Histological and morphological evaluation of condylar cartilage of young rats in response to stimulation of mandibular protrusion

Milena Peixoto Nogueira de Sá¹, Adilson Luiz Ramos¹, Jacqueline Nelises Zanoni¹, Fabricio Dias de Souza², Daniele Esteves Pepelascov¹ and Raquel Sano Suga Terada*¹

¹Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá, Paraná, Brazil. ²Universidade Estadual de Pernambuco, Recife, Pernambuco, Brazil. *Author for correspondence. E-mail: rssterada@uem.br

ABSTRACT. The aim of this study was to evaluate the histological and morphological aspects of the condyle in response to mandibular protrusion induced by composite resin occlusal planes. Thirty-five-week-old male Wistar rats were selected and randomly divided in two groups. One group received a composite resin type of occlusal plane that induced mandibular protrusion, and the other was the control group, without treatment. Animals were euthanized after experimental time intervals of 7, 21 and 30 days. For histological analysis, thickness of the proliferative, serial and hypertrophic layers was measured, as well as each layer separately. The highest difference in cartilage thickness was observed at day 21, showing a significant increase of the proliferative layer. There were also other histomorphological changes related to occlusal plane interference. Condylar forward repositioning induced by composite occlusal planes influenced the endochondral ossification, increasing the proliferative layer.

Keywords: articular cartilage, mandibular condyle, angle class II malocclusion.

Introduction

Biomechanical forces produced by postural movement of the mandible and/or orthopedic-orthodontic treatments may induce an adaptive response from the condylar cartilage (Shen & Darendeliler, 2005; Cholasueksa, Warita & Soma, 2004; Fuentes et al., 2003a; Fuentes et al., 2003b; Gribel, 1999; Lam, Sadowsky & Omerza, 1999; Nerder, Bakke & Solow, 1999; Hesse, Artun, Joondeph & Kennedy, 1997). This condylar remodeling and adaptation is inherent to the type of secondary cartilaginous tissue, accompanying the normal process of development of the stomatognathic system (Petrovic, Stutzmann, & Oudet, 1975).

The positioning of the mandible is constantly explored during orthopedic-orthodontic treatment, by means of using fixed or removable devices when seeking to achieve a correct maxillomandibular relationship. The cooperation of patients as regards the use of removable appliances may be a limiting factor for successful treatment. Therefore, fixed alternatives are preferred. The selective wear technique and the use of occlusal planes made of resin bound to the teeth or the Planas Direct Tracks (PDT) (Planas, 2013; Belanger, 1992; Simões, 1981; Thilander, Wahlund, & Lennartsson, 1984; Buck, 1970) are an alternative that promotes mandibular advancement, seeking a response of anterior growth (Planas, 2013; Hesse et al., 1997; Simões, 1981).
particular, the inclined resin bonded planes are a simple alternative to induce mandibular anterior repositioning (Planas, 2013; Chibinski, Czlushniak, & Melo, 2005; Gribel & Gribel, 2005; Ramirez-yañez, 2003; Gribel, 2002). The presence of advancement devices seems to induce adaptive changes in the condylar cartilage (Liu, Kaneko, & Soma, 2007a; Sato, Muramoto, & Soma, 2006), glenoid fossa and articular eminence (Liu, Kaneko, & Soma, 2007b; Tuominen, Kantoma, Pirttiniemi, & Poikela, 1996; Woodside, Metaxis, & Altuna, 1987), but little has been described about the effects of PDT.

Therefore, the aim of the present study was to evaluate the rat mandibular condyle response to mandibular protrusion induced by the fixed inclined plane made of composite resin.

Material and method

Thirty male Wistar rats, 5 weeks old were randomly divided into 2 groups: Control (C) and Experimental (E). The animals in both groups were fed a pasty diet and water ad libidum. Light was controlled (simulating a night-day cycle) and a temperature of 23°C was monitored.

Animals of the Group E received an intraoral device (an inclined occlusal plane made of resin), capable of promoting mandibular advancement and changing functional occlusion (Figure 1). For this purpose, impressions were taken of the animals' mouths to allow fabrication of the devices and their inclination to be adjusted on the plaster casts. After this, a matrix was manufactured in acetate in order to allow standardization of the technique. The devices were made of resin composite Z-250 (3M Dental Products, St. Paul, MN/USA), on the maxillary and mandibular incisors.

Animals were anesthetized with a combination 1:1 of ketamine (10%) + xylazine (2%), via intramuscular injection, at the dose of 0.5 mg 100⁻¹ g. With sedated animals, enamel etching was performed, with subsequent application of light polymerizing adhesive, insertion and light polymerization of a thin layer of resin, filling the acetate matrix with resin, and adaptation of the appliances to the teeth for final light polymerization. After this, accompanying the inclination attributed to the maxillary incisors, a polyester strip was adapted with the purpose of adding resin composite to the mandibular incisors. Thus, an inclined plane was established, capable of inducing the advanced position of the mandible.

The presence of the devices and their inclination prevented the mouth from closing in habitual occlusion, which caused mandibular protrusion (Figure 2).

The animals in Group C did not receive any device, and was the Control Group (Figure 3). This study was approved by the Institutional Review Board State of the University of Maringá (UEM), 080/2008.

Tissue preparation

Animals were euthanized by deep anesthesia after time intervals of treatment of 7, 21 and 30 days. Immediately after death, the heads of the animals were sectioned, dissected and immersed in fixative solution (Bouin) for 4 days. After consecutive changes of alcohol in a series of increasing concentrations, the regions of the temporomandibular joint (TMJ) were dissected. After this, the parts were decalcified in a solution containing 20% sodium citrate, formol and distilled water, for 5 days. To remove the decalcifying agent, the parts were washed in running water for 4 hours. The histological routine was performed, and after
Response to stimulation of mandibular protrusion

After stimulation of mandibular protrusion, the parts were embedded, following the same plane of orientation as the ramus parallel to the surface of the block.

Semi-serial sections, 7 μm thick, were made in the parasagittal plane of the condyle region, using a Leica RM 145® microtome, and stained with hematoxylin and eosin, mounted on slides with the use of Permount. Because this concerned a symmetrical protrusion of the two condyles, only the TMJs of the right side were sectioned.

**Morphometric Analysis**

Images were captured in high definition format by an optical microscope (MOTIC BA400) and sent to a computer. With the image analysis software - Image-Pro-Plus 4 (Media Cybernetics, USA), the thicknesses of the proliferative, serial and hypertrophic layers were measured, providing 6 cuts from each animal. In each cut, 5 measurements were made in each layer, totaling 30 measurements for each animal (Figure 4).

**Figure 4. A) 7 μm histological section of the mandibular condyle of a Wistar rat in the control group, after 7 days. Note the demarcated area where the images were captured for analysis (B). Measurements were made following the direction of the dashed line. B) Posterior region and part of the median region of the condyle, where total (yellow), proliferative (blue), hyaline (green) and hypertrophic (red) layers were measured.**

**Statistical Analysis**

This was a double-blind study and the results were analyzed in the software STATISTICA 7. Data distribution was not normal, so that non parametric tests were used. The groups were evaluated by the Kruskal-Wallis test.

**Results**

There were significant differences in the total thickness of cartilage in all the experimental time intervals (Table 1). In Group E, after 7 days of placing the devices, alterations in the arrangement and characteristics of the cells were found (Figures 5A and B); the total thickness reduced by 33% when compared with Group C (Table 1).

![Image](image.png)

**Figure 5. Distribution of relative frequencies of the different layer thicknesses: (A) Total, (B) Hypertrophic, (C) Hyaline, (D) Proliferative, control group after 7 (C7), 21 (C21) and 30 (C30) days and experimental after 7 (E7), 21 (E21) and 30 (E30) days.**

Although the proliferative layer of Group E was slightly higher (Figures 5A and B), with an increase of 33% (Table 1), the hypertrophic and serial layers showed a reduction in thickness of 55 and 33%, respectively (Table 1), when compared with Group C (Figures 6B and D).

After 21 days, in Group E, it was possible to observe considerable predominance of cells of the proliferative layer (Figures 6B and D), according to the data shown in Table 1 and Figures 6B and D.

Although the proliferative layer of Group E was slightly higher (Figures 5A and B), with an increase of 33% (Table 1), the hypertrophic and serial layers showed a reduction in thickness of 55 and 33%, respectively (Table 1), when compared with Group C (Figures 6B and D).

At the experimental time interval of 30 days, Groups C and E (Figures 5E and F) were shown to be close with regard to the behavior of condylar cartilage, however, there was a small increase in the proliferative layer in Group E (close to 12% more than in Group C) (Table 1).

The growth without interference revealed by Group C demonstrated a reduction in total thickness (including the sub-layers) of the condylar cartilage (Figures 5A, C and E) over time.
Discussion

In Class II, due to mandibular deficiency (Hägg et al., 2008), the goal of an orthopedic-orthodontic treatment is to favor mandibular growth, although the extent to which this may be possible, beyond the genetic thresholds, is still controversial. The signs of malocclusion are already present in primary dentition, persist in mixed dentition and tend to become consolidated as the patient gets older (Baccetti, Franchi, McNamara, & Tollaro, 1997). In view of the body of current evidence, the most adequate time (time-benefit) to promote this correction occurs during the pubertal phase, involving the growth spurt (Baccetti et al., 1997;
Mcnamara & Bryan, 1987) although it is also possible to obtain response to mandibular advancement at earlier stages.

The animals used in this study attained the period of rat life between 5 and 9 weeks, corresponding to the prepubertal phase up to the pubertal growth spurt (Shen & Darendeliler, 2005). The experimental model of the TMJ of rats is well accepted and has been widely studied due to the similarity to the human TMJ (Ren, Maltha, & Kuipers-Jagtman, 2004).

Orthodontic or orthopedic devices that promote changes in mandibular posture include fixed or removable devices, with the fixed type being more efficient because it does not depend on cooperation from the patient. In the present study, fixed inclined planes were used, which promoted mandibular advancement when the rats occluded. Classical studies (Charlie, Petrovic, & Herrmann-Stutzmann, 1969; Petrovic, 1972; Stutzmann & Petrovic, 1979; Vardimon, Stutzmann, Graber, Voss, & Petrovic, 1989) examined the histological effects of mandibular protrusion on the condylar cartilage of rats and demonstrated acceleration of its growth. They concluded that, firstly, an increase would occur in the prechondroblastic layer, followed by increase in the hypertrophic layer, increasing the endochondral condylar growth. When used bone markers, it was also observed a condylar increase in response to advancement in rats aged 4 weeks (Petrovic, Stutzmann, & Gasson, 1981), and an increase in the proliferative, pre- and chondroblastic layers, resulting in total mandibular augmentation, beyond that of the control rats. In spite of these results, other studies have demonstrated a reduction in the hypertrophic layer in comparison with the total layer, in response to mandibular advancement in rats, corroborating the findings of the present study. Ghafari & Degroote (1986) verified an increase in the rest layer and acceleration of ossification in the hypertrophic layer, which was less thick in the experimental group, with regard to total cartilage.

Various experimental models that have evaluated mandibular growth in response to advancement have been published (Carlson, 1999; Mcnamara & Bryan, 1987; Mcnamara & Carlson, 1979; Meikle, 2007). Analyses were made by means of radiographic (Liu et al., 2007a), histological (Liu et al., 2007b), and immunohistochemical studies (Sato et al., 2006), comparison of different consistencies of food (Bresin & Kiliaridis, 2002; Maki, Nishioka, Shioiri, Takahashi, & Kimura, 2002), calcium restriction (Watanabe, Imamura, Uchikanbori, Fujita, & Maki, 2008), Type X collagen expression (Rabies & Hägg, 2002; Rabie, Xiong, & Hägg, 2004; Shen, Zhao, Kaluarachchi, & Rabie, 2006), Type II collagen expression (Rabies et al., 2004; Tuominen et al., 1996), transcription factor Sox 9 (Papadopoulou et al., 2007; Rabie, She & Hägg, 2003a) and vascular endothelial growth factor – VEGF (Rabies & Hägg, 2002; Rabie, Shum, & Chayanupatkul, 2002b). Some studies have demonstrated that forward positioning of the mandible causes cellular responses capable of promoting increase in cartilage by endochondral ossification, culminating in bone neoformation (Leung, Rabie, & Hägg, 2004; Rabie, Leung, Chayanupatkul & Hägg, 2002a; Rabie et al., 2002b; Rabie et al., 2003a; Shen et al., 2006). In the present study, it was possible to observe changes in the cartilage thickness of rats submitted to mandibular advancement in comparison with the controls. In a time interval of 7 days, a reduction in total thickness was verified, however, the thickness in the proliferative layer increased when the Groups E were compared with Groups C. It is supposed that the advancement promotes stretching of the muscle fibers, and the condyle is positioned more anteriorly in the glenoid fossa, elongating the tendons and stimulating the region of the retrodiscal pad. According to Rabie and Hägg (2002), due to the high adaptive capacity, an increase in cell responses occurs, leading to the proliferation of new blood vessels, a fact confirmed by the high level of expression of vascular endothelial growth factor (VEGF), a potent regulator of neovascularization, present in the region of the condyle and articular fossa (Leung et al., 2004).

After the experimental time interval of 21 days, it was possible to find a significant increase in the condylar cartilage in Group E, particularly in the proliferative layer. Other studies (Rabies, Wong, & Tsai, 2003c; Shen & Darendeliler, 2005) have demonstrated that mandibular advancement accelerates and increases condylar growth, by the differentiation of mesenchymal cells into chondrocytes, leading to the formation and increase in the amount of cartilaginous matrix. To regulate this process, some genetic control mechanisms, such as transcription factor Sox 9 (Rabies et al., 2003a), mediator of mechanical Ihh - Indian hedgehog - signal transduction (Tang, Rabie, & Hägg, 2004) and negative feedback involving PTH-RP - parathyroid hormone related protein (Rabies, Tang, Xiongi, & Hägg, 2003b), are closely related.

Collagen Type X is specifically expressed in hypertrophic cartilage, confirming its role in the terminal stage of chondrocyte maturation (Fukada, Shibata, Suzuki, Ohya, & Kuroda, 1999). Rabie and

---

Acta Scientiarum. Health Sciences

Hägg (2002) observed a significant increase in the expression of this protein when the mandible was stimulated in the position of advancement, and verified an important correlation between the synthesis of this protein and the quantity of endochondral ossification. Among the properties of Collagen Type X, the facility to be resorbed is outstanding, with it being replaced by bone matrix and interfering in the calcification process during endochondral ossification (Bonen & Schmid, 1991).

After 30 days, it was possible to register a greater similarity between the condylar cartilage in Group C and E, however, the hypertrophic layer was smaller in Group E. It is interesting to point out a similarity between the two groups, but in Group E, the mandible continued to be supported in a forward position. Some authors (Leung et al., 2004; Rabie et al., 2002a; Rabie et al., 2002b; Shen et al., 2006) have found that in this period, replacement of cartilage by bone occurs. The transition from chondrogenesis to osteogenesis occurs in the erosive zone, where the chondrocytes begin to synthesize alkaline phosphatase, and concomitantly, the cartilaginous matrix undergoes calcification (Meikle, 2007). In a stage of calcification, the matrix inhibits the diffusion of nutrients into the tissue, culminating in the death of the chondrocytes. With the death of the chondrocytes, a large portion of the matrix becomes segmented, forming a series of gaps in the region. The discontinuity of the intercellular matrix and the spaces formed by the gaps create a situation that favors vascular invasion. The capillary vessels that occupy the region bring progenitor bone cells and bone marrow cells that will differentiate into osteoblasts (Cancedda, Castagnola, Cancedda, Dozin, & Quarto, 2000; Garant, 2003). The osteoblasts assume their positions on the spicules and cartilaginous remnants, initiate the synthesis of bone or osteoid tissue, which will subsequently be calcified, resulting in bone neoformation (Gartner & Hiatt, 1997). Therefore, the similarity of the results between Group C and E is considered favorable for mandibular growth, considering that it would be indicative of acceleration of ossification in the experimental group. Further studies with markers and measurement of the total mandibular length may confirm this fact.

Conclusion

After histological and morphometrical evaluation, changes were observed in the condylar cartilage during the experimental time interval of 30 days. The mandibular advancement position proposed in this experimental model was capable of changing the thickness and characteristics of condylar cartilage. The stimulation promoted cell histodifferentiation and proliferation, initiating the cascade of endochondral ossification events.

References


*Received on May 11, 2015* .

*Accepted on September 2, 2015* .

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.