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ORIGINAL ARTICLE

Effect of periosteal stimulation therapy on bone formation in orthopedically expanded suture in rats

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Structured Abstract

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Objective – The aim of this experimental study was to evaluate the effects of periosteal stimulation therapy (PST), on bone regeneration in response to expansion of the interpremaxillary suture, in rats.

Material and Methods – Sixteen male, 50–60 days old Wistar rats were divided into two equal groups (control and experimental). Both groups were subjected to expansion for 5 days, and 30 cN of force was applied to the maxillary incisors with helical spring. On the 2nd day of the expansion procedure in the experimental group, the periosteum over the interpremaxillary suture was stimulated locally by intraperiosteal penetrations with a hypodermic needle. Bone regeneration in the interpremaxillary suture was evaluated by bone histomorphometry and the new bone area, bone perimeter, feret's diameter and new bone/old bone percentage parameters were evaluated. Mann–Whitney *U*-test was used for statistical evaluation at $p < 0.05$ level.

Results – Significant differences were found between groups for all investigated histomorphometric parameters. New bone area ($p < 0.01$), bone perimeter ($p < 0.05$), feret's diameter ($p < 0.01$) and new bone/old bone percentage ($p < 0.01$) measurements were significantly higher in the experimental group than that in the control. Bone histomorphometric measurements revealed that bone architecture in the PST group was improved.

Conclusions – Local stimulation of the periosteum of the sutural area during expansion stimulates bone formation and improves healing.

Key words: image analysis; maxillary expansion; osteogenesis; periosteum; rats

Introduction

Widening of the midpalatal suture by rapid maxillary expansion (RPE) during orthodontic treatment increases the width of posterior dentition rapidly, which is followed by active bone formation in the suture (1–4). It is well known that even after a retention period, the expanded maxilla has a strong tendency to rebound to its previous form (5, 6). The extent of this relapse may be as much as 90% (7). Reorganization of hard tissue in the suture starts by the end of active treatment phase (8, 9). Haas (8) concluded that ossification of the suture margins is completed in 60–90 days.

Although the reason for the relapse is not fully understood, rate and quality of bone formation in the midpalatal suture during and after expansion may affect the post-treatment relapse (1). Therefore, it would be potentially beneficial to accelerate bone formation in the midpalatal suture during and after expansion for preventing relapse of the skeletal base and shortening of the retention period (1, 2).

Goldman and Smukler (10, 11) indicated that lifting of or trauma to the periosteum would result in typical responses of the tissues to injury. The repair phase was accompanied by marked regenerative activity of the cells of the periosteum, resulting in the formation of new connective tissue and bone. It has been determined that during the healing of fractures, the osteogenic cells of the periosteum and the cells of the endosteum play an important role in the repair process (12). The periosteum has osteogenic potential because, under appropriate stimulation, mesenchymal progenitors adjacent to the bone surface can differentiate into osteoblasts (13).

For fracture healing or distraction osteogenesis (DO) protocols, various experimental studies have been carried out to stimulate the callus mechanically, such as micromovements applied in the direction axial to the callus (14), static compression for shortening the callus after callotasis (15), as well as electrical stimulation (16), and electromagnetic stimulation (17). So far, no studies in the literature have used controlled periosteal stimulation therapy (PST) during DO or maxillary expansion procedures.

There have been few studies that attempted to change bone regeneration capacity in midpalatal suture during maxillary expansion. Sawada and Shimizu (2) investigated the expression of transforming growth factor-β1 (TGF-β1) during expansion of the midpalatal suture to evaluate its synergetic effects on bone formation and found that application of TGF-β1 during the early stage was essential to attain the most effective bone formation. Saito and Shimizu (1) evaluated the effects of low-power laser irradiation on bone regeneration during expansion of the midpalatal suture in rats and suggested that laser therapy could have a therapeutic benefit in inhibiting relapse and shortening the retention period through acceleration of bone regeneration. Uysal et al. (3) evaluated the effects of dietary boron on bone formation in response to expansion of interpremaxillary suture during different retention periods in rabbits and concluded that dietary boron had positive effects on early phase of bone regeneration in the interpremaxillary suture. In a recent study, Uysal et al. (4) evaluated the effects of ED-71, a new active vitamin D analog, on bone regeneration of the interpremaxillary suture in rats and found that locally administered ED-71 had a marked stimulatory effect on bone formation in a particular time.

The aim of this experimental study was to evaluate the effects of PST, on bone regeneration during expansion of the interpremaxillary suture in rats. These effects were evaluated with quantitative bone histomorphometric examination. For this study, the null hypothesis assumed that PST has no stimulatory effect on bone formation in the suture subjected to forced expansion, in rats.

Materials and methods

Animals and groups

Sixteen male, 50–60 days old Wistar rats weighing 184.65 ± 17.95 g were used in this study. All animals were housed in polycarbonate cages in a 12-h light/dark environment at the constant temperature of 23°C and fed a standard pellet diet (Expanded pellets; Stepfield, Witham, Essex, UK) with tap water *ad libitum*. The experimental protocol was approved by the University of Erciyes, Regional Animal Research Ethics Committee. This study was organized as a parallel group design with one group receiving the experimental protocol and the other receiving the control. The power analysis was performed with G*POWER Ver. 3.0.10. (Franz Faul, Universität Kiel, Germany) software. Based on 1:1 ratio between groups, a sample size of 14 animals would give more than 85% power to detect significant differences with 0.40 effect size and at $\alpha = 0.05$ significance level. Animals were randomly divided into two groups (control and experimental) of eight rats each.

Appliance placement

Expansion appliance comprised of helical springs that fabricated from 0.014-inch, stainless steel wires. Springs were placed on a grid and activated on a single arm with pliers. The force was measured with a gauge (30 cN), and the springs were not reactivated during

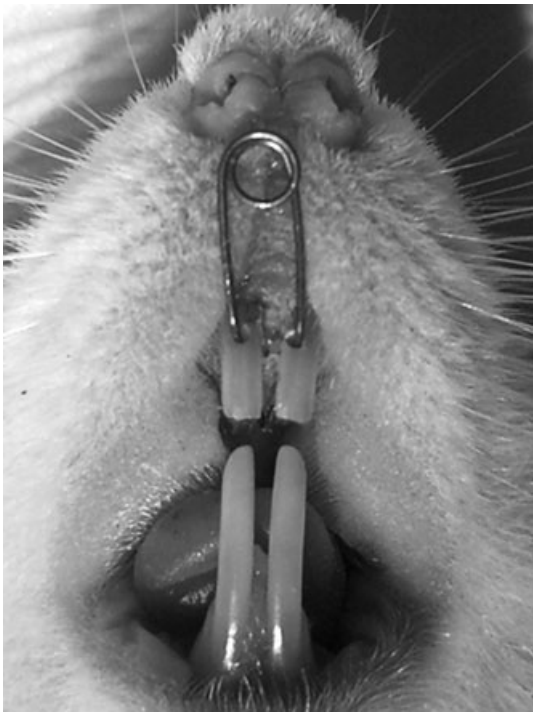

Fig. 1. Appliance *in situ.*

the expansion period. Appliances were attached to maxillary incisors of all animals under anesthesia (Xylasine + Ketamine combination, 0.5 and 1 ml/kg intramuscular, respectively). A hole was drilled in both incisors at the lingual-gingival level and springs were inserted into the holes, buccally (Fig. 1).

Both groups were subjected to expansion for 5 days. Then, the springs were removed and a piece of rectangular wire was inserted into the holes between the two incisors for retention. Tooth separation was maintained during the retention phase for 10 days. The distance between the mesial edges of the maxillary incisors was measured at the beginning and on the fifth day of the expansion with a digital caliper (MSI-Viking, SC, USA). Occlusal radiographs were taken at baseline, end of expansion and at the end of retention period.

Periosteal stimulation

The periosteum over the expansion area was stimulated as previously described by Goldman and Smukler (10, 11). On the 2nd day during the expansion procedure, intraperiosteal penetrations were made with a No. 25 gauge hypodermic needle, attached to a syringe for better leverage. The instrument penetrated the mucosa at an angle acute to its surface and engaged the underlying bone firmly. Multiple penetrations, in a 'pincushion' fashion, were carried out in each selected area (10 mm apart from the sutural line in left and right direction). No surgical dressing was placed after the procedure.

Specimen preparation

After a 10-day retention period, the rats were sacrificed with an overdose of Ketamine/Xylasine combination, and their premaxillae were dissected and placed in bottles containing 10% formalin. During decalcification, the solution was changed three times a day. After fixation, the retaining wires were removed, and the premaxillae were decalcified with 5% formic acid for 3 days. The decalcified premaxillae were fixed again in the same manner and sectioned. The maxillary incisor acted as the primary guide for orienting the sections. The section was cut perpendicular to the sagittal plane and was determined by two points; one was at the alveolar crest and the other was 4 mm apically. This plane passed through the center of the incisor's crown at its gingival portion. The tissues were fixed in 10% neutral-buffered formalin, processed and embedded in paraffin. Standard 5-μm thick sections were obtained from paraffin-blocks for each sample.

Image acquisition and histomorphometric analysis

Histological sections were stained with hematoxylin–eosin prior to microscopic examination (Fig. 2–4). As bone formation of the surface area was sometimes irregular and not suitable for quantitative measurement, histomorphometric measurements were performed only on a selected area localized 200 μm deeper from the surface of the osseous palate facing the oral cavity. Measurements were realized using an open source java-based image analysis program. For this purpose, a microscope and digital camera system (Olympus CX41/DP25 Research System; Olympus Corp, Tokyo, Japan) were utilized.

Histomorphometric measurements were performed double-blinded by two of the contributors, and final results were an average of values from these two separate evaluations. Two histological sections were analyzed for each animal. The relevant areas on the slides were pre-defined, and two representative digital images were captured under 400× magnification. Computer-assisted

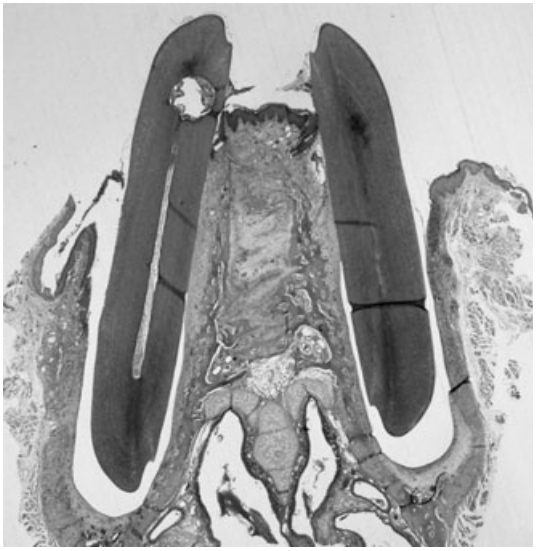

Fig. 2. Hematoxylin–eosin staining of the histological sections prior to optical-microscope examination (40× magnification).

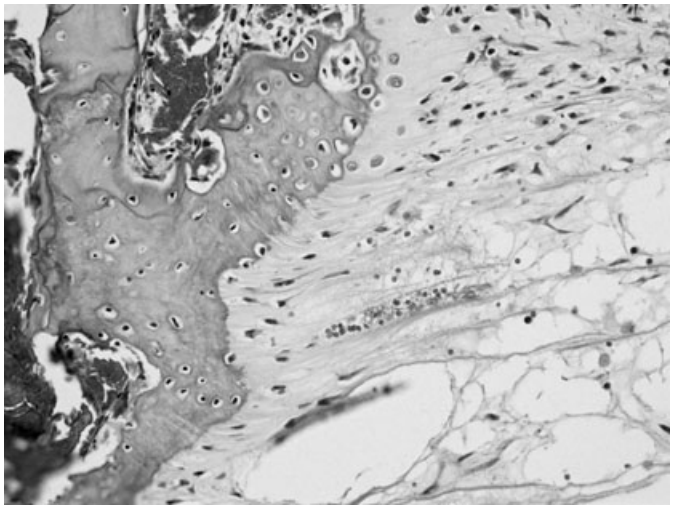

Fig. 3. Photomicrograph of a section in the expansion area of control group showing abundant formation of bone trabeculae. Large connective tissues indicate the beginning stages of bone formation (HE 200× magnification).

image analysis software, IMAGE-J (18), was used to make measurements histomorphometrically. For this purpose, two separate image analysis macro have been prepared by one of the authors (Y.K.).

Statistical analysis

All data were analyzed with the statistical package for social sciences, 13.0 (SPSS for Windows; SPSS Inc, Chicago, IL, USA). Descriptive statistics were given as mean, standard deviation, minimum and maximum. The group differences were studied by the Mann–Whitney *U*-test. When the *p*-value was <0.05, the statistical test was determined as significant.

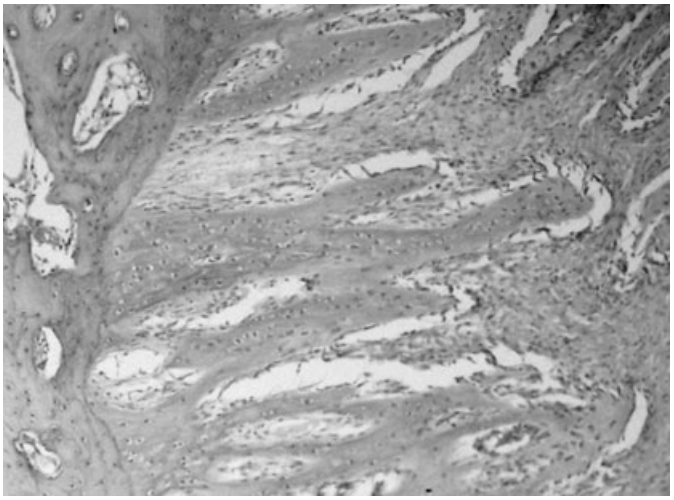

Fig. 4. Photomicrograph of a section in the expansion area of experimental group (Group IV) showing larger masses of new bone trabeculae. New bone became attached to old bone at the site of expansion. Large amounts of new bone forming area indicate the later stages of bone formation (HE 200× magnification).

Results

All animals survived to the end of the study. However, deep mucosal infection/dehiscence was observed in one animal in the experimental group, and this animal was excluded from the study. The expansion appliance was well tolerated, and the animals gained weight. The body weight of one rat in experimental group decreased during the expansion period, but subsequently recovered. No statistically significant changes in body weight were observed between groups during expansion and retention periods (Table 1).

Biometric analysis for the amount of expansion was performed by image analysis software at the most anterior part of the premaxilla on histological sections. Suture width measurements from histological sections showed that the interpremaxillary suture was expanded following the application of the activated helical loop (Fig. 2). The results indicated that the mean amount of expansion was less in the PST group (248.21 ± 23.31 μm) than that in the control (276.46 ± 24.56 μm). However, the statistical analysis showed no statistically significant differences ($p > 0.05$) (Table 2).

Statistical analysis showed significant differences between the two groups for all investigated histomorphometric parameters. New bone area ($p < 0.01$), bone

Table 1. **Body weight changes (kg) between groups during expansion and retention periods**

		T1–T0		T2–T0		Significance	
Groups	N	Mean	Standard deviation	Mean	Standard deviation	T1–T0	T2–T0
Periosteal stimulation	7	0.278	0.196	0.321	0.124	NS	NS
Control	8	0.286	0.164	0.312	0.145	NS	NS

T0, start of experiment; T1, end of expansion (5th day); T2, end of retention (15th day).

Table 2. **Results and statistical comparisons of biometric analysis for determination of the amount of expansion (μ)**

Groups	N	Mean (μ)	Standard deviation	Minimum	Maximum	Mann–Whitney *U*	Significance
Periosteal stimulation	7	248.21	23.31	227.35	278.61	0.769	NS
Control	8	276.46	24.56	243.82	299.93		

Table 3. **Histomorphometric comparison between groups of amount of new bone formation**

	Periosteal stimulation (n = 7)				Control (n = 8)					
Histomorphometric measurements	Mean	Standard deviation	Minimum	Maximum	Mean	Standard deviation	Minimum	Maximum	Mann–Whitney *U*	*p*
New bone area (μm^2)	102.256	11.027	85.650	11.567	57.640	19.979	28.900	89.570	2.000	0.004*
Bone perimeter (μm)	189.670	44.988	122.768	23.608	127.077	52.091	82.465	23.619	5.000	0.022**
Feret's diameter (μm)	54.247	12.184	26.330	85.194	15.214	2.950	12.083	17.148	0.000	0.002*
New/old bone (%)	68.420	6.070	60.000	74.730	34.857	11.895	20.000	55.550	0.000	0.002*

n, sample size; *$p < 0.01$; **$p < 0.05$.

perimeter ($p < 0.05$), feret's diameter ($p < 0.01$) and new bone/old bone percentage ($p < 0.01$) measurements showed statistically significant differences (Table 3). For all histomorphometric parameters, the PST group showed more positive results than the control, related to the new bone formation and revealed that bone architecture in the treatment group was improved.

Discussion

To our knowledge, this study is the first to report faster healing of bone in the interpremaxillary suture area during expansion, by applying local periosteal stimulation. After the application of expansion strain, more stable and larger callus could be achieved by this stimulation. Also, more new bone formation in the expansion region could be observed, leading to a more advanced stage of bone healing (Figs 3 and 4).

In the medical field, mechanical stimulation and application of pharmacological agents to increase the bone formation are well-known applications. In the orthodontic literature, few studies have been carried out to stimulate regeneration in the interpremaxillary suture after expansion. Sawada and Shimizu (2) applied a single dose of TGF-β1; and Saito and Shimizu (1) evaluated low-power Ga-AI-As diode laser irradiation for stimulation of the expanding midpalatal suture in rats. Both studies found significantly stimulated bone regeneration in the midpalatal suture. In recent studies, Uysal et al. (3) evaluated the stimulatory effects of dietary boron in rabbits and locally administered ED-71 in rats (4), on bone formation in response to expansion of the interpremaxillary suture and found that these agents could stimulate bone regeneration during

expansion and retention periods. In the current study, the effects of surgical stimulation of the periosteum on bone regeneration in response to expansion of the interpremaxillary suture was investigated in rats, and increase in newly formed, mineralized bone area in the suture with PST was demonstrated.

Maxillary expansion causes a multifactorial adaptive response within the midpalatal suture. Mechanical expansion results in distortion of the sutural structure, inducing a biologic chain of events leading to osseous modeling that allows the suture to restore itself to its original architecture (19). In this study, the stimulatory effect of PST on bone regeneration in the interpremaxillary suture in response to expansion was investigated by using a histomorphometric method. This method is a reliable technique that is frequently used in quantitative evaluation of bone remodeling, *in vivo* and *in vitro* conditions (20).

The nature of the effects of force on the rate of bone mineralization can be undertaken by experimental studies on animals. While monkey and cat have maxillary sutures similar in most aspects to that of man and have been used in maxillary expansion experiments, the ideal animals with which to obtain a clear picture of bone and suture changes under stress are rabbits and rats (21). Thus in this study, according to the ethical considerations, the smallest animal model was chosen to test stimulation method in bone modeling.

In this study, local stimulation of periosteal tissues over the suture and its effect on bone regeneration were evaluated during a maxillary expansion procedure. To minimize systemic adverse effects and to support bone formation in a definite time interval and in a definite area, it is important to apply a stimulus or agent locally. Thus, the present method seems to be suitable to evaluate the pure effects of testing the mechanical stimulation technique, on bone regeneration.

The normal interpremaxillary suture width in young rats is approximately 20–60 μm. Burstone and Shafer (22) found that expansion of the suture by rubber wedges over a period of 5 days resulted in an opening of the suture to an average width of 377 ± 104 μm. In this study, occlusal radiographs showed a wide separation of the premaxillary bones after 5 days of expansion, and the suture width measurements were found in range between 227.35 and 299.93 μm. The amount of expansion in all groups was similar and showed no statistically significant differences. Less suture width in the experimental group indicates new bone formation along the medial margins of bone segments.

Gradual traction can be applied not only to long bones but also to the maxillofacial area, usually to form new bone (23, 24). In most studies of DO for long bones or mandible lengthening, the authors have reported new bone formation by intramembranous ossification (23, 24). In this study, no cartilaginous or fibrocartilaginous tissue was found in newly formed bone, and the ossification was defined as intramembranous.

Possibly the disturbance of the blood supply to the mucosa and interpremaxillary suture by the PST caused increased vascularization. On the basis of healing studies in experimental animals, Melcher and Accursi (13) suggested that osteoperiosteal flaps would have fibrogenic and osteogenic capacities to provide superior donor tissue in flap surgery. Following the same train of thought, Goldman and Smukler (10) demonstrated that it is possible to stimulate the periosteum surgically into activity. It is well established that the body responds to injury by accumulating into the wound area, cells which have the potential to ensure repair (10). These descendants of the hemopoietic stem cells, thymocytes and lymphocytes are very important repair elements and can be considered to have played a part in the osteogenesis seen in this study. In this study, lifting of or trauma to the periosteum resulted in typical responses of the tissues to injury. The repair phase was accompanied by marked regenerative activity by the cells of the periosteum, resulting in the formation of new connective tissue and bone. These events confirm previous studies (10, 11) which indicate that inactive periosteal cells retain the potential for generative activity. Similar osteogenic capacities are exhibited by the endosteal cells lining the marrow spaces even in areas distant to the actual 'stimulation sites'.

Histomorphometrical analysis showed that the quantity of newly formed bone in PST specimens was greater than in controls. The marked increase in histomorphometric parameters was found statistically significant. Histopathological examination showed that the trabecular structure was increasing gradually; the histomorphometrical result can be explained as a characteristic of the bone healing of the rats. Also, there could have been resorption as a result of bone remodeling. Further experimental research is necessary to determine the optimum conditions for osseous healing of expansion areas.

Conclusion

These findings suggest that locally applied PST can stimulate bone regeneration in the orthopedically expanded interpremaxillary suture, during expansion and retention periods.

Clinical relevance

This study confirmed that inactive periosteum could be nudged into fibrogenic and osteogenic activity by means of controlled injury. It was demonstrated that local stimulation of the periosteum of the interpremaxillary suture area during expansion, stimulates bone formation and improves healing. This principle could potentially be applied during distraction osteogenesis or for the treatment of patients with long bone fractures fixed by external devices and might be particularly helpful in treating fractures with delayed union and non-union.

References

1. Saito S, Shimizu N. Stimulatory effects of low-power laser irradiation on bone regeneration in midpalatal suture during expansion in the rat. *Am J Orthod Dentofacial Orthop* 1997;111:525–32.
2. Sawada M, Shimizu N. Stimulation of bone formation in the expanding mid-palatal suture by transforming growth factor-beta 1 in the rat. *Eur J Orthod* 1996;18:169–79.
3. Uysal T, Ustdal A, Sonmez MF, Ozturk F. Stimulation of bone formation by dietary boron in an orthopedically expanded suture in rabbits. *Angle Orthod* 2009;79:984–90.
4. Uysal T, Amasyali M, Enhos S, Sonmez MF, Sagdic D. Effect of ED-71, a new active vitamin D analog, on bone formation in an orthopedically expanded suture in rats. A histomorphometric study. *Eur J Dent* 2009;3:165–72.
5. Krebs AA. Midpalatal suture expansion studied by the implant method over a seven-year period. *Trans Eur Orthod Soc 2* 1964;40:131–42.
6. Vardimon AD, Graber TM, Voss LR. Stability of magnetic versus mechanical palatal expansion. *Eur J Orthod* 1989;11:107–15.
7. Timms DJ. Long term follow-up of cases treated by rapid maxillary expansion. *Trans Eur Orthod Soc* 1976;52:211–5.
8. Haas AJ. The treatment of maxillary deficiency by opening the midpalatal suture. *Angle Orthod* 1965;35:200–17.
9. Cleall JF, Bayne DI, Posen JM, Subtelny JD. Expansion of the midpalatal suture in the monkey. *Angle Orthod* 1965;35:23–35.
10. Goldman HM, Smukler H. Controlled surgical stimulation of periosteum. *J Periodontol* 1978;49:518–22.
11. Goldman HM, Smukler H, Lugo-Romeu F, Swart N, Bloom A. Stimulated osteoperiosteal pedicle grafts in dogs. *J Periodontol* 1983;54:36–43.
12. Axhausen W. Osteogenic phases of regeneration of bone. *J Bone Joint Surg* 1956;38A:593.
13. Ogita M, Rached MT, Dworakowski E, Bilezikian JP, Kousteni S. Differentiation and proliferation of periosteal osteoblast progenitors are differentially regulated by estrogens and intermittent parathyroid hormone administration. *Endocrinology* 2008;149:5713–23.
14. Kassis B, Glorion C, William T, Blanchard O, Pouliquen J. Callus response to micromovement after elongation in the rabbit. *J Pediatr Orthop* 1996;16:480–3.
15. Hamanishi C, Yoshii T, Totani Y, Tanaka S. Lengthened callus activated by axial shortening: histological and cytomorphometrical analysis. *Clin Orthop* 1994;307:250–4.
16. Pepper JR, Herbert MA, Anderson JR, Bobechko WP. Effect of capacitive coupled electrical stimulation on regenerate bone. *J Orthop Res* 1996;14:296–302.
17. Van Roermund PM, Ter Haar Romeny BM, Hoekstra A, Schoonderwoert GJ, Brandt CJ, van der Steen SP et al. Bone growth and remodeling after distraction epiphysiolysis of the proximal tibia of the rabbit: Effect of electromagnetic stimulation. *Clin Orthop* 1991;266:304–12.
18. Rasband WS. *Image-J*. Bethesda, Maryland, USA: U.S. National Institutes of Health; http://rsb.info.nih.gov/ij/, 1997–2008.
19. Chang HN, Garetto LP, Potter RH, Katona TR, Lee CH, Roberts WE. Angiogenesis and osteogenesis in an orthopedically expanded suture. *Am J Orthod Dentofacial Orthop* 1997;111:382–90.
20. Eriksen EF, Axelrod DW, Melson F. Bone histology and histomorphometry. In: Axelrod DW, Eriksen EF, Melson F, (eds). *Bone Histomorphometry*. New York: Raven Press; 1994. pp. 33–8.
21. Storey E. Tissue response to the movement of bones. *Am J Orthod* 1973;64:229–47.
22. Burstone CJ, Shafer WG. Sutural expansion by controlled mechanical stress in the rat. *J Dent Res* 1959;38:534–40.
23. Kostopoulos L, Karring T. Role of periosteum in the formation of jaw bone: an experiment in the rat. *J Clin Periodontol* 1995;22:247–54.
24. Cope JB, Samchukov ML. Mineralization dynamics of regenerate bone during mandibular osteodistraction. *Int J Oral Maxillofac Surg* 2001;30:234–42.