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Midpalatal suture expansion: an in vivo histological and immunohistochemical study of the impact of force magnitude

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ABSTRACT

Although there is sufficient clinical evidence regarding the effectiveness of rapid expansion of the middle palate, some of the biological responses to variables such as the magnitude of the expansive force are still unknown. ***Aim*****:** *The aim of this study was to conduct a qualitative and quantitative analysis of the response of midpalatal suture tissues in growing rats exposed to expansive forces of different magnitudes.* ***Materials and Method:*** *Twenty-four 7-week-old male Wistar rats were divided into 3 groups: a control group (C) and two experimental groups to which 60 g or 90 g expansive forces were applied for 7 days (E60 and E90, respectively). Frontal sections of the upper maxillae were stained with haematoxylin-eosin, Masson's trichrome and PAS-Alcian Blue. The expression of runt-related transcription factor 2 (Runx2) and receptor activator of nuclear factor kappa B ligand (RANKL) was evaluated by immunohistochemical staining.* ***Results:*** *The degree of expansion and the biological response of the cells involved in the suture ossification process were evaluated histomorphometrically. ANOVA was used for statistical comparisons. Both the expansive forces applied caused significant increases in maxillary width and suture area. Group E60 showed qualitative changes in the composition of the cartilage extracellular matrix, a significant increase in the percentage of Runx2+ mesenchymal-like cells, and a significant reduction in the number of RANKL+ chondrocytes/mm².* ***Conclusions*****:** *These results show that lower expansive forces would stimulate osteogenesis in a direct manner not associated to the endochondral ossification of suture secondary cartilage, enabling partial elucidation of how bone resorption and formation are regulated during rapid maxillary expansion.*

Keywords: *maxillary expansion - sutures - orthodontic force - animal models - histology*

Expansión de la sutura palatina: Estudio histológico e inmunohistoquímico *in vivo* del impacto de la magnitud de la fuerza

RESUMEN

Existen suficientes pruebas clínicas sobre la eficacia de la expansión rápida del paladar medio; sin embargo, aún se desconocen algunas de las respuestas biológicas a variables como la magnitud de la fuerza expansiva. ***Objetivo*****:** *El objetivo de este estudio fue realizar un análisis cualitativo y cuantitativo de la respuesta de los tejidos de la sutura medio-palatina en ratas en crecimiento expuestas a fuerzas expansivas de diferentes magnitudes.* ***Métodos*****:** *Veinticuatro ratas Wistar macho de siete semanas de edad fueron divididas en tres grupos: un grupo de control (C) y dos grupos experimentales a los que se aplicaron fuerzas expansivas de 60 g o 90 g respectivamente durante siete días (E60 y E90). Se obtuvieron cortes frontales del maxilar superior que fueron teñidos con hematoxilina-eosina (H&E), tricrómico de Masson y PAS-Alcian Blue. También se evaluó la expresión del factor de transcripción Runx2 y del ligando activador del receptor nuclear kappa B (RANKL) mediante inmunohistoquímica.* ***Resultados*****:** *se evaluaron histomorfométricamente el grado de expansión y la respuesta biológica de las células implicadas en el proceso de osificación de la sutura. Se utilizó ANOVA para las comparaciones estadísticas. Ambas fuerzas expansivas aplicadas provocaron aumentos significativos en el ancho maxilar y el área de la sutura. El grupo E60 mostró cambios cualitativos en la composición de la matriz extracelular del cartílago, un aumento significativo en el porcentaje de células tipo-mesenquimales Runx2+ y una reducción significativa en el número de condrocitos RANKL+/mm2.* ***Conclusiones*****:** *estos resultados muestran que las fuerzas expansivas más leves estimularían la osteogénesis de forma directa, sin relación con la osificación endocondral del cartílago secundario de la sutura, lo que permite elucidar parcialmente cómo se regulan la resorción y la formación óseas durante el proceso de la expansión rápida del maxilar.*

Palabras clave: *expansión maxilar – suturas - fuerza ortodóntica - modelos animales - histología*

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INTRODUCTION

In orthodontics, the usual treatment for narrow upper maxilla is rapid midpalatal expansion (RME), which involves opening the midpalatal suture, with ensuing formation of new bone tissue[1,2]. Although there is sufficient clinical evidence on the effectiveness of this procedure[3,4], it is of particular interest to learn more about the biological response of underlying tissues to the different variables involved, such the magnitude of the expansive force, the time for which the force is applied, the activation protocols, and patient age at the time of implementation. Expansion protocols may use stronger forces for quick expansion or lighter forces for stable expansion.

Various experimental animal models of midpalatal suture expansion have been developed to evaluate the effect of this treatment[5-7]. The present study applied a model of midpalatal suture expansion in rats using a stainless-steel wire spring which generates a calibrated preload expansive force that is equivalent over the entire zone of application.

Histologically, the midpalatal suture in rodents consists of secondary cartilage containing mesenchymal-like cells and osteochondroprogenitor cells[8]. The latter have high proliferative activity and the unique ability to differentiate into chondrocytes or osteoblasts, depending on external biomechanical factors[9]. The midpalatal suture responds to lighter expansive forces by stimulating osteogenic differentiation of this secondary cartilage, generating endochondral ossification[9,10]. Initially, the secondary cartilage present in the suture opens and separates towards the laterals of the palatal bone with active proliferation of chondrocytes. Subsequently, osteoprogenitor cells of the periosteum migrate towards the interior of the suture and then differentiate into osteoblasts[6,10,11].

The endochondral ossification process also involves recruitment of osteoclasts to resorb hypertrophied calcified cartilage, which will subsequently be replaced by new bone. Important in this mechanism are factors that regulate bone resorption and formation such as RANKL and transcription factor Runx2. Hypertrophic chondrocytes express RANKL, thereby regulating precursor recruitment and osteoclast formation[12]. Maxillary suture expansion yields increased expression of the bone remodelling markers such as RANK, RANKL and OPG[13,14]. Runx2 regulates the final differentiation of chondrocytes during endochondral ossification by inducing the passage from chondrocyte in proliferation to hypertrophic chondrocyte, which is necessary for this ossification to proceed normally. This is why Runx2, through activation of the Wnt/β-catenin signalling pathway, is the main transcriptional factor associated with osteoblastic differentiation and endochondral ossification[15,16]. Cartilage extracellular matrix components mediate suture expansion osteogenesis through the Wnt/β-catenin signalling activated Runx2 pathway in osteoblasts[17].

Many of the cell responses in the osteogenesis process triggered by expansive forces, and whether lower-magnitude forces might generate a preferable response pattern, remain unknown to date. Therefore, to better understand the cell responses involved in expansion, as well as the effect of expansive forces of different magnitudes on the response of suture cartilage, the aim of this study was to conduct a qualitative and quantitative analysis of the response of midpalatal suture tissues in growing rats exposed to expansive forces of different magnitudes.

MATERIALS AND METHOD

Experimental units

Twenty-four 7-week-old male Wistar rats (250 to 270g bw) were randomly divided into 3 groups: a control group (C, n=8) and two experimental groups in which rats were equipped with an expansion spring exerting an initial force of either 60 g (E60, n=8) or 90 g (E90, n=8). The rats were housed in galvanized steel cages, with three animals per cage, at 21–24 ºC and 52–56% humidity, under 12h light/dark cycles. The animals had free access to food (standard diet rat-mouse chow, Cooperación) and water.

The experimental protocol was approved by the Ethics Committee of Buenos Aires University's School of Dentistry (Res CICUAL 003/2023) and complied with the ARRIVE guidelines and the National Institutes of Health Guide for the Care and Use of Laboratory Animals[18].

Maxillary expansion protocol

The expansive forces were achieved by means of an orthodontic appliance consisting of a stainless-steel wire spring 30 mm long (Strainght Wire Ortho Organizers, Inc., USA) with an expansion loop

in the centre that determined a 10 mm aperture at its free ends (Fig. 1a-b). Springs made of .014" and .016" stainless-steel wires receive a preload force of approximately 60g (E60) and 90g (E90), respectively. The force was calibrated with an *ad hoc* device consisting of an electronic scale and a dynamometer.

The appliances were installed while the animals were under general anaesthesia by intraperitoneal (IP) injection of ketamine at a dose of 50 mg/kg bw (5 %) and xylazine at a dose of 20 mg/kg bw (2%).

The springs used to generate expansive forces on the midpalatal suture were attached to the palatal surfaces of the upper molars using the direct bonding technique. The procedure for installing the springs involved cleaning the molars manually with pumice powder and a brush, rinsing with water, aspiration, drying with air jet, and acid etching with 37% phosphoric acid on the palatal surfaces of the first, second and third right and left molars of the maxilla. The expander spring was positioned with the loop towards the incisors. Light-curing adhesive (Transbond XT, 3M Unitek) was used with its applicable primer (MIP, 3M Unitek), covering the palatal and occlusal molar surfaces. The animals in group C underwent the same procedures except for installation of the expander spring.

Seven days after installation, the animals were anesthetized by intraperitoneal injection of xylazine (5 mg/kg, König Laboratory) and ketamine (50 mg/kg, Holliday Laboratory) and euthanized by intracardiac injection of 0.2 ml of euthanyl (Brouwer Laboratory). The upper maxillae were resected for further processing.

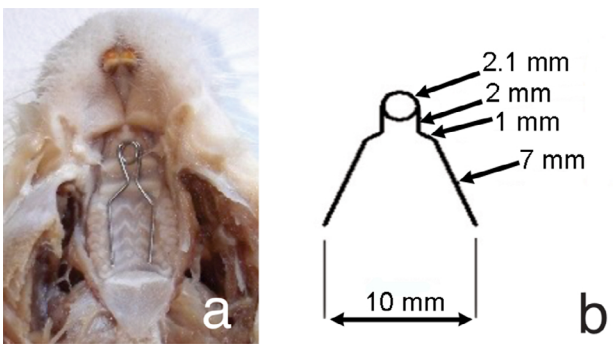

Fig. 1: Methodology: Maxillary expansion
a) Occlusal view of rat upper maxilla with device installed, resected post-euthanasia. b) Diagram showing the attached loop and force direction. The diagram shows the original design with the measurements in millimeters for each section of the spring.

Sample processing

Specimens were fixed in 10% formaldehyde-buffer solution pH 7.4 at 4 ºC and then decalcified in 4M EDTA in 0.4M NaOH (Anedra) pH 7.2 at 4 ºC for 60 days, after which they were processed histologically and embedded in paraffin. Frontal sections 7-8 µm thick were cut at the level of the first upper molars. Histological sections were stained with haematoxylin-eosin (H&E), Masson's trichrome and Periodic Acid-Schiff-Alcian blue stain (PAS-AB). The expression of runt-related transcription factor 2 (Runx2) and of receptor activator of nuclear factor kappa B ligand (RANKL) in the suture tissue was evaluated by immunohistochemical staining.

The sections for immunohistochemical examination were deparaffinized in xylol for 15 min and hydrated in decreasing concentrations of ethyl-alcohol to a final concentration of 70%. For antigen retrieval, the sections were incubated with 0.1% trypsin in tris-maleate buffer, pH 7, at 37 °C for 10 min. The sections were then incubated in 3% hydrogen peroxide in methanol to block endogenous peroxidase. To block nonspecific binding, excess fluid was drained, and the sections were dried and incubated with 1% BSA in PBS 0.01M in a humidity chamber at room temperature for 1 h. The sections were then washed in 0.01M PBS for 5 min, and in normal horse serum (Vector Laboratories, Burlingame, CA, USA) for 10 min. The reaction was detected using a peroxidase-biotin streptavidin system (PK-7800, Vector Laboratories, Burlingame, CA, USA). After that, the sections were incubated in streptavidin peroxidase complex in a humidity chamber at room temperature for 5 min and washed twice in 0.01M PBS at 50 rpm for 5 min each time. The primary antibody was developed with DAB brown stain (DAB substrate kit for peroxidase, SK-4100, Vector Laboratories, Burlingame, CA, USA), and haematoxylin (RANKL) and methyl green (Runx2) counterstain, following the manufacturer's instructions.

RANKL was detected by incubation with primary anti-rat anti-RANKL antibody (AF462, R&D Systems Inc., Minneapolis, MN, USA) at a concentration of 5 µg/mL. RUNX2 was detected by incubation with primary anti-RUNX2 antibody ([EPR14334] Abcam, UK) 1:1000 dilution in PBS 0.01M, in a humidity chamber at 4 °C for 12 h. Negative controls were created by replacing the primary antibody with normal serum (BioGenex

Lab., CA, USA). Bone specimens with proven positive staining by our laboratory were used as positive control.

Histological and histomorphometric evaluation

Photomicrographs of the histological sections were acquired using a light-field microscope (Axioskop 2; Carl Zeiss, Jena, Germany) and a digital camera (Nikon CoolPix 12 Mp, Japan), and analysed histomorphometrically with Image Pro Plus Software®, version 5.1 (Media Cybernetics).

Histological sections cut at the level of the middle roots of the first upper molars were analyzed by light microscopy to define the study area and identify the sites where the measurements were performed (Fig. 2):

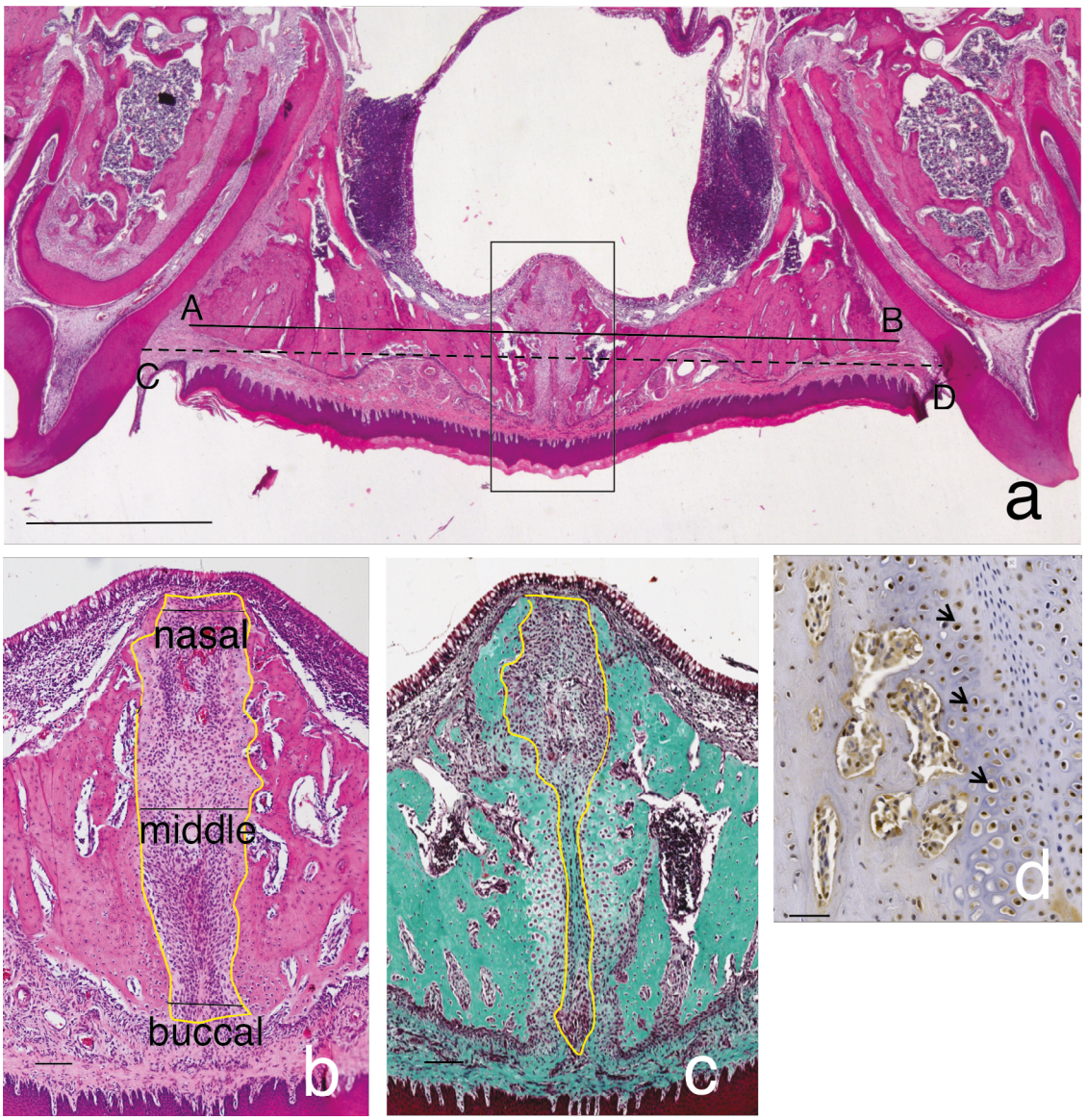

Fig. 2: Methodology: Histomorphometry
a) Photomicrograph of a section at the level of the middle roots of the first upper molars showing right and left molars and the study area of the midpalatal suture (rectangle) observed by light microscopy. H&E, original magnification 10X. Measurements: Maxillary width (µm): continuous line between points A and B, Intermolar width (µm): dashed line between points C and D. Scale 1000 µm. b) Photomicrograph of a section of the midpalatal suture. H&E, original magnification 20X. Measurements: Suture area (µm2): area marked by the yellow perimeter, Suture width (µm): measured at three different levels: nasal, middle and buccal. Scale 100 µm. c) Photomicrograph of a section of the midpalatal suture. Masson's trichrome, original magnification 20X. Measurements: Fibrous area (µm2): area marked by the yellow perimeter. Scale 100 µm. d) Photomicrograph of a section of the midpalatal suture. Runx2 immunostaining contrasted with haematoxylin, original magnification 40X. Chondrocytes with brown stained nuclei were considered positive (arrow). Scale 50 µm.

- Transverse expansion, determined by measuring maxillary and intermolar widths:
  a) Maxillary width (μm): distance between the points determined at the level of the bone crests of the palatal alveolar cortical plates of the right and left first molars (point A to point B) (Fig. 2a)
  b) Intermolar width (μm): distance between the points determined at the level of the cementoenamel junctions on the palatal surfaces of the right and left first molars (point C to point D) (Fig. 2a)
- Suture area (μm^2) (Fig. 2b)
- Suture width (μm) (Fig. 2b) measured at three different levels: nasal, middle and buccal
- Fibrous area (μm^2) (Fig. 2c): fibrous tissue inside the suture area.

Immunohistochemical staining was quantified twice by one examiner with standardized training. The following parameters were determined on immunohistochemically stained sections by direct visualization under a light microscope:

- % Runx2+ C: The percentage of Runx2-positive chondrocytes in the cartilage area was calculated based on the total number of chondrocytes (Runx2-positive plus Runx2-negative chondrocytes). Chondrocytes with brown stained nuclei were considered positive. An average of 60-80 chondrocytes from the total suture cartilage were analysed per sample (Fig. 2d).
- N Runx2+C/mm^2: The number of Runx2-positive chondrocytes per mm^2 in the cartilage area was counted.
- Percentage of Runx2+ (% Runx2+ MC): The percentage of Runx2-positive mesenchymal-like cells in the fibrous area of the suture was calculated based on the total number of mesenchymal-like cells (Runx2-positive plus Runx2-negative mesenchymal-like cells). An average of 120-160 mesenchymal-like cells from the total suture fibrous area were analysed per sample.
- N Runx2+MC/mm^2: The number of Runx2-positive mesenchymal-like cells per mm^2 in the cartilage area was counted.
- N RANKL+C/mm^2: The number of RANKL-positive chondrocytes per mm^2 in the cartilage area was counted.

Statistical analysis

Results were expressed as mean ± standard deviation. The data were analysed statistically by one-way ANOVA (Tukey's multiple comparisons test) to compare groups, using GraphPad Prism Software. Values of $p<0.05$ were considered statistically significant.

RESULTS

Maxillary expansion in groups E60 and E90 were observed in the histological images of sections stained with haematoxylin and eosin (Fig. 3). In the expanded groups, greater intermolar width and a larger suture area were observed in relation to the control group. No obvious inflammatory reaction was found in the area of the midpalatal suture in any of the groups. Figure 4 illustrates with higher magnification the histological and immunohistochemical changes observed after application of the expansive forces. Masson's trichrome stained sections showed that the normal midpalatal suture in group C consisted of a uniform mass of cartilage containing chondrocytes covering the edges of the palatal bone. On the nasal and oral surfaces of the palatal bone, there was a fibrous layer with periosteal cells. After the expansion in groups E60 and E90, the layers of cartilage tissue were forced apart laterally by the mechanical tension force, and the fibrous tissue from the periosteum (with a large number of mesenchymal-like cells) penetrated and migrated into the suture. Cartilage tissue was marked using PAS-Alcian blue staining (PAS-AB). The acid mucin content in the cartilage matrix was high in the central portion of the suture (AB+), especially in group C, while in the periphery of the cartilaginous matrix and the central portion of the suture in groups E60 and E90, there was an increase in PAS+ marking. Midpalatal sutures of animals from groups E60 and E90 showed an increase in Runx2+ mesenchymal-like cells compared to the control, while midpalatal sutures of animals in group E60 clearly showed smaller quantity of RANKL+ chondrocytes compared to animals from groups C and E90.

Transverse measurements showed that in the expansion groups (E60 and E90), both maxillary width and intermolar width increased significantly after suture expansion (Fig. 5a-b). Suture width increased significantly in the central area in group E90 compared to the control (Fig. 5c-e). Suture

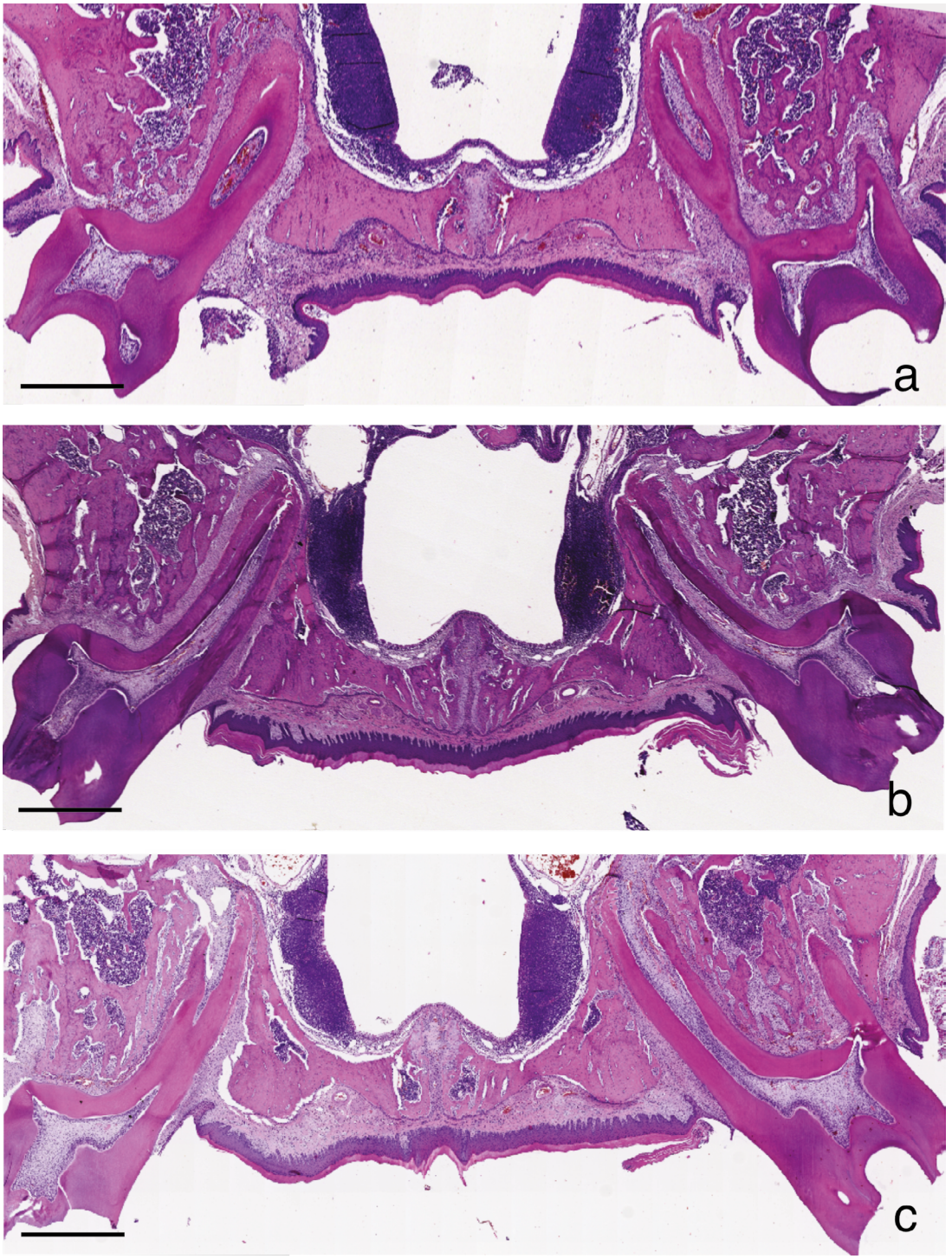

Fig. 3: Midpalatal suture frontal sections stained with H&E.
a-c) Histological changes in the cartilage of the midpalatal suture after force application. Frontal photomicrographs of rat mid-palatal suture in a control animal (a) and animals subjected to 60g (b) and 90g (c) expansive forces. In the expanded groups, greater intermolar width and a larger suture area were observed in relation to the control group. Stained with H&E, original magnification 10X. Scale 1000 µm.

area was significantly greater in group E60 than in group C, and the fibrous area associated with the suture was significantly greater in both experimental groups, E60 and E90, than in C (Fig. 5f-g).

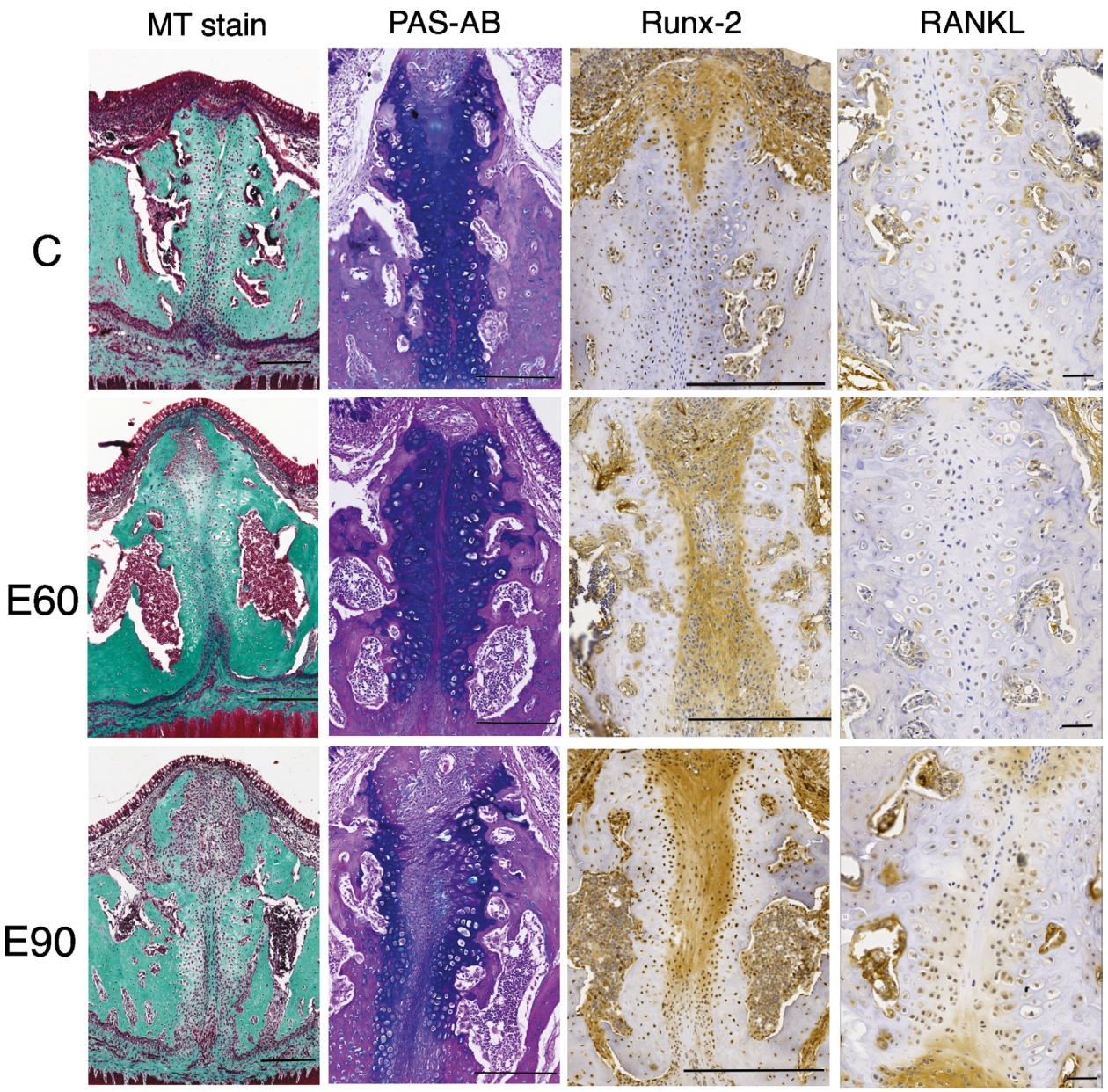

Fig. 4: Histological and immunohistochemical analysis of midpalatal sutures.
MT stain: Masson's trichrome stain. Images show palatal process bone tissue in turquoise, epithelium and connective tissue in red, and suture cartilaginous tissue in light green. Experimental animals (E60 and E90) show an increase in suture area, and greater fibrous tissue infiltrate within the suture. Original magnification 20X. Scale 200 µm.
PAS-AB: The images show bone tissue in pink, (PAS+, neutral mucins) periphery of suture cartilage in fuchsia, and (AB+, acid mucins) central portion of the cartilaginous matrix of the suture in blue. Animals subjected to expansive forces (E60 and E90) show an increase in suture area and width, with changes in the distribution of cartilaginous matrix components, displaying an increase in PAS+ matrix. Periodic Acid-Schiff-Alcian blue stain. Original magnification 20X. Scale 200 µm.
Runx-2 immunohistochemistry: Photomicrographs of midpalatal sutures of animals from groups C, E60 and E90 with Runx2 immunodetection, showing an increase in Runx2 positive mesenchymal-like cells in the experimental groups (E60 and E90) compared to the control. Original magnification 10X. Scale 300 µm.
RANKL immunohistochemistry: Photomicrographs at higher magnification of midpalatal sutures of animals in groups C, E60 and E90 with immunodetection for RANKL, clearly showing the smaller quantity of RANKL+ chondrocytes in animals from group E60 compared to animals from groups C and E90. Original magnification 40X. Scale 50 µm.

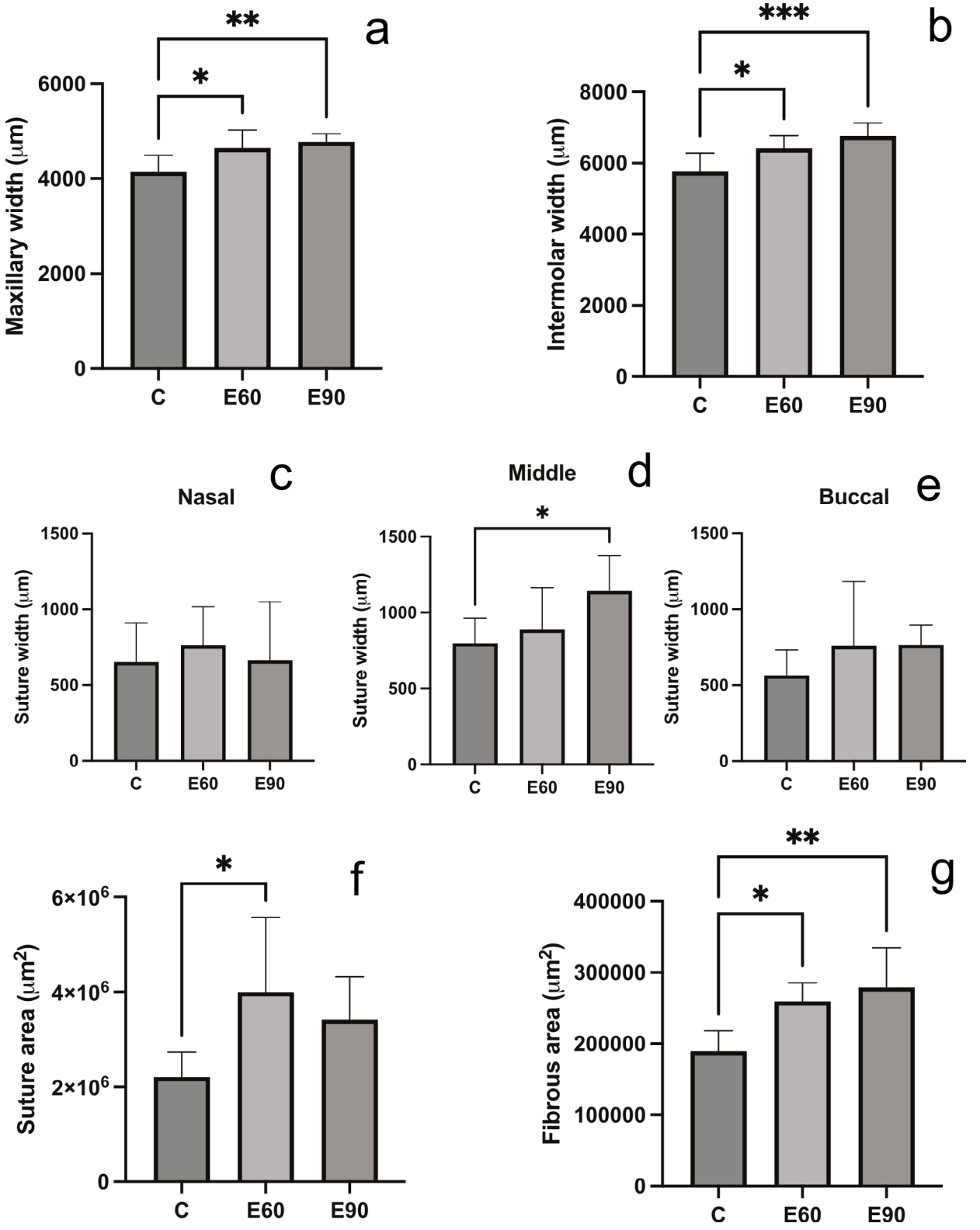

Fig. 5: Histomorphometric evaluation.
*a) Maxillary width, b) Intermolar width, c) Suture width at nasal level, d) Suture width at middle level, e) Suture width at buccal level, f) Suture area, g) Fibrous area inside the cartilage of the suture. Results are expressed as mean±SEM, n=5-7 animals per group. One-way ANOVA in conjunction with Tukey's post-test were performed, *p<0.05, **p<0.01, ***p<0.001.*

The percentage and number of Runx2+ chondrocytes/mm^2 did not differ significantly among groups (Fig. 6a-b). However, the percentage and number of Runx2+mesenchymal-like cells/mm^2 were significantly greater in the two experimental groups than in the control (Fig. 6c-d). The number of RANKL+ chondrocytes/mm^2 was significantly lower in the suture of the animals in group E60 than in groups C and E90, which did not differ significantly from each other (Fig. 6e).

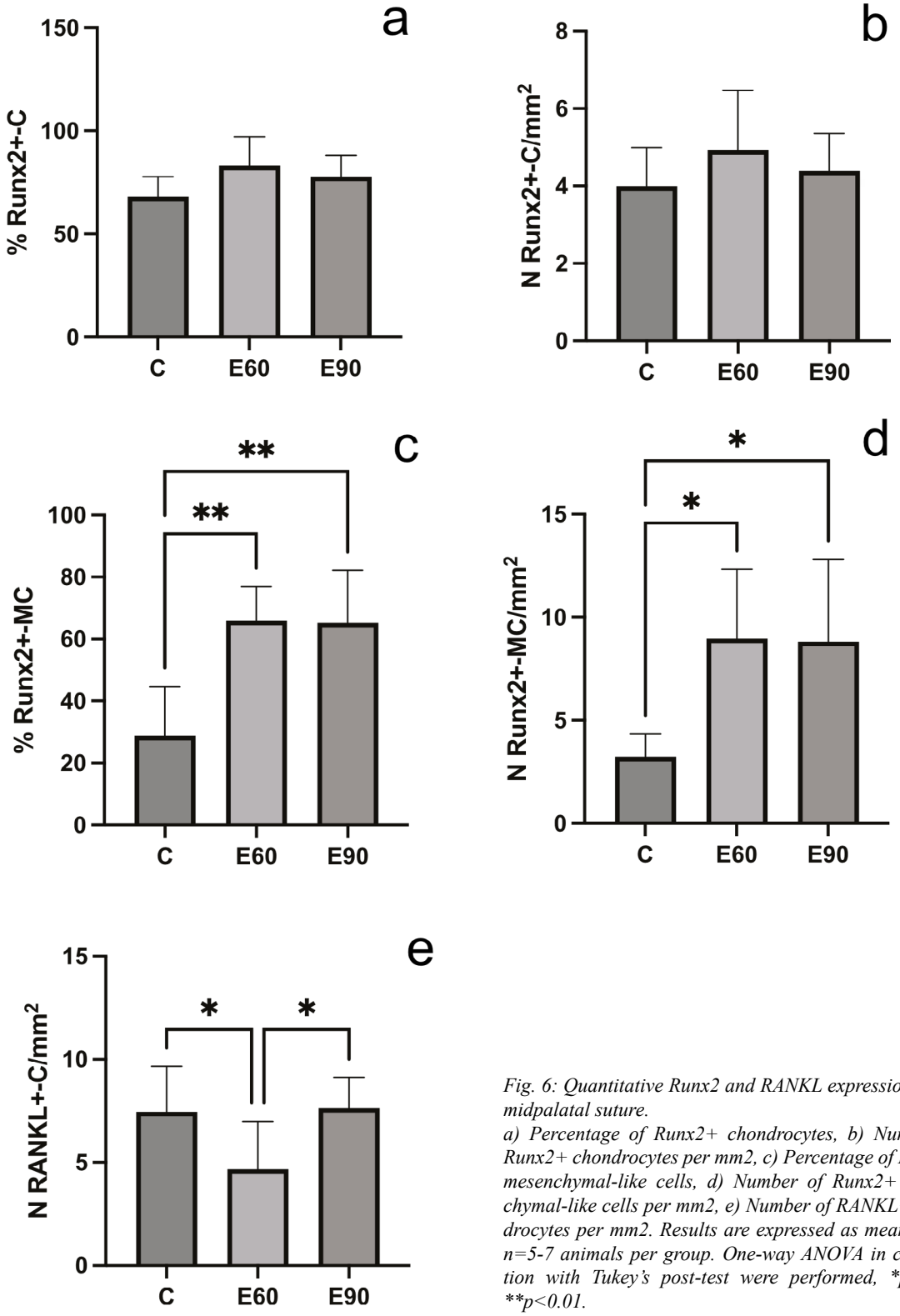

Fig. 6: Quantitative Runx2 and RANKL expression in the midpalatal suture.
*a) Percentage of Runx2+ chondrocytes, b) Number of Runx2+ chondrocytes per mm2, c) Percentage of Runx2+ mesenchymal-like cells, d) Number of Runx2+ mesenchymal-like cells per mm2, e) Number of RANKL+ chondrocytes per mm2. Results are expressed as mean±SEM, n=5-7 animals per group. One-way ANOVA in conjunction with Tukey's post-test were performed, *p<0.05, **p<0.01.*

DISCUSSION

Sutures allow stress distribution and bone remodelling during growth through sutural distraction osteogenesis. Palatal expansion through midpalatal suture distraction achieved by applying tensile forces is the most effective treatment for correcting maxillary constriction. Since most of the mechanisms involved are not yet fully understood, especially in relation to the magnitude of the force applied, it is crucial to investigate the intrinsic mechanisms that drive ossification of the suture as a function of the application of forces of different magnitudes. The present study tested expansive forces of different magnitudes, and found that when the forces were within a physiological range that did not generate inflammation or areas of hyalinosis/necrosis in the expanded zone, the degree of suture expansion was similar, with active participation of key molecules responsible for modulating the bone remodelling process. However, considering that the responses were similar, maxillary expansion with light, continuous force is the option of choice because the molars maintained healthy periodontal support as the alveolar process was expanded[19]. Other authors emphasize the use of light forces to promote chondrocyte proliferation and induce a better suture cartilage response pattern compared to stronger expansion[11].

In rat midpalatal suture, suture cartilage ossification has generally been observed as endochondral bone formation at the boundary between the maxillary bone and the cartilage[8,11,19,20]. However, regarding ossification, the results of histological and histomorphometric observations indicate the invasion and proliferation of fibrous tissue from the periosteum, with the presence of abundant mesenchymal-like cells, suggesting that intramembranous bone formation is induced at the boundary between the layers of cartilage following expansion. These observations are consistent with the descriptions in the first studies of maxillary expansion in rats[9], in which the application of tensile force to the midpalatal suture cartilage changed the phenotypic expression of osteochondroprogenitor cells in the secondary cartilage, indicating pathway differentiation. The tensile forces applied during expansion induce the formation of new bone in the mid-palatal suture, and there is suture remodelling throughout the expansion procedure, involving bone resorption, bone formation and fibre reorganization[21,22].

According to the above, the increase Runx2 expression in mesenchymal-like cells in the expanded midpalatal suture suggests that the osteochondroprogenitor cells differentiated into preosteoblasts in response to the expansive force. Runx2 is considered an essential transcription factor for osteoblast differentiation, which is determinant in early osteogenesis[16]. In the midpalatal suture samples, the expression of osteoblast markers, including Runx2, increased significantly in the expanded suture tissues as determined by qPCR at 7 and 14 days, suggesting an increase in osteoblastic differentiation and activity[14]. Yi et al.[23] reported similar results at 7 days of 50g expansive force application. The current study found increased Runx2 expression in mesenchymal-like cells, which is related to direct differentiation into osteoblasts in the initial phase of osteogenic induction. Studies by Takahashi et al.[9], published several decades ago, already suggested that the evidence of type I collagen and the decrease in cartilaginous extracellular matrix rich in type Il collagen during the early stages were caused by inhibition of the chondrogenic differentiation of progenitor cells and the differentiation into preosteoblasts in response to the expansive force in rats. In a recent study of a control suture consisting of cartilage-covered palatine connected by a thin band of fibrous tissue, it was found that after one week of expansion, there was an increase in proliferation of osteoblast-like cells, considered periosteal cells, indicating active bone formation[24]. The observations in the current study strongly suggest that rather than being endochondral, bone formation is intramembranous, occurring concomitantly with changes in the composition of the cartilaginous matrix produced by new osteoblasts derived from mesenchymal-like progenitor cells that invade the suture, arising from nearby periosteum during the earliest stages of expansion.

Receptor activator of nuclear factor-kappa B ligand (RANKL), an osteoclastogenesis regulatory molecule, plays an important role in inducing bone remodelling. Arnez et al.[13] showed that rapid maxillary expansion in rats upregulated the expression of RANKL at 3 and 7 days, and downregulated it at 10 days. Guerrero et al.[14] verified an increased expression of Rank, Rankl and Opg at suture sites under mechanical force, although they found no difference in osteoclast

numbers. Yi et al.[23] found an increase in RANKL expression immunohistochemically at 7 days with a 50g expansive force, resulting in enhanced osteoclast differentiation and activation during expansion. Similar results were reported by Chen et al.[15], where RANKL and OPG increased after rapid maxillary expansion. Hypertrophic chondrocytes are a source of the RANKL required to induce osteoclastogenesis and formation of the marrow space during endochondral ossification. Indeed, in Rankl$^{-/-}$ knockout mice, calcified cartilage resorption was prevented by reducing RANKL expression in hypertrophic cells[25]. In our study, the number of RANKL+ chondrocytes/mm^2 was significantly lower in group E60 than in groups C and E90, suggesting that lighter expansive forces would reduce osteoclast recruitment and differentiation towards the interior of the suture cartilage, inhibiting bone resorption and preventing its endochondral ossification.

Human and rat midpalatal suture differ mainly in structure and growth. While both serve as palatal growth centres, rat midpalatal suture contains secondary cartilage that will be replaced by endochondral ossification[6], in contrast to the intramembranous ossification that occurs in the humans[1]. Nevertheless, the rat model is useful and valuable for studying the maxillary expansion and bone remodelling mechanism, particularly in relation to bone formation and the analysis of osteoblast response.

CONCLUSIONS

The reduction in RANKL expression in chondrocytes and the increase in Runx2 expression in mesenchymal-like cells after expansion suggest that lighter expansive forces would stimulate osteogenesis in a direct manner not associated to the endochondral ossification of the secondary cartilage present in the suture. The results of this study contribute novel data on cell response to expansive forces of different magnitudes, enabling partial elucidation of how the processes of bone resorption and formation involved in rapid maxillary expansion are regulated, independently of the maturation of the tissue present in the suture.

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CONFLICT OF INTERESTS

All the authors declare that there is no conflict of interest regarding the publication of this manuscript.

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