

Periosteum response to static forces stimulates cortical drifting: A new orthopedic target



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ABSTRACT

Background: The mechanism of cortical bone adaptation to static forces is not well understood. This is an important process because static forces are applied to the cortical bone in response to the growth of soft tissues and during Orthodontic and Orthopedic corrections. The aim of this study was to investigate the cortical bone response to expanding forces applied to the maxilla.

Methods: Overall, 375 adult Sprague-Dawley rats were divided into three groups: 1) static force group, 2) static force plus stimulation group, and 3) sham group. In addition to static force across the maxilla, some animals were exposed to anti-inflammatory medication. Samples were collected at different time points and evaluated by micro-computed tomography, fluorescence microscopy, immunohistochemistry, and gene and protein analyses.

Results: The application of expansion forces to the maxilla increased inflammation in the periosteum and activated osteoclasts on the surface of the cortical plate. This activation was independent of the magnitude of tooth movement but followed the pattern of skeletal displacement. Bone formation on the surface of the cortical plate occurred at a later stage and resulted in the relocation of the cortical boundary of the maxilla and cortical drifting.

Conclusions: This study demonstrates that cortical bone adaptation to static forces originates from the periosteum, and it is an inflammatory-based phenomenon that can be manipulated by the clinician. Our findings support a new theory for cortical adaptation to static forces and an innovative clinical approach to promote cortical drifting through periosteal stimulation. Being able to control cortical drift can have a significant impact on clinical orthodontic and dentofacial orthopedics by allowing corrections of severe deformities without the need for maxillofacial surgery.

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1. Introduction

It has been shown that trabecular bone can sense dynamic forces. In response to low-magnitude dynamic forces, quick changes in trabecular density give the skeleton a fast adaptation

ability [1,2]. Similarly, cortical bone demonstrates sensitivity to dynamic forces; however, in comparison with trabecular bone, the changes in cortical bone take place over a longer period of time and are initiated in response to higher dynamic forces [3–6]. These characteristics make cortical bone the prominent load-bearing bone in our skeleton [7].

Interestingly, neither trabecular nor cortical bones show sensitivity to static forces [2,4,8,9], which can explain why lack of activity can cause trabecular bone loss even when the static force of gravity is present [10].

Previously, we and others have demonstrated that the mechanism of trabecular bone and cortical bone adaptation to dynamic forces originated either indirectly from strain-induced-changes in the matrix recognized by the bone cells [11–16] or by direct recog-

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dition of this mechanical stimulation by the cells themselves [2,17]. Signals produced by static forces may be too short in duration to be able to stimulate bone cells directly or indirectly, which could explain why both trabecular and cortical bone are not responsive to static forces [18,19].

While static forces are not osteogenic, in many clinical scenarios, the application of force by a growing pathology could stimulate bone formation. For example, an increase in the size of a chronically growing cyst or tumor can cause bone formation [20–22]. Considering these pathologies grow very slowly, the force produced by these growing structures can be considered mostly continuous, uninterrupted, or static. While some experimental models show that static forces may modify the shape of the cortical bone [23,24], those observations suggest that the target of these forces is not the bone itself.

Therefore, the rationale for our study is to investigate the role of periosteum on maxillary cortical bone adaptation to static forces, and how we can harness periosteum response to deliberately stimulate cortical bone changes in our patients.

2. Methods and materials

2.1. Animal study

Adult Sprague-Dawley rats (375 animals with average body weight 360 g, 120 days of age) were treated according to the protocol approved by the New York University Institutional Animal Care and Use Committee (protocol 121009 approved November 2013). All animals were housed in polycarbonate cages in a 12-hour light/dark environment at the constant temperature of 23°C and fed a standard pellet diet (Stepfield, Witham, Essex, United Kingdom) with tap water ad libitum. Animals were randomly divided into different groups and treated with static force, static force plus periosteal stimulation (bilateral or unilateral), static force and anti-inflammatory medication, or sham (appliance installed with no activation).

In some animals, the anti-inflammatory drug diclofenac (5 mg/kg) was injected intramuscularly daily with changing injection sites to prevent additional discomfort. Animals were weighed daily to calculate the dose of the medication for each animal accurately.

Bone labeling was performed using an intraperitoneal injection of Calcein green (15 mg/kg) on days 0, 28, and 54. Animals were euthanized by carbon dioxide narcosis on days 0, 1, 3, 7, 14, 28, and 56.

2.2. Application of static force

On day 0, animals were anesthetized with an intraperitoneal injection of Ketamine and Xylazine (0.09 mL/100 g). Anesthesia was verified by lack of response to toe-pinch. The static force was delivered by a calibrated custom-designed expanding spring that applied a 100 cN force to the molars. This force was selected based on previous studies demonstrating that 100 cN induces cellular activity in the maxillary sutures [25,26]. This force is not considered excessive when compared with regular vertical chewing forces of the rats that, on average, are around 54 to 76 N.

Expanding springs were fabricated from 0.016" stainless steel wires (3M Unitek, Monrovia, CA), bent into a single helix placed mesial to first molars while the arms engaged around maxillary molars (Fig. 1A). The springs were secured with flowable resin around the molars. The springs were calibrated to produce 100 cN force (50 cN on each side) using a digital force gauge. The application of static forces was carried out for 56 days, as noted above.

Animals and the integrity of the springs were monitored daily under inhalation anesthesia (isoflurane–nitrous oxide). If springs were dislodged, they were reinstalled at that time.

2.3. Periosteal stimulation

Stimulation of the periosteum was accomplished with a custom-designed appliance with two rows of four needles. The stimulation was done in an area extending from the distal of the first molar to the mesial of the third molar, bilaterally or unilaterally, at day 0 and day 28. Stimulation was accomplished by gently perforating the gums and periosteum until the needles reached the cortical bone surface.

2.4. Histology, immunohistochemistry, and fluorescence microscopy

Maxillae were demineralized in 14% ethylenediaminetetraacetic acid for 3 to 4 weeks at 4°C and dehydrated in ethanol gradients and xylene before embedding in paraffin. The sample embedded in paraffin was cut into 5 µm occlusal sections using Leica Biosystems RM2265 Fully Automated Rotary Microtome. Five consecutive sections were used for osteoclast staining.

For identification of osteoclasts, sections were immunostained using Vectastin ABC kit (Vectastin ABC kit, Vector Laboratories, Burlingame, CA) with an antibody for tartrate-resistant acid phosphatase (TRAPcP-5b; Zymed antibodies; Invitrogen, Carlsbad, CA), a marker of osteoclasts. As a negative control, sections were exposed to pre-immune serum. Sections of each sample were scanned on a Scan Scope GL series optical microscope (Aperio, Bristol, United Kingdom) and analyzed at 20× magnification.

Osteoclasts were defined as TRAP-positive multinuclear cells on the surface of cortical bone of maxillary alveolar bone between the mesial border of the middle roots of the first molars and distal border of the distal roots of the second molars. They were quantified as the mean of three measurements per section in a fixed frame (3.5 mm × 0.5 mm) (Fig. 1B). Five animals were used for each group, and two examiners completed all histological quantifications.

For fluorescence microscopy, fixed maxillae were washed overnight in running water, dehydrated in an alcohol series, cleared with xylene, and embedded in methyl methacrylate according to the method of Erben. Samples were sectioned at 5-µm thickness on an RM 2265 Leica microtome (Leica Biosystems, Buffalo Grove, IL), viewed, and photographed (Leica DMRX/E Universal Microscopy, Turboscan software, Cambridge, United Kingdom).

2.5. Micro-computed tomography imaging

The entire rat heads were collected and fixed for 72 hours with 4% (w/v) paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, followed by storage in 70% ethanol. The samples were scanned in a Scanco Micro-computed tomography (micro-CT) (µCT40, Scanco Medical AG, Bassersdorf, Switzerland). The skulls were scanned at an energy of 70 kV and intensity of 114 mA, with 300 ms integration time, resulting in a 16-mm isotropic voxel size. Results were analyzed utilizing µCT V6.0 software on the HP open platform (OpenVMS Alpha Version 1.3-1 session manager). Frontal sections of the scanned maxilla were compared at the area of mesial roots of the second molars.

The width of the palate (distance between the palatal walls at the level of intersection between palate and alveolar) was measured in the micro-CT images at the level of the mid-coronal plane of the maxillary second molar (Fig. 2A). The magnitude of unilateral tooth movement was calculated by subtracting palate width

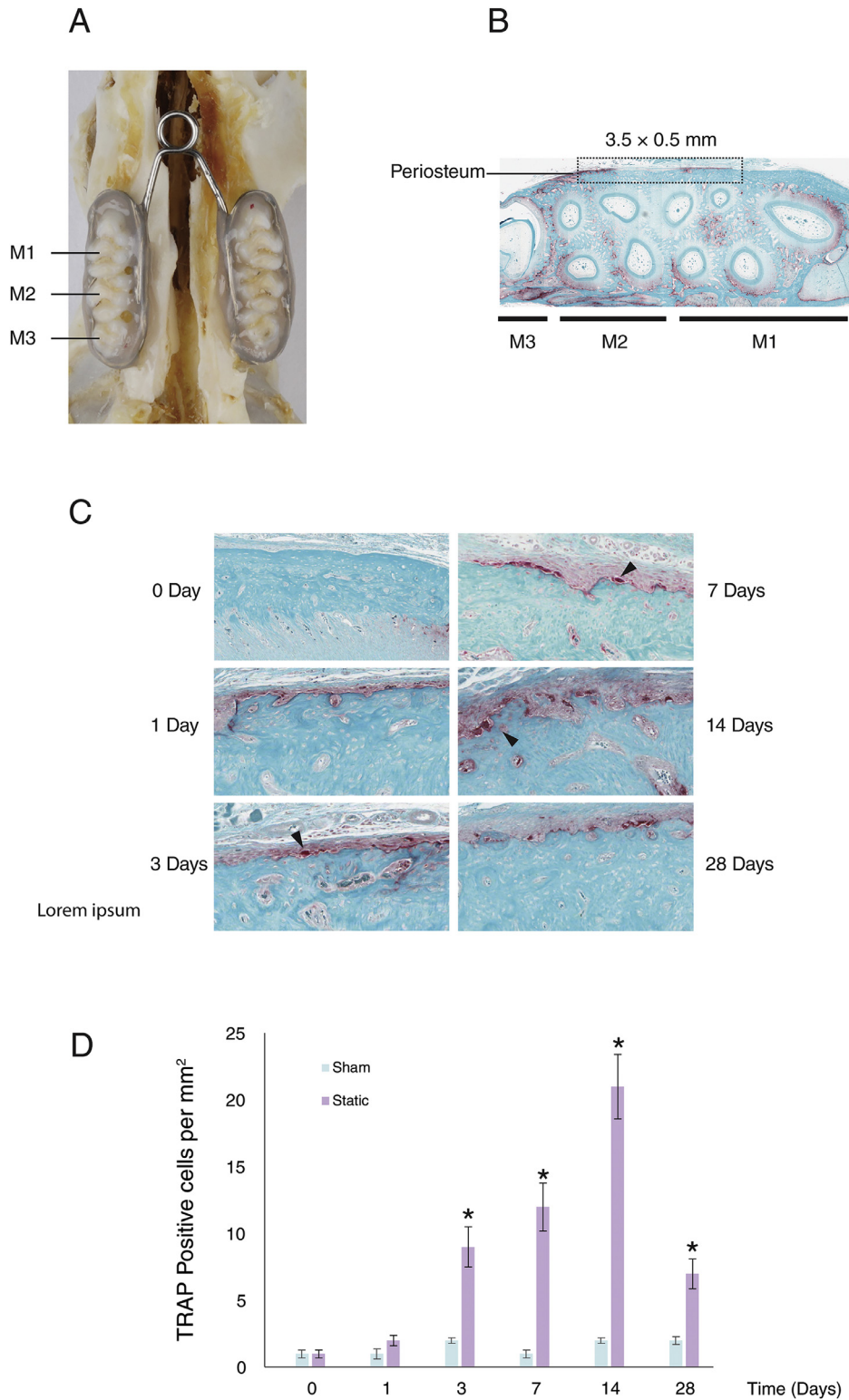
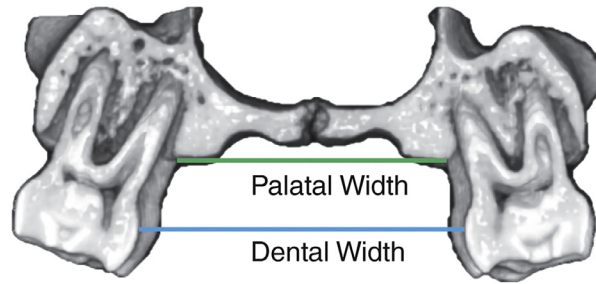
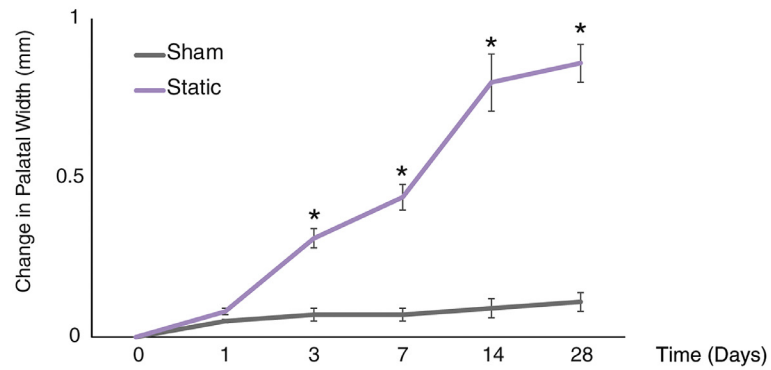


Fig. 1. Static forces increase the number of osteoclasts in periosteum. Calibrated springs used to produce static forces on the rat maxilla were fabricated from 0.016" stainless steel wires (3M Unitek, Monrovia, CA) and secured to maxillary molars by flowable resin (A). Immunohistochemistry for TRAP was performed in paraffin sections of the rat maxilla to identify active osteoclasts on the surface of the cortical maxillary bone in an area extending from the mesial border of the middle roots of the first molars and distal border of the distal roots of the second molars, in a fixed frame of 3.5 mm × 0.5mm (B). Light microphotographs show TRAP-positive osteoclasts in the surface of cortical bone at different time points (C). Osteoclasts are stained as dark red cells (black arrowheads, magnification 10×). Osteoclasts were quantified at different time points, on the cortical surface of maxillary alveolar bone in the area defined in B. Each value represents the mean ± SD of five animals (D).

A



B



C

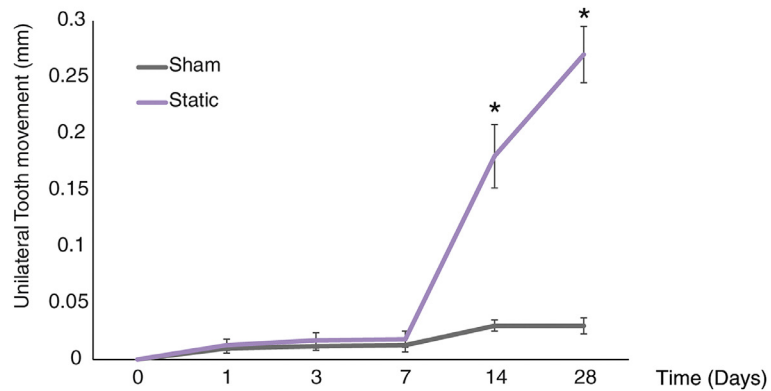


Fig. 2. Increase in osteoclasts in the periosteum follows palatal width increase but precedes tooth movement. Palatal width and dental width of the maxilla were measured using micro-CT 3D reconstructed images, and sections at the level of the mid-coronal plane of the maxillary second molar. Green line shows the width of palate (distance between the palatal walls at the level of intersection between palate and alveolar walls), and red line shows the dental width (distance between height of contour of second molars) (A). The palatal widths were measured over time in both Static and Sham maxillae (B). Unilateral tooth movement was measured as described in Methods and Materials section at different time points (C). Data expressed as the mean \pm SD of distances in mm. Each number represents the average of five samples. *Static group significantly different from sham, $P < 0.05$.

from the inter-dental width (distance between the height of contour of second molars) and dividing the difference by two (Fig. 2A).

2.6. RNA analysis

For total RNA extraction, five animals from each group were sacrificed at different time points by carbon dioxide narcosis at 24 hours, and the soft tissue surrounding the buccal surface of alveolar bone including periosteum was dissected and frozen in liquid nitrogen. After RNA extraction, Real-time polymerase chain reaction for bone and inflammatory markers was performed with primers specific for rat genes, with a QuantiTect SYBR Green RT-PCR kit (Qiagen, Valencia, CA) on a DNA Engine Optican 2 System (MJ Research, Waltham, MA). A messenger ribonucleic acid (mRNA) pool for each group was tested three times. Relative levels of mRNA were calculated and normalized to the level of GAPDH (Glyceraldehyde 3-Phosphate Dehydrogenase) and acidic ribosomal protein mRNA.

2.7. Protein analysis

Levels of inflammatory marker interleukin (IL)-1 β were measured by enzyme-linked immunosorbent assay (ELISA). The soft tissue covering the buccal cortical plate of alveolar bone was dissected from five animals, frozen in liquid nitrogen, and pulverized. Lysates were prepared, and total protein was quantitated using a BCA protein assay kit (Pierce, Rockford, IL). The concentration of IL-1 β (Thermo, Rockford, IL) was determined by ELISA. Data were analyzed in comparison with a standard curve.

2.8. Sample size calculation and statistical analysis

The sample size was calculated based on the results of our previous animal studies [25,26], assuming an estimated 50% difference in the expression of inflammatory markers and osteoblast markers. Type I error was set at 5% and the power of the statistical test was set at 90% (power = 0.9, b = 0.1). Based on this calculation, a sample size of four per group was suggested. We decided to increase the sample size to five to account for attrition.

After confirming the normal distribution of samples by the Shapiro-Wilk test, group comparisons were assessed by ANOVA. Pairwise multiple comparison analysis was performed with Tukey's post hoc test. Two-tailed *P* values were calculated; *P* < 0.05 was set as the level of statistical significance.

3. Results

3.1. Osteoclasts appear in the periosteum in response to static forces

The health status and body weight of the rats were evaluated daily, and no significant differences were observed among the groups. To investigate if static forces across the maxilla induced changes in alveolar bone, we quantified the number of TRAP-positive osteoclasts in the surface of cortical bone at different time points in an area defined along the roots of second and first molars (Fig. 1B). In response to application of transverse static forces, osteoclasts appeared in the buccal periosteum at day 1 and increased overtime from day 1 to day 28, with a peak on day 14 (Fig. 1C and D). The increase in the number of osteoclasts in the static force group at day 1 was not statistically significant in comparison with sham group (*P* > 0.05) (Fig. 1D). However, the increase in osteoclast numbers was statistically significant for all other time points, when compared with sham group (*P* < 0.01). By day 28 a trend towards a decrease in the number of osteoclasts was observed (*P* < 0.05) (Fig. 1C and D).

3.2. Osteoclast appearance in the periosteum follows palatal width increase but precedes tooth movement

In response to static transverse forces across the maxilla, the width of the palate increased, and the molars moved laterally (Fig. 2A). Palatal width increased significantly on days 3, 7, 14, and 28, with the peak increase on day 14, which in comparison with the sham group was statistically significant (*P* < 0.01) (Fig. 2B). The appearance of osteoclasts in the periosteum followed the pattern of increase in palatal width (compare Fig. 1D with Fig. 2B). However, the appearance of osteoclast in the periosteum occurred ahead of tooth movement (compare Fig. 1D with Fig. 2C) and it was not related to magnitude of tooth movement. The magnitude of unilateral tooth movement in the experimental group in comparison with the sham group was statistically significant only on days 14 and 28 (*P* < 0.01) (Fig. 2C). No significant amount of tooth movement was observed on days 1, 3, and 7 and in none of the time points studied did the tooth movement reach the cortical plate.

3.3. Activation of osteoclasts is an inflammatory-based phenomenon

We hypothesize that the change in alveolar bone position during buccal or lingual displacement in response to static forces can traumatize the periosteum and therefore, the appearance of osteoclasts in the periosteum is a trauma-based phenomenon. To investigate this hypothesis, we studied the expression of the inflammatory cytokine, IL-1 β , in the soft tissue covering the buccal cortical plate 28 days after the application of static forces (Fig. 3A). This soft tissue included the buccal periosteum. IL-1 β was present in soft tissue at all time points during static force application and its expression was significantly higher in comparison with the sham group (*P* < 0.05). Levels of this cytokine peaked twice in the periosteum, once on day 1 and again on day 14. This expression gradually decreased until day 28 (Fig. 3A).

To investigate if inflammation is the signal-stimulating osteoclast activity in the periosteum, animals were exposed to similar static forces in the presence or absence of anti-inflammatory medication. In the presence of anti-inflammatory medication, no osteoclast activity was observed in periosteum at day 7 (Fig. 3B) even though the alveolar bone was exposed to static forces.

3.4. Cortical bone resorption is followed by cortical bone formation

To evaluate if osteoclast activity at the surface of cortical bone will cause the destruction of alveolar bone, micro-CT scans were completed at days 28 and 56. While static forces caused a significant decrease in bone density of the buccal plate of the alveolar bone at day 28, the buccal cortical plate was restored by day 56 (Fig. 4A).

Gene expression studies confirmed that osteoblastic activity followed the osteoclastic activity. Indeed, expression of the early osteogenic marker, collagen type I (Fig. 4B), and the late osteogenic marker, osteocalcin (Fig. 4C) were observed in the periosteum with peak expression at day 14 or 28 respectively.

3.5. Periosteal stimulation increases inflammation and osteoclast numbers

If the increase in bone formation in the cortical plate is osteoclast-dependent, one would expect that in the presence of the same magnitude of static forces, increasing the number of osteoclasts in the area by controlled trauma to the periosteum would stimulate further cortical bone formation.

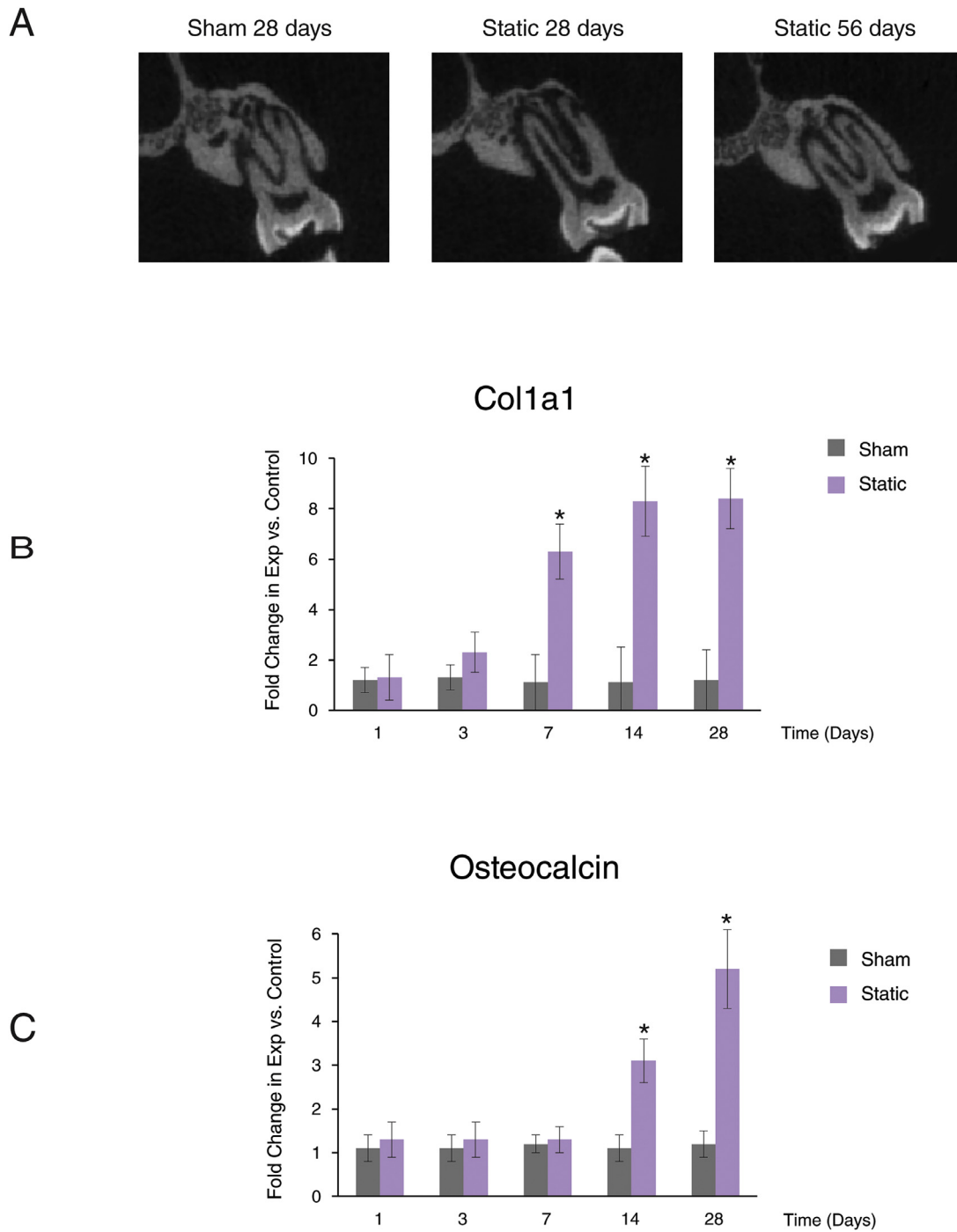


Fig. 4. Static forces activate osteoblasts in periosteum resulting in bone formation. Micro-CT images of coronal section of maxilla at the area of second molars were compared between sham and static groups. Static group demonstrate significant decrease in bone density in area of second molars at day 28. However, after 56 days, the animals that received static force showed significant reconstruction of buccal cortical plate (A). Change in expression of osteogenic markers collagen type 1 (B) and osteocalcin (C) in soft tissue covering the cortical plate of posterior teeth at different time points was measured by RT-PCR. Data are expressed as mean \pm SD “fold change” in expression in comparison with control. Each value represents the average of five samples. (*significantly different from sham group, $P < 0.05$). RT-PCR, reverse transcriptase-polymerase chain reaction

In our experimental model, the static transverse forces that are applied perpendicular to the alveolar bone activated the osteoclast on the cortical buccal plate. Further evaluation of the periosteum in the buccal cortical plate showed an increase of inflammatory markers in the periosteum, coinciding with the movement of the alveolar bone towards its periosteal envelope. It should be emphasized that the application of a static force to hemimax-

illa is significantly different from the application of static forces to long bones. If the force can only bend the bone, the magnitude of trauma to the periosteum is much less in comparison with conditions where application of a static force, in addition to the bending effect, can also displace the bone towards its periosteal envelope. In the maxilla, because of the existence of surrounding sutures, the hemimaxilla can be displaced in the direc-

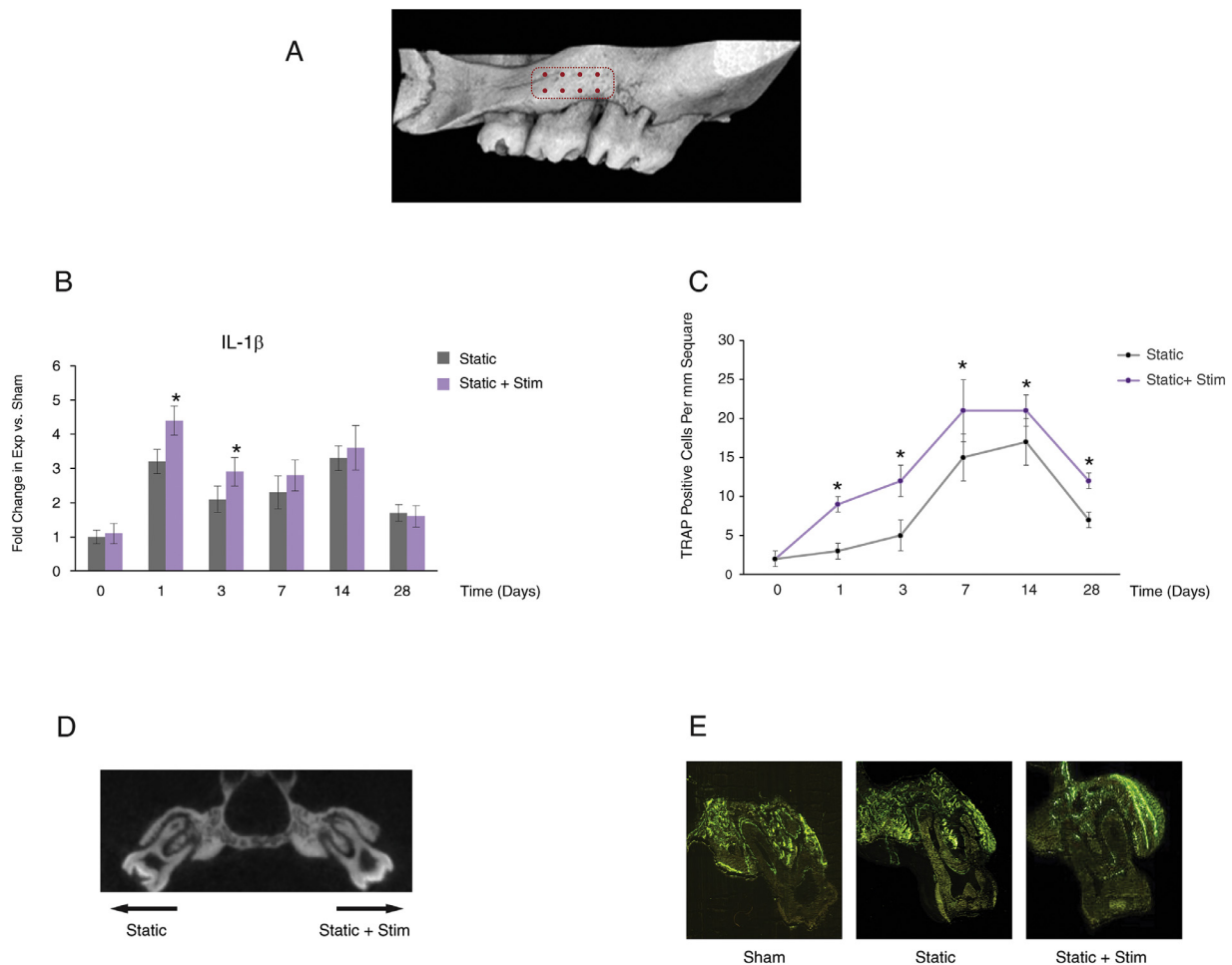


Fig. 5. Periosteal stimulation increases inflammation, osteoclasts numbers and cortical bone formation. To study the effect of osteoclast on cortical bone formation we increased the number of TRAP-positive cells in the periosteum by periosteal stimulation with small needles in the area of the second maxillary molar (A). Change in expression of IL-1 β in the soft tissue covering the cortical plate of posterior teeth was measured by RT-PCR at different time points. Data are expressed as mean \pm SD “fold change” in expression in comparison with sham. Each value represents the average of five samples. (*significantly different from static group at the same time point, $P < 0.05$) (B). Immunohistochemistry for TRAP was performed in paraffin section of both “static” and “static + stim” groups to identify TRAP-positive cells in the periosteum of maxillary alveolar bone. Graph shows mean numbers of TRAP-positive cells at different time points, in the area between the mesial border of the mesial roots of the first molars and distal border of the distal roots of the second molars. Each value represents the mean \pm SD of five animals (*significantly different from static group at the same time point, $P < 0.05$) (C). The effect of periosteal stimulation on bone formation was compared by applying static forces and stimulating periosteum in one side of the maxilla. Micro-CT image of coronal sections of maxilla in the area of second molars shows asymmetrical bone formation 56 days after stimulation (D). Representative fluorescent microscopy images of coronal section of maxilla of Sham, Static, and Static + Stim at the area of second molars at day 56 show significant increase in fluorescence in response to periosteal stimulation. (E). Bone labeling was performed by Calcein Green on days 0, 28, and 54. RT-PCR, reverse transcriptase-polymerase chain reaction

tion of force. This movement follows the pattern of the opening of the suture as we have previously demonstrated [25,26]. In the current experiments, the inflammatory markers peaked at the time this movement was maximum. This observation suggests that the periosteum is traumatized by the displacement of the alveolar bone.

Here, we demonstrated that static forces applied to the cortical bone can stimulate osteoclasts in the periosteum through an inflammatory reaction. The reaction of the periosteum in these experiments was similar to the reaction of the periodontal ligament during orthodontic tooth movement when static orthodontic forces stimulate osteoclast recruitments and activity all around the roots of the moving tooth [29]. Similar to what we observed in our tooth movement model, the presence of these cells in the periosteum could be inhibited by anti-inflammatory drugs.

To further investigate the role of trauma to the periosteum, caused by maxillary displacement, in the appearance of osteoclasts

we investigated if this phenomenon was related to the teeth moving. Because the static transverse forces in our model were applied through the teeth to the alveolar bone it has been assumed that movement of the tooth toward the buccal cortical plate will push the tooth out of the alveolar bone and cause resorption of the buccal cortical plate [30,31]. However, our observations reject this hypothesis because osteoclast appearance occurred much earlier than tooth movement and even occurred in the area of the maxilla where there were no teeth (data not shown). Furthermore, in none of our samples did the teeth reach to buccal cortical plate in 28 days.

Next, we evaluated if, after static force application, osteoclast activation, and bone resorption, the cortical bone would be re-stored. As expected, osteoblasts were indeed, activated after a delay and created a new buccal cortical plate. This new cortical plate, in comparison with the original position of the buccal cortical plate, showed a lateral drift and an increase in size, with no loss of cortical bone in its horizontal or vertical dimensions. This phenomenon

is similar to bone formation observed during chronic pathologies such as cysts and tumors [20–22].

Two different bone movements should be identified in these studies. First, the displacement of the cortical plate laterally because of movement of the hemimaxilla in the same direction of the static force which occurs after the opening of the sutures. This is accompanied by an increase in the width of the palate observed in a cross-section of alveolar bone and palate [25,26]. This movement is not considered cortical drifting. Second, is the drift of the cortical bone laterally that can be observed in the fluorescent microscopy images. This cortical drifting was not observed in the first month after application of static forces, but was visible in the second month, which argues that bone formation on the cortical plate is a delayed phenomenon. This observation has two new important clinical applications. First, the buccal cortical plate can go through significant drifting and is not a fixed boundary of the jaws as previously believed. Second, the formation of a new buccal cortical plate is a delayed reaction, and perhaps the clinical radiographic evaluation of this bone should not be completed right after the application of static forces but, especially in humans, should be evaluated months after the application of the force. It is logical to assume that if the formation of cortical bone is a delayed reaction, then the area should not be disturbed surgically during this phase of modeling.

To further demonstrate that bone formation in the periosteum was related to osteoclast activity, we increased the number of osteoclasts at the surface of bone by direct trauma to the periosteum. This is important because while we show that static forces produced by displacement of bone can traumatize the periosteum and stimulate osteoclast activity, we did not explain how bone formation in response to the static forces occurred.

Previous studies demonstrated that osteoclasts, by secreting different factors such as $TGF\beta 1$, can recruit the osteoblasts to the bone surface and increase bone formation [32–38]. Furthermore, our findings are in agreement with studies that demonstrated that the adaptation of cortical bone to mechanical stimulation originates from periosteum [33,39–41].

However, in previous studies, the mechanical stimulation was dynamic, while in our studies, we focused mostly on static forces, which had not been studied before.

Our study can explain previous observations that show that the periosteum plays a significant role in the growth of the cortical bone [42–45]. Forces produced by growth are partly static forces because of increases in the mass of soft tissues, changes in the rest position of muscles, or many other examples, and partly dynamic forces because of an increase in the magnitude of forces of muscles during function. In this study we did not investigate the effect of dynamic forces on the periosteum but, we demonstrated that the static forces, as long as they are applied slowly, can have an osteogenic effect on cortical bone, a mechanism that is perhaps used during growth of bone, regardless whether it is physiological or pathological growth. It is interesting to mention that a similar mechanism has been observed during endochondral growth, where the periosteum and perichondrium interact with cartilage and affect cartilage growth [46,47].

Cortical drifting has been reported during the growth of the skeleton when different patterns of osteoclast and osteoblast activity have been reported on different surfaces of cortical bone [48]. Because muscles and soft tissue are connected to bone through sharp fibers [49] crossing the periosteum to the bone, one cannot help but think that an increase in size and function of soft tissue, perhaps by increasing the magnitude of static and dynamic forces on the surface of the bone, increase insult on the periosteum and therefore stimulate bone formation. This is very important clinically,

as it provides evidence supporting the hypothesis that growth of cortical bone depends on the growth of soft tissues [50–52]. More studies in this area are necessary to further understand this important phenomenon.

Due to the limited length of our study we were not able to investigate the long-term changes in the shape of the alveolar bone, but we believe that the changes in morphology of bone occurring during application of static forces and periosteum remodeling may be maintained afterward by normal function of muscles and jaw activity. In presence of normal function, the bone restores its original shape as has been observed during fracture healing of long bones [53]. In absence of supporting function, there is no need for the body to maintain the new bone. Therefore, we hypothesize that periosteal stimulation and cortical bone formation may result in stable new bone formation when accompanied by proper change in function. At this moment we do not have enough data to support this view.

Understanding the mechanism of periosteal stimulation during application of static forces also can explain the mechanism of bone formation during distraction osteogenesis where periosteum is exposed to relatively static forces [54].

Our research demonstrates how both dynamic and static forces by influencing different targets can reshape the cortical form [2]. Cortical bone is the main factor in sculpturing the general form of our skeleton. Therefore, different disciplines of science, when studying the form of the skeleton should pay attention not only to dynamic forces and their direct effect on bone, but should also consider static forces that indirectly affect the cortical bone and therefore our form.

5. Conclusion

In conclusion, while osteocytes and periosteum osteoclasts can play a significant role in recognizing dynamic mechanical stimulation directly and initiate adaptation of the skeleton, inflammation-based activation of osteoclasts plays a significant role in the response of cortical bone to static forces. In both cases, periosteum contains all the required progenitor cells to allow these mechanical bone adaptations. This study demonstrates that cortical plate of the alveolar bone can be remodeled in adult rats in response to the static forces. Static forces induce a transient inflammatory reaction in the periosteum, followed by activation of osteoclasts independent of the magnitude of tooth movement. Bone formation in response to static forces is a delayed phenomenon during which osteoblasts restore the cortical bone, resulting in lateral movement of the cortical plate in space, referred to as cortical drifting. Furthermore, localized periosteal stimulation can enhance cortical drifting during static force application.

There are two main aspects to the innovations discussed in this article. First, we propose a new mechanism for how bones respond to static force, and their mechanism of adaptation, suggesting a role for cortical drifting during growth and the creation of our final skeletal form. Second, this study demonstrates for the first time how periosteal stimulation (using small needles) amplifies the bone formation at the surface of the cortical bone and can be used to promote cortical drifting and reshaping of the cortical bone, which can have a significant impact in clinical orthodontics and dentofacial orthopedics.

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CRedit author statement

CT and MA were responsible for conceptualization of the project, securing funding, developing the research methodology, supervision of all experiments, data analysis, writing, and editing the manuscript; FA and MA were responsible for project administration, executing experiments, and data collection; SPO was responsible for creating and testing the device for periosteal stimulation; CS had a role in the writing, creating figures, reviewing and editing of the manuscript.

References

- [1] Rubin C, Turner AS, Bain S, Mallinckrodt C, Anabolism McLeod K. Low mechanical signals strengthen long bones. *Nature* 2001;412:603–4.
- [2] Alikhani M, Khoo E, Alyami B, et al. Osteogenic effect of high-frequency acceleration on alveolar bone. *J Dent Res* 2012;91:413–19.
- [3] Frost HM. Wolff's law and bone's structural adaptations to mechanical usage: an overview for clinicians. *Angle Orthod* 1994;64:175–88.
- [4] Sugiyama T, Price JS, Lanyon LE. Functional adaptation to mechanical loading in both cortical and cancellous bone is controlled locally and is confined to the loaded bones. *Bone* 2010;46:314–21.
- [5] Tomlinson RE, Li Z, Li Z, et al. NGF-TrkA signaling in sensory nerves is required for skeletal adaptation to mechanical loads in mice. *Proc Natl Acad Sci U S A* 2017;114:E3632–41.
- [6] Brodt MD, Silva MJ. Aged mice have enhanced endocortical response and normal periosteal response compared with young-adult mice following 1 week of axial tibial compression. *J Bone Miner Res* 2010;25:2006–15.
- [7] Johannesdottir F, Thrall E, Muller J, Keaveny TM, Kopperdahl DL, Bouxsein ML. Comparison of non-invasive assessments of strength of the proximal femur. *Bone* 2017;105:93–102.
- [8] Alikhani M, Lopez JA, Alabdullah H, et al. High-frequency acceleration: therapeutic tool to preserve bone following tooth extractions. *J Dent Res* 2016;95:311–18.
- [9] Srinivasan S, Balsiger D, Huber P, et al. Static preload inhibits loading-induced bone formation. *JBM Plus* 2019;3:e10087.
- [10] Kazakia GJ, Tjong W, Nirody JA, et al. The influence of disuse on bone microstructure and mechanics assessed by HR-pQCT. *Bone* 2014;63:132–40.
- [11] Rubin CT, Lanyon LE. Regulation of bone mass by mechanical strain magnitude. *Calcif Tissue Int* 1985;37:411–17.
- [12] Pollack SR, Salzstein R, Pienkowski D. Streaming potentials in fluid-filled bone. *Ferroelectrics* 1984;60:297–309.
- [13] Weinbaum S, Cowin SC, Zeng Y. A model for the excitation of osteocytes by mechanical loading-induced bone fluid shear stresses. *J Biomech* 1994;27:339–60.
- [14] Oxlund BS, Ørtoft G, Andreassen TT, Oxlund H. Low-intensity, high-frequency vibration appears to prevent the decrease in strength of the femur and tibia associated with ovariectomy of adult rats. *Bone* 2003;32:69–77.
- [15] Qin YX, Kaplan T, Saldanha A, Rubin C. Fluid pressure gradients, arising from oscillations in intramedullary pressure, is correlated with the formation of bone and inhibition of intracortical porosity. *J Biomech* 2003;36:1427–37.
- [16] Malone AMD, Batra NN, Shivaram G, et al. The role of actin cytoskeleton in oscillatory fluid flow-induced signaling in MC3T3-E1 osteoblasts. *Am J Physiol Cell Physiol* 2007;292:C1830–6.
- [17] Garman R, Gaudette G, Donahue LR, Rubin C, Judex S. Low-level accelerations applied in the absence of weight bearing can enhance trabecular bone formation. *J Orthop Res* 2007;25:732–40.
- [18] Robling AG, Turner CH. Mechanical signaling for bone modeling and remodeling. *Crit Rev Eukaryot Gene Expr* 2009;19:319–38.
- [19] Robling AG, Duijvelaar KM, Geevers JV, Ohashi N, Turner CH. Modulation of appositional and longitudinal bone growth in the rat ulna by applied static and dynamic force. *Bone* 2001;29:105–13.
- [20] Waldman S, Shimonov M, Yang N, et al. Benign bony tumors of the paranasal sinuses, orbit, and skull base. *Am J Otolaryngol* 2022;43:103404.
- [21] Sarrafpour B, El-Bacha C, Li Q, Zoellner H. Roles of functional strain and capsule compression on mandibular cyst expansion and cortication. *Arch Oral Biol* 2019;98:1–8.
- [22] High AS, Hirschmann PN. Symptomatic residual radicular cysts. *J Oral Pathol* 1988;17:70–2.
- [23] Raab-Cullen DM, Akhter MP, Kimmel DB, Recker RR. Bone response to alternate-day mechanical loading of the rat tibia. *J Bone Miner Res* 1994;9:203–11.
- [24] Meade JB, Cowin SC, Klawitter JJ, Van Buskirk WC, Skinner HB. Bone remodeling due to continuously applied loads. *Calcif Tissue Int* 1984;36(1 Suppl. 1):S25–30.
- [25] Mani Alikhani SA, Al Jearah MM, Gadhavi N, et al. Osteoclasts: the biological knife in sutural responses to mechanical stimulation. *Innovation* 2018;1:e1.
- [26] Alikhani M, Alansari S, Al Jearah MM, et al. Biphasic sutural response is key to palatal expansion. *J World Fed Orthod* 2019;8:9–17.
- [27] Erben RG. Embedding of bone samples in methylmethacrylate: an improved method suitable for bone histomorphometry, histochemistry, and immunohistochemistry. *J Histochem Cytochem* 1997;45:307–13.
- [28] Frost HM. Mechanical determinants of bone modeling. *Metab Bone Dis Relat Res* 1982;4:217–29.
- [29] Teixeira CC, Khoo E, Tran J, et al. Cytokine expression and accelerated tooth movement. *J Dent Res* 2010;89:1135–41.
- [30] Cardinal L, da Rosa Zimmermann G, Mendes FM, Andrade Jr I, Oliveira DD, Dominguez GC. Dehiscence and buccal bone thickness after rapid maxillary expansion in young patients with unilateral cleft lip and palate. *Am J Orthod Dentofacial Orthop* 2022;162:16–23.
- [31] Lin L, Ahn HW, Kim SJ, Moon SC, Kim SH, Nelson G. Tooth-borne vs bone-borne rapid maxillary expanders in late adolescence. *Angle Orthod* 2015;85:253–62.
- [32] Deng R, Li C, Wang X, et al. Periosteal CD68+ F4/80+ macrophages are mechanosensitive for cortical bone formation by secretion and activation of TGF- β 1. *Adv Sci (Weinh)* 2022;9:e2103343.
- [33] Gao B, Deng R, Chai Y, et al. Macrophage-lineage TRAP+ cells recruit periosteum-derived cells for periosteal osteogenesis and regeneration. *J Clin Invest* 2019;129:2578–94.
- [34] Cho SW, Soki FN, Koh AJ, et al. Osteal macrophages support physiologic skeletal remodeling and anabolic actions of parathyroid hormone in bone. *Proc Natl Acad Sci U S A* 2014;111:1545–50.
- [35] Chang MK, Raggatt LJ, Alexander KA, et al. Osteal tissue macrophages are intercalated throughout human and mouse bone lining tissues and regulate osteoblast function in vitro and in vivo. *J Immunol* 2008;181:1232–44.
- [36] Sinder BP, Pettit AR, McCauley LK. Macrophages: their emerging roles in bone. *J Bone Miner Res* 2015;30:2140–9.
- [37] Vi L, Baht GS, Whetstone H, et al. Macrophages promote osteoblastic differentiation in-vivo: implications in fracture repair and bone homeostasis. *J Bone Miner Res* 2015;30:1090–102.
- [38] Wang B, Pourshafeie A, Zitnik M, et al. Network enhancement as a general method to denoise weighted biological networks. *Nat Commun* 2018;9:3108.
- [39] Debnath S, Yallowitz AR, McCormick J, et al. Discovery of a periosteal stem cell mediating intramembranous bone formation. *Nature* 2018;562:133–9.
- [40] Duchamp de Lageneste O, Julien A, Abou-Khalil R, et al. Periosteum contains skeletal stem cells with high bone regenerative potential controlled by Periostin. *Nat Commun* 2018;9:773.
- [41] Zannit HM, Silva MJ. Proliferation and activation of Osterix-lineage cells contribute to loading-induced periosteal bone formation in mice. *JBM Plus* 2019;3:e10227.
- [42] Foolen J, van Donkelaar CC, Ito K. Intracellular tension in periosteum/perichondrium cells regulates long bone growth. *J Orthop Res* 2011;29:84–91.
- [43] Gigante A, Chillemi C, Quaglino D, Miselli M, Pasquali-Ronchetti I. DL-penicillamine induced alteration of elastic fibers of periosteum-perichondrium and associated growth inhibition: an experimental study. *J Orthop Res* 2001;19:398–404.
- [44] Schumacher B, Albrechtsen J, Keller J, Flyvbjerg A, Hvid I. Periosteal insulin-like growth factor I and bone formation. Changes during tibial lengthening in rabbits. *Acta Orthop Scand* 1996;67:237–41.
- [45] Kuijpers-Jagtman AM, Bex JH, Maltha JC, Daggars JG. Longitudinal growth of the rabbit femur after vascular and periosteal interference. *Anat Anz* 1988;167:349–58.
- [46] Bertram JEA, Polevoy Y, Cullinane DM. Mechanics of avian fibrous periosteum: tensile and adhesion properties during growth. *Bone* 1998;22:669–75.
- [47] Crilly RG. Longitudinal overgrowth of chicken radius. *J Anat* 1972;112:11–18.
- [48] Enlow DH, Moyers RE, Merow WW. *Handbook of facial growth*. 2nd ed. Philadelphia, PA: W B Saunders Co. Ltd.; 1982.
- [49] Evans SF, Chang H, Knothe Tate ML. Elucidating multiscale periosteal mechanobiology: a key to unlocking the smart properties and regenerative capacity of the periosteum? *Tissue Eng Part B Rev* 2013;19:147–59.
- [50] Bromage TG. The functional matrix hypothesis revisited. 5. Orofacial capsular matrices defined. *Innovation* 2022;1:e1.
- [51] Botzenhart UU, Keil C, Tsagkari E, Zeidler-Rentsch I, Gredes T, Gedrange T. Influence of botulinum toxin A on craniofacial morphology after injection into the right masseter muscle of dystrophin deficient (mdx-) mice. *Ann Anat* 2021;236:151715.
- [52] Moss-Salentijn L, Melvin L. Moss and the functional matrix. *J Dent Res* 1997;76:1814–17.
- [53] Egawa S, Miura S, Yokoyama H, Endo T, Tamura K. Growth and differentiation of a long bone in limb development, repair and regeneration. *Dev Growth Differ* 2014;56:410–24.
- [54] Kadlub N, Dallard J, Kogane N, Galliani E, Boisson J. Mandibular magnetic distractor: preclinical validation. *Br J Oral Maxillofac Surg* 2022;60:767–72.