incised, Plate-Occlusive)

In region R1, new bone was observed in subregion R1a towards the hinge of the device and in subregion R1b distally from the distraction screw (Figure 3a). The thickness of bone in subregion R1b was always greater than in subregion R1a. The newly formed bone consisted of woven bone reinforced by parallel-fibered bone and contained some bone marrow cavities. In the central region around the distraction screw, signs of both bone resorption and bone apposition were observed. At the periphery of the distraction plate (region R2), a thick layer of new bone was observed in subregions R2a and R2b (Figure 3b). The new bone contained large cavities of immature bone marrow. More bone formation was found on the sections outside the plate (region R3) with the greatest thickness observed towards the hinge (Figure 3c). The thickness of the new bone layer at this site was about three times greater than that of the old calvarial bone. The layer of new bone was more mature towards the calvarial surface than towards the skin (Figure 4a), where many osteoblasts and osteoid indicated ongoing bone formation (Figure 4b).

Group II (Bone-Perforated, Periosteum-Intact, Plate-Perforated)

In region R1, new bone formation was observed in subregion R1a at bone perforation sites and in subregion R1b (Figure 5a). More bone formation was observed in cases where the perforated bone possessed large bone marrow cavities (Figure 5b). The distraction gap between new bone and the plate was occupied by a loose connective tissue that contained remnants of the coagulum. In regions R2 and R3, new bone was observed all along the original bone surface (Figure 5, c and d).

Group III (Bone-Perforated, Periosteum-Incised, Plate-Occlusive)

Little new bone formation was found in region R1, when the perforations in the old bone coincided with the absence of bone marrow (Figure 6a). The space between the distraction plate and bone was occupied by a highly vascularized loose connective tissue. Some bone formation was found distally, in subregion R1c (not shown). A contiguous layer of new bone was observed on top of the old bone at region R2 (Figure 6b) and in region R3 (Figure 6c).

Group IV (Bone-Intact, Periosteum-Intact, Plate-Occlusive)

There was almost no new bone formation observed in subregion R1a (Figure 7a). In contrast, a thick layer of new bone was observed in subregion R1b and extended distally into subregion R1c. In region R2, a layer of new bone with almost uniform thickness was found (Figure 7b). An exceptionally thick layer of new bone was consistently observed in region R3, outside the distraction plate (Figure 7c). A clear gradient of bone maturation was evident. While the new bone was more mature towards the calvarium, elongated trabecules of woven bone oriented parallel to the distraction vector

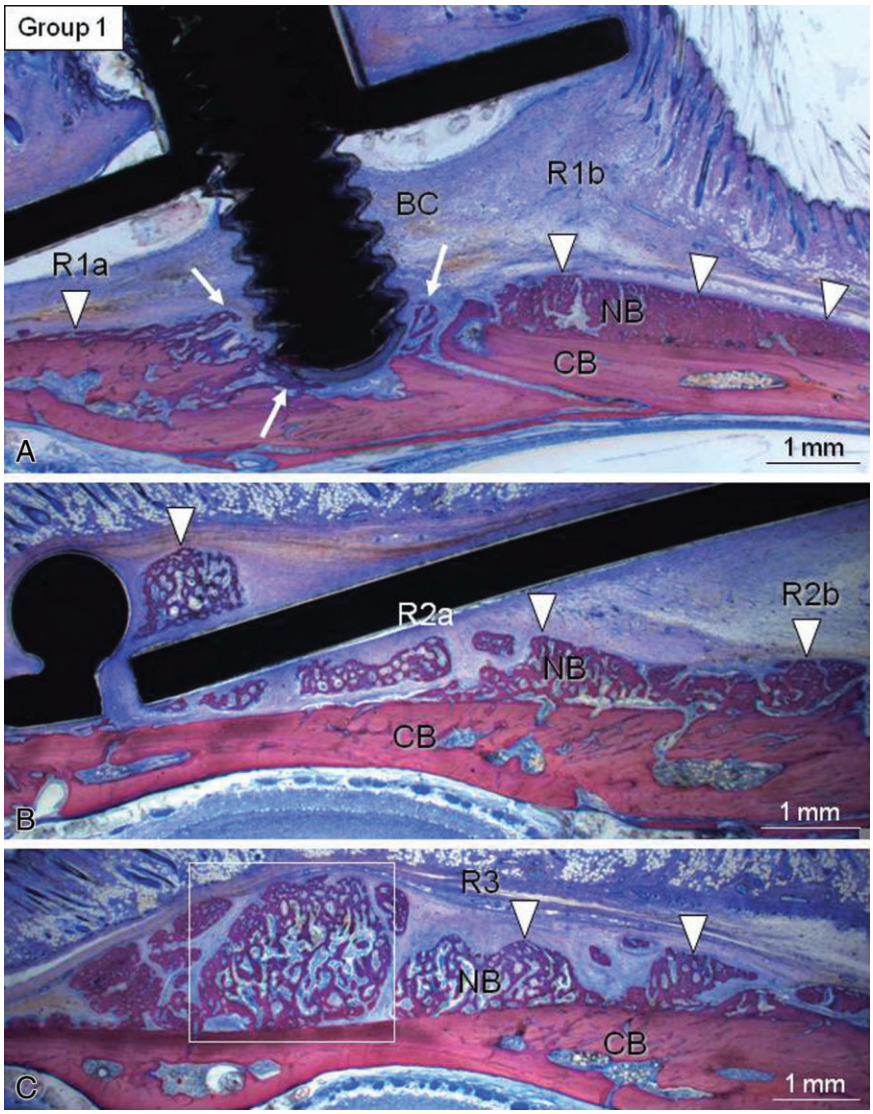

Figure 3 *A*, Midaxis section of region R1. Bone resorption and little new bone formation (arrows) is evident where the screw is in contact with the calvarial bone (CB). Right adjacent to the resorption site, new bone (NB; arrowheads) is formed on top of the old bone. Remnants of the blood clot (BC) are evident between the new bone and the distraction plate. *B*, In region R2, new bone formation is observed all along the old bone surface. *C*, Most new bone is found outside of distraction plate in region R3 with the greatest thickness observed close to the hinge. The thickness at this site is up to three times greater then the thickness of the original calvarial bone.

were indicative of ongoing bone formation (Figure 7, c and d).

Group V (Bone-Intact, Periosteum-Incised, Plate-Perforated)

In the midaxis of the distraction device, there was more new bone in subregion R1b than in R1a (Figure 8a). In region R2, new bone was found in both subregions R2a (Figure 8b) and R2b (Figure 8c). In subregion R2a, the new bone was even seen penetrating through the perforation holes of the distraction plate. Outside the distraction device in region R3, the layer of new bone was slightly thicker than that underneath the plate in region R2 (Figure 8d).

Group VI (Bone-Intact, Periosteum-Intact, Plate-Perforated)

In the midaxis section of the distraction device, more new bone was found in subregion R1b than in subregion R1a (Figure 9, a and b). In region R2, the difference in the height of new bone between subregions b and a was much more pronounced than in region R1 (Figure 9c).

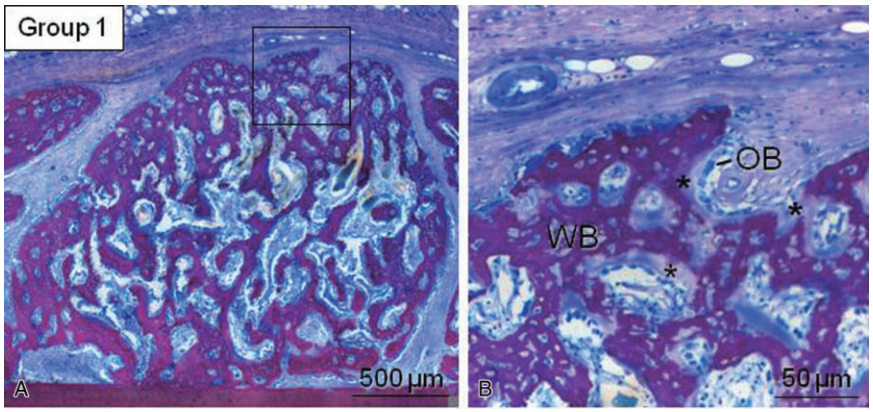

Figure 4 *A*, High magnification of the area outlined in Figure 3c. The boxed area in (a) is enlarged in (b). Towards the skin, a fine trabecular network of woven bone (WB) is indicative of ongoing bone formation. While this new bone was more mature close to the old calvarium bone, new bone formation is still ongoing at the periphery. Osteoid (*) and osteoblasts (OB) are clearly visible at the leading front of bone formation.

Outside the distraction plate in region R3, there was a moderately thick and uniform layer of new bone observed (Figure 9d).

Group VII (Bone-Perforated, Periosteum-Incised, Plate-Perforated)

In the midaxis of the distraction device, there was almost the same height of new bone in subregions R1a and R1b (Figure 10a). Instead of a bone layer of uniform thickness, a patch-wise deposition of bone corresponding to the bone perforations was observed. New bone was associated with a bone marrow (Figure 10b). In region R2, new bone formation was observed on the old calvarial bone and the thickness of this new bone was greater in subregion R2a than in subregion R2b (Figure 10c). An almost uniform layer of new bone was observed outside of the distraction plate in region R3 (Figure 10d).

Group VIII (Bone-Perforated, Periosteum-Intact, Plate-Occlusive)

In the midaxis of the distraction device, new bone was observed on top of the calvarial bone (Figure 11a). Most new bone was present at the perforation sites of the calvarial bone (Figure 11b). In region R2, new bone was slightly ticker in subregion R2a (Figure 11, c and d) than

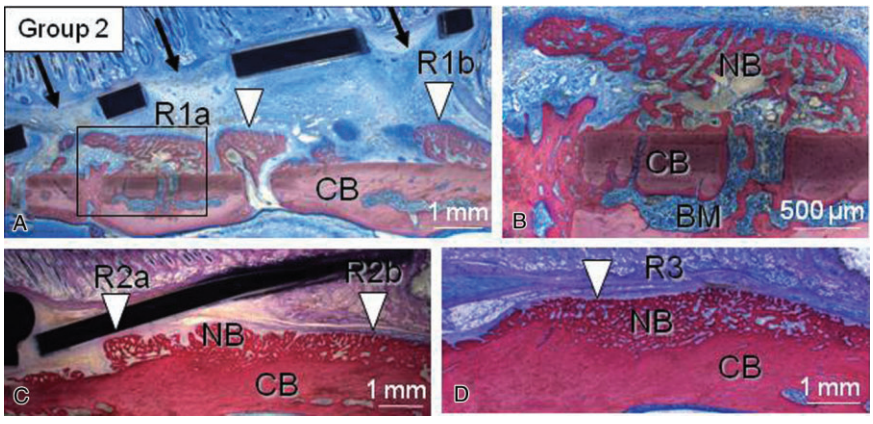

Figure 5 *A*, Midaxis section of region R1. Perforations (arrows) are seen in the distraction plate and in the calvarial bone (CB) in subregion R1a. In subregions R1a and R1b, new bone (arrowheads) is observed on top of the calvarial bone. Loose connective tissue and remnants of the blood clot are seen between new bone and the distraction device. *B*, High magnification of the area outlined in (a). New bone (NB) seems to have emerged from the cavities of bone marrow (BM) at the bone perforations. *C*, In region R2, new bone has formed all along the surface of the calvarial bone. *d*, Likewise, a contiguous layer of new bone is also present all along the calvarium in region R3.

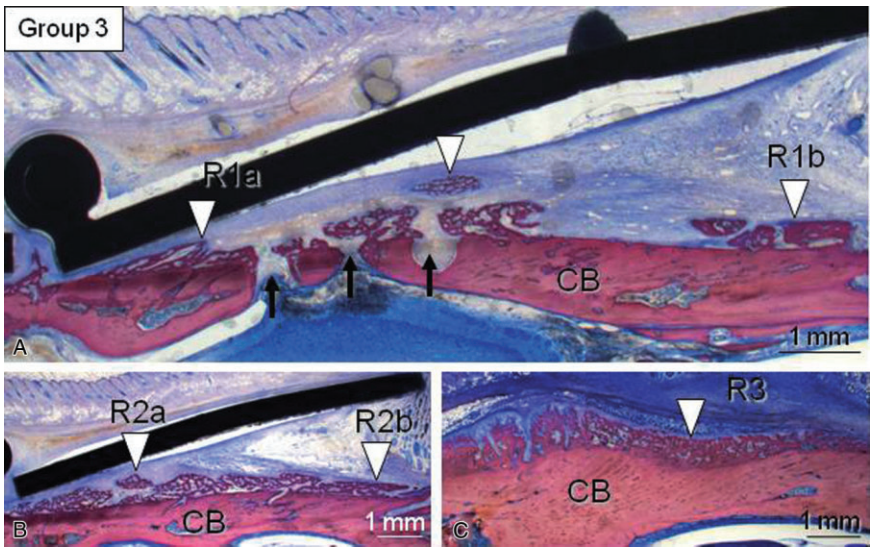

Figure 6 *A*, Histological section next to the midaxis in region R1 illustrating the perforated (arrows) calvarial bone (CB). Isolated deposits of woven bone emerge from the calvarium (arrowheads). There is less new bone formation observed at site where the calvarial bone lacks bone marrow. *B*, In region R2, an almost contiguous layer of new bone is deposited on the calvarial bone. *C*, Like in region R2, a layer of new bone is found on top of old bone outside of distraction plate in region R3.

in subregion R2b (not shown). The gap between the basal plate and distraction plate was filled with new bone and a thin layer of new bone was also found extending over the distraction plate (see Figure 11, c and d). In this group, such thin deposits of new bone were occasionally observed in other groups and no association between the occurrence of this phenomenon and the treatment group could be made.

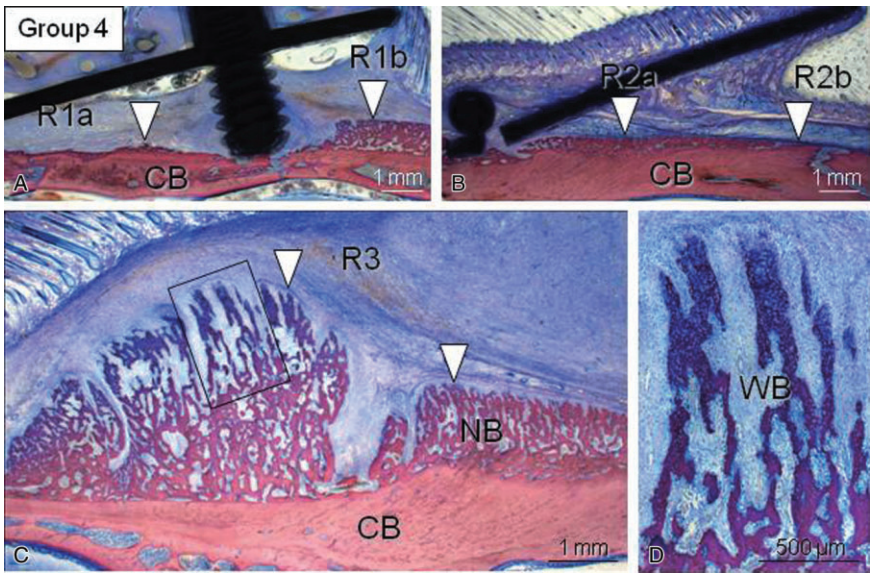

Figure 7 *A*, Section through the midaxis of distraction device in region R1. While in subregion R1a almost no new bone is found adjacent to the calvarial bone (CB), a thick layer of new bone is deposited in subregion R1b and extending outside the distraction device (arrowheads). Some bone resorption is found peripheral to the distraction screw. *B*, A layer of new bone with an almost uniform thickness is present at the periphery of the device at region R2. *C* and *D*, The boxed area in (c) is enlarged in (d). An exceptionally thick layer of new bone (NB) is found outside the distraction plate in region R3. While the new bone is more mature towards the calvarium, the superficial layer is indicative of ongoing woven bone (WB) formation. Fine trabucules of woven bone are oriented parallel to the distraction vector.

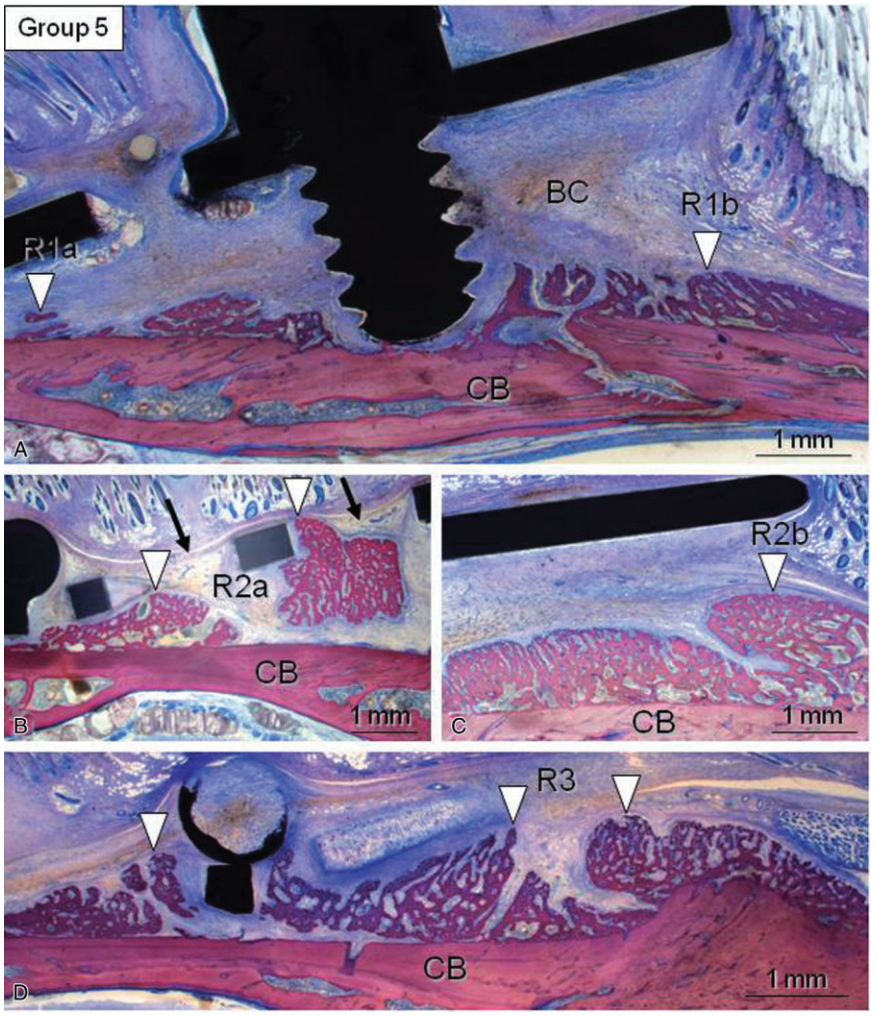

Figure 8 *A*, Midaxis section of region R1 showing new bone formation (arrowheads) between the calvarial bone (CB) and the distraction plate. The newly formed bone layer is slightly thicker in subregion R1b then in subregion R1a. A little bone resorption is seen around the distraction screw. Remnants of blood clot (BC) are seen between the new bone and distraction device. *B*, In subregion R2a, new bone is found in the distraction gap and extending into the plate perforations (arrows). *C*, A thick layer of new bone is seen in the distraction gap between the calvarial bone and the distal end of the distraction plate. *D*, Lateral to the distraction device in region R3, an irregularly thick layer of new bone covers the old calvarial bone.

Histomorphometric Analysis

The difference in the mean maximum new bone height between the eight groups was statistically significant ($p < .001$). When only a single treatment modality was evaluated without influence the other two modalities, more bone formation was found when original the bone surface was not perforated ($p < .001$), the lateral periosteum was left intact ($p = .0418$), or the distraction plate was perforated ($p = .982$).

Mean maximum new bone height for regions and subregions is shown in Table 2. When determined histomorphometrically, maximal bone height in region R1a was statistically greater in group VII than in groups I ($p = .019$), IV ($p = .027$) and VIII ($p = .014$). Maximum bone height in subregion R2a was statistically bigger for group V than for group VI ($p = .021$). In subregion R2b, no statistically significant differences were found.

In subregion Rc, the higher maximal bone height was found in group IV in comparison to group III ($p = .018$) and in group VI in comparison to groups III ($p = .006$), VII ($p = .017$) and VIII ($p = .031$). No significant differences were found between groups in region R3.

DISCUSSION

A gradual distraction of the periosteum from the original bone surface is expected to result in new bone formation. The model of PDO initially established in rats

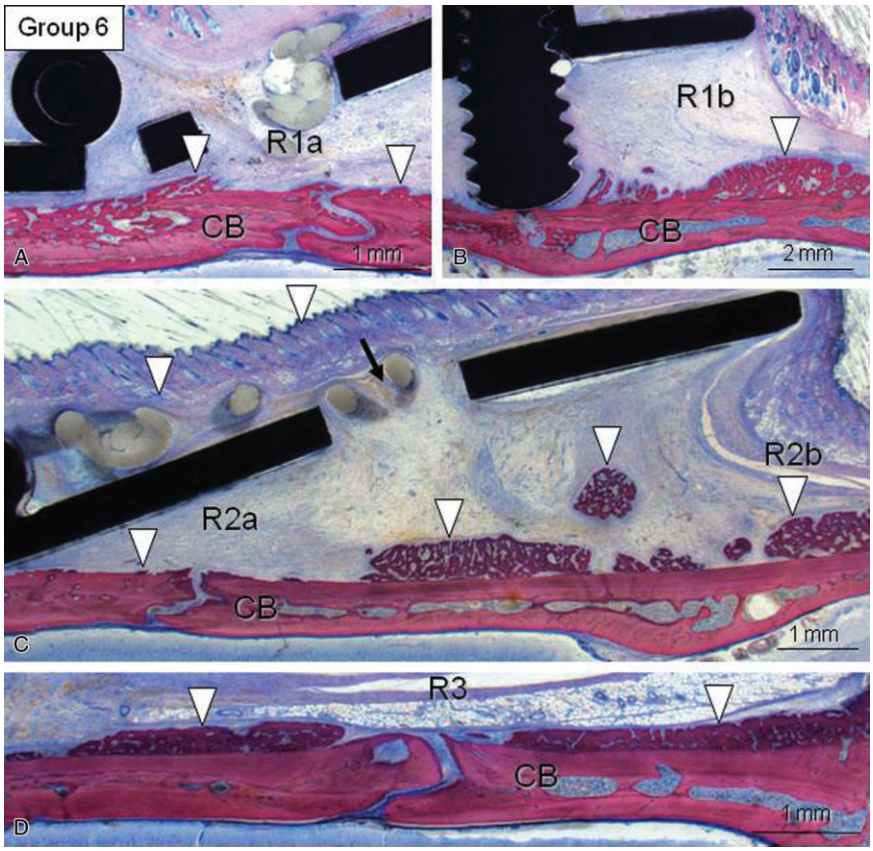

Figure 9 *A*, Section through the midaxis of distraction device illustrating new bone formation (arrowheads) adjacent to the calvarial bone (CB). *B*, Note that the thickness of new bone is greater in subregion R1b then in subregion R1a. *C*, This difference between subregions is much more pronounced in region R2. The seemingly isolated area of new bone may have formed in association with the resorption processes observed lateral to the distraction screw. *D*, New bone layer of moderate thickness is observed outside the distraction device in region R3, except the site where a bone suture reached the bone surface.

demonstrated successful augmentation induced by the mechanical activation of the periosteum.[15] Periosteal distraction in the present study resulted in new bone formation in all groups. To identify the origin of new bone, the biological activity of each of the two potential osteogenic tissues was selectively influenced in this study. The features of the present rat model though demonstrated several limitations to answer the main question. Due to the small surface of the calvarium, treatment modalities could have been insufficiently refined; cortical bone perforations were located next to region R2. An effort was thus made to avoid possible mutual influences of three different modalities by precise depiction of the three regions and three subregions on the histological sections. The area of new bone formation next to the distraction screw influenced by bone resorption was excluded from the histomorphometric analysis. Because of the uncertain detection of subregions in region R3, only one maximal value per region was chosen. The general finding was the bone formation from the original bone surface, but the trend in subregions/regions cannot support this conclusion. The results of the present study, however, confirmed differences between groups, when all three modalities were treated in the opposite way.

At the conventional DO site, the major source of vascular cells arises mainly from the original cortical bone and not from the periosteum.[27] As previously confirmed at the later consolidation periods of PDO,[17] the results of the present study indicated the benefit of the openings of the vessel-rich spongy bone. However, the main limitation to confirm this hypothesis was the incongruent presence of the bone marrow within the thin calvarial bone. Impaired new bone formation was obvious by the absence of bone marrow cavities. A negative influence of cortical bone perforations on new bone formation was statistically confirmed by exclusion of the lateral and apical periosteum. The influence of intact cortical bone on new bone formation cannot be completely excluded. Reflection of the periosteum damages

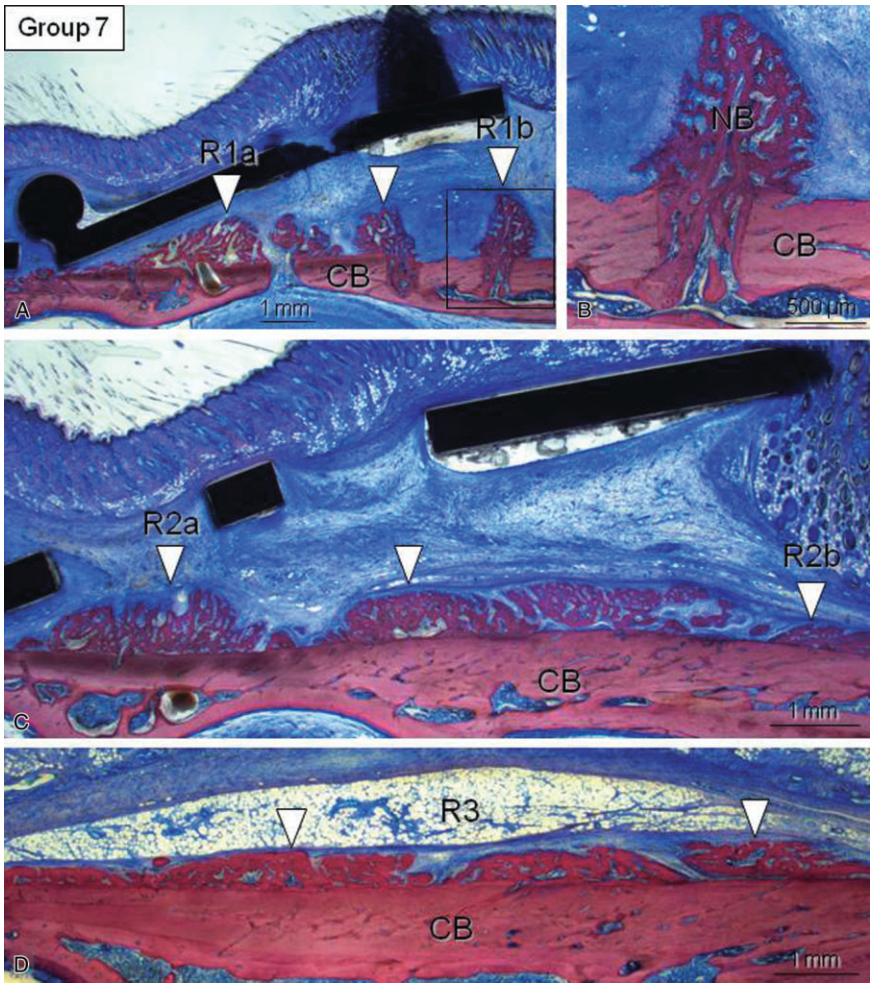

Figure 10 *A*, Midaxis section through the distraction device showing new bone (arrowheads) emerging from the perforations in the calvarial bone (CB). *B*, High magnification of boxed area in (a) illustrating new bone (NB) emerging from the opened bone marrow space in the calvarial bone. *C*, The new bone formed in the distraction region R2 is thicker in subregion R2a compared to subregion R2b. *D*, A layer on top of old bone is also found external to the distraction device in region R3.

small vessels and spontaneous perforations induced by widening Volkman's vascular channels.[19] When the bone is thinner (0.4–0.8 mm), the bone sinusoids and intraosteal vessels are reduced or even missing.[28] This corroborate the present findings. Significantly higher maximal new bone values in the R1 found in group VII then in groups I and IV indicate the importance of the old bone stimulation in the presence of periosteum. Considering the differences between group VII in comparison to groups VIII and III ($p = .055$) (perforated calvarium, occlusive plate) in contrast to groups V and VI (intact calvarium, perforated plate), it is possible that the presence of a periosteum in the present model might be more important than perforating the calvarial bone.

Because of the location of periosteal incision and calvarial bone perforations, region R2 might be considered most crucial in the present study. However, the interpretation of significant differences in region R2 on the basis of the present data is highly speculative. New bone found external to the distraction plate in subregion R2a (see Figure 3b) was previously demonstrated in PDO.[25,29] These values were excluded from the statistical analysis, while likely originating from the bone that formed in region R3 (see Figure 3c). It is uncertain how incision of the periosteum in region R2 affected the conditions for periosteal distraction in regions R1 and R3.

Periosteum is considered a major source of new callus in DO[1,22,27] The importance of the periosteum in the present study was identified in subregion Rc. In contrast to the DO, the periosteum in PDO is surgically elevated from the bone surface and sutured over the calvarial bone and distraction device. Exfoliated and returned periosteum promotes the process of new bone

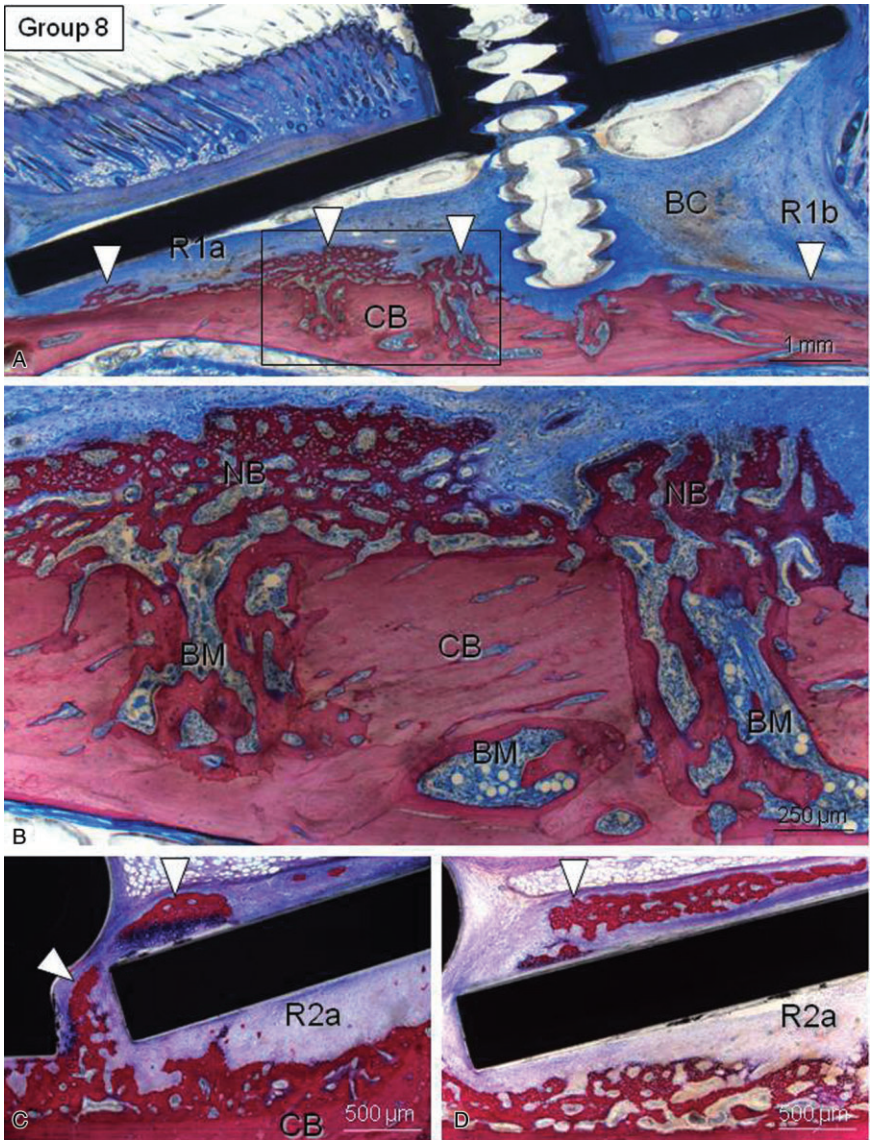

Figure 11 *A*, In the midaxis section, new bone (arrowheads) is observed in subregion R1a and R1b over the calvarial bone (CB). There is still some residual blood clot (BC). *B*, Higher magnification of the boxed area in (a) showing new bone (NB) at sites where the bone marrow (BM) is perforated. *C* and *D*, In group VIII, as in some other groups, new bone formation is also observed in the gap between the basal plate and the distraction plate and external to the distraction device without making contacts with the metal surface.

formation[30] by the mutual interaction of the bone matrix and osteoprogenitor cells in the periosteum.[31] The results of Zhang and colleagues[32] on the isografts in mice showed that the expansion of early mesenchymal progenitor cells from the periosteum accounts for 70% of bone formation on the graft. In the present study, the true viability of the periosteum was not evaluated. Maximal amount of bone was found laterally to the distraction plate, with the typical trabecular orientation parallel to the distraction vector.[1,24,25] Several factors may be attributed to the lack of significant differences between the groups for region R3. Following elevation of periosteum, the vascularization remains preserved mainly at the periphery.[33] Periosteal microvasculature on the calvarium of rats shows the presence of large afferent and efferent vessels[28] and a great abundance of pericytes, which may serve as a supplementary source of osteoblasts.[34] While the periosteum in the present study was incised only along the distraction plate, a complete periosteal excision instead of incision would possibly enhance the differences between the groups for region R3.

The osteogenic calvarial cell population might be very sensitive to biomechanical stimuli, since the

TABLE 2 Values of Maximal Bone Height Distributed Per Regions and Subregions (All Values in mm, Expressed as Means ± SD)

Group	R1a	R1b	R2a	R2b	Rc	R3
I	0.33 ± 0.06	0.44 ± 0.19	0.74 ± 0.41	0.38 ± 0.25	0.33 ± 0.16	0.84 ± 0.55
II	0.49 ± 0.24	0.73 ± 0.26	0.66 ± 0.24	0.69 ± 0.21	0.37 ± 0.14	0.79 ± 0.37
III	0.39 ± 0.05	0.35 ± 0.08	0.59 ± 0.38	0.57 ± 0.34	0.20 ± 0.13	0.71 ± 0.41
IV	0.41 ± 0.20	0.71 ± 0.21	0.64 ± 0.40	0.70 ± 0.32	0.49 ± 0.23*	1.13 ± 0.58
V	0.54 ± 0.07	0.69 ± 0.14	0.83 ± 0.39*	0.75 ± 0.35	0.43 ± 0.26	0.74 ± 0.26
VI	0.66 ± 0.26	0.80 ± 0.09	0.23 ± 0.17	0.93 ± 0.49	0.55 ± 0.27*	0.63 ± 0.39
VII	0.80 ± 0.09*	0.78 ± 0.23	0.68 ± 0.19	0.57 ± 0.11	0.26 ± 0.14	0.50 ± 0.33
VIII	0.38 ± 0.19	0.51 ± 0.29	0.77 ± 0.40	0.64 ± 0.34	0.28 ± 0.17	0.97 ± 0.68

*Statistically significant difference at $p < .05$.

collagen fibers originating from the periosteum transverse the cambium layer and pass to the bone surface between the osteoblasts.[35] The presence of the periosteal insertion on the old bone outside the distraction plate apparently affected an incongruent amount of newly formed bone in the present study (see Figure 7c). The conventional DO in rats showed the typical pattern of bone healing for daily rates up to 0.5 mm.[36,37] Besides the comparison of new bone formation by immediate and gradual elevation of the periosteum,[24,25,29] no experiment was performed to compare different distraction rates in PDO. By the angular movement of the distraction plate, the distraction rate gradually decreases towards the hinge. As previously demonstrated in long bones DO, the continuation of the periosteum might be most relevant for its function; the amount of stretch may be of minor importance, since the periosteum can locally grow.[38] Due to the frequent incidence of device exposure, a decreased rate of distraction using additional treatment modalities[39] might be considered for future studies on PDO in the rat calvaria.

The early consolidation period in the present study was used to locate a source of new bone. Considering the results of the rat model on conventional DO,[36,37] complete bone formation after 1 week of consolidation in the present study was not expected. The persistence of a prominent coagulum bordered by a granulation tissue had likely delayed new bone formation.[40] It is possible that the highly vascularized tissue will diminish with time, whereas the presence of osteoid and new blood vessels could be seen even 6 weeks after reposition of the periosteum on the calvaria of rats.[30]

As in the previous reports on PDO,[9–15,24,25] the apposition of new bone from the existing calvarial bone was confirmed by the activation of periosteum in the present model. Two potentially osteogenic tissues were selectively treated, with the aim to identify the origin of new bone. Within the limitations of the present model, a positive effect was achieved by creating an access to the endosseal compartment, but the periosteum could outrange the influence of calvarial bone perforations. Besides the lack of statistical significance between the groups for region R3, the gain in bone thickness indicates the powerful potential of PDO. According to the present state of knowledge, PDO might be still away from a clinical application. The advantages of PDO in comparison to more demanding surgical procedures pointed towards further evaluation of this treatment modality. Knowledge of the relative contributions made to bone formation by the bone base and the periosteal layer will serve as a basis for specifically improving PDO and enhancing its efficacy. The evaluation of this promising treatment modality includes the investigation of highly specific therapeutic measures such as the application of osteoinductive growth factors at defined topographic locations.

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