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Effect of Different Types of Force on the Amount of Tooth Movement, Hyaline Areas, and Root Resorption in Rats

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Abstract

Aims: This study evaluated the effects of using continuous, continuous interrupted, and intermittent forces on the roots and periodontal ligaments in the first molars of rats. **Materials and Methods:** The right first molars of 54 Wistar rats were moved with continuous force (CF group), continuous interrupted force (CIF group), or intermittent force (IF group) for 5, 7, and 9 days. Ankylosed incisors were used as anchors for the orthodontic tooth movement. To establish the different types of force, NiTi springs of 50 cN were maintained, deactivated, or removed for certain periods. Amounts of tooth movement, hyaline areas, and root resorption levels were analyzed using analysis of variance (ANOVA) and post-hoc Tukey's test with a significance level of 5%. **Results:** There was no difference in the amount of tooth movement between the CF and CIF groups, which differed significantly from the IF group. The CF group demonstrated a significant formation of hyaline areas, nearly 5 times more, mainly on the fifth day. **Conclusions:** Continuous force produced more hyaline areas with greater probability of generating root resorption. Continuous interrupted force enabled better periodontal ligament repair and more efficient elimination of hyaline areas.

Keywords: Ankylosis, hyaline, rats, root resorption, tooth movement

Introduction

The orthodontic tooth movement (OTM) is greatly influenced by the duration and characteristics of the applied force, which generates varied reactions in the tissues.[1] There are three types of force applied in orthodontics. The "continuous force (CF)" is produced by wires and springs with superelastic characteristics (high limits of elasticity and shape memory), with the goal of maintaining the initial strength over a long period between activations. The "continuous interrupted force (CIF)" is obtained when wires or springs with reduced limits of elasticity and shape memory are used; the force gradually decreases until it reaches a level at which it is incapable of producing tooth movement. In contrast, an "intermittent force (IF)" acts over a shorter period and is completely eliminated with the removal of the force-generating device. IFs are commonly seen with the use of removable extraoral devices and intermaxillary elastics.[2] The combined effects of the treatment duration, applied force, direction of tooth movement, and method of force application render interrupted and IFs as less aggressive than CFs.[3]

The application of mechanical forces to induce OTM produces regions of stress or tension in the periodontal ligament (PDL), establishing areas of traction, or stretching and areas of compression of the periodontal fibers. Clast recruitment and activation induce bone removal from the area adjacent to PDL compression, whereas, on the tension side, osteoblasts encourage bone development.[3-5] These processes of bone resorption and apposition lead to tooth repositioning.[6,7]

Investigations of continuous and IFs of different intensities during OTM have shown that the type of force has a greater influence on tooth movement than does the magnitude of the force.[8] Biologically, not only the intensity of the force but also its duration and tissue response to the application of the same are important for tooth movement.[9] Some authors

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have stated that smooth CFs result in desirable biological responses,[10,11] and are often associated with direct bone resorption.[12,13] The initiation and maintenance of high stress on the PDL produces hyaline areas and indirect bone resorption. The hyaline area is a homogeneous region of the PDL that is free of nuclei or cells, due to cell migration or necrosis. The formation and subsequent elimination of hyaline areas promote an interruption of tooth movement.[2,12-15] Excessive stress or tension in the PDL generates hypoxia or anoxia in the cells and consequent root resorption that begins after the death of the root-lining cementoblast layer.[4,5] The clasts begin the root resorption process in the periphery of the hyaline areas.[12,14] Therefore, verification of the amount of root resorption and hyaline areas may show what the best type of force to be used in OTM.

There is a lack of consensus on the best type of force to produce tooth movement, or even, what tissue reactions are expected with the use of them. Few studies have been able to establish and compare the three types of force in orthodontics. Therefore, the scope of the study was to establish three types of force and to compare the amount of tooth movement, hyaline areas, and root resorption using ankylosed incisors as anchors to move the upper first molars of Wistar rats. The null hypothesis was that there would be no difference in the tissue responses between the three types of force.

Methodology

The study employed 54 male albino Wistar rats (*Rattus norvegicus*), ~90 days old and weighing ~300 g (Vivarium of name omitted), which were maintained at 22°C ± 2°C with 6 animals per cage, with crushed food and unrestricted water were provided (protocol #2008-004689).

The experimental models comprised three groups of 18 animals each. Animals were subjected to OTM by CF groups, CIF groups, or IF groups. Each group was divided into three subgroups with six animals each, to evaluate changes occurring at periods of 5, 7, and 9 days, Groups were subdivided by day and identified as CF5, CF7, CF9, CIF5, CIF7, CIF9, IF5, IF7, and IF9. In all groups, the first upper right molars were subjected to OTM, anchored to the upper incisors on the same side.

Experimental procedures were performed with the animals under intramuscular anesthesia, obtained with 80 mg/kg ketamine hydrochloride (Dopalen, Sespo Ind. and Co., Ltd., Jacareí, SP, Brazil) and 10 mg/kg xylazine hydrochloride (Anasedan, Agribrands of Brazil, Ltd., Paulínia, SP, Brazil), according to manufacturer's instructions. To provide stable anchorage and eliminate undesirable effects consequent to continuous eruption, ankylosis was induced in the upper right incisors of each animal 15 days before installation of the coil springs.[16,17]

For OTM, the device designed by Heller and Nanda[18] was used, modified by replacing the stainless steel by NiTi spring of 50 cN (Sentalloy, GAC, NY, USA) and inserting photopolymerizable resin (Z100, 3M, St. Paul, MN, USA) into the cervical region of the incisor. The packed resin was used to improve retention of the steel wire in the cervical region of the previously reimplanted incisor and to enable (de) activation of the spring. The first upper right molar was involved in spring installation. The spring was installed by using a steel wire (0.20-mm diameter; Morelli, Sorocaba, SP, Brazil), attached to a closed-section spring (3-mm length) that released a CF of 50 cN.[19-22] When stretched to 3 mm in length and attached to the cervical resin of the upper right incisor with 0.25-mm-diameter steel wire (Morelli, Sorocaba, SP, Brazil), the spring established a CF for 5, 7 and 9 days. For the CIF groups, the springs were activated and deactivated on alternate days until the animals were killed on days 5, 7, and 9. The springs were deactivated and kept passive until the next activation, with the attaching wire left on the incisor. For the IF groups, the springs were installed and removed on alternate days until the animals were killed on days 5, 7, and 9 [Figure 1].

The amount of OTM was determined as the difference between the right and left sides of the distance from the mesial face of the first upper molar to the distal face of the third upper molar.[21,22] Two properly calibrated evaluators performed the measurements with a digital caliper (Mitutoyo, São Paulo, SP, Brazil) and a magnifying eyepiece with ×4 magnification (Intex, Valinhos, SP, Brazil).

After 5, 7, and 9 days of experimentation, the animals were killed by anesthetic overdose and decapitation. Transversal cuts of 6 μm in thickness were made at the cervical and mid-root level of the molars, to the separation of all roots, and the sections were stained with hematoxylin and eosin (HE). The images were captured by a digital camera connected to the microscope (Zeiss Axiophot, Carl Zeiss, Göttingen, Germany). The histometric measures were made with Imaging Systems (Software Release 4.8.2, AxioVision, Carl Zeiss, Göttingen, Germany). The hyaline areas were outlined on the pressure sides (mesial) of the intermediate buccal (IB) roots and quantified as a percentage of the total area of the PDL).

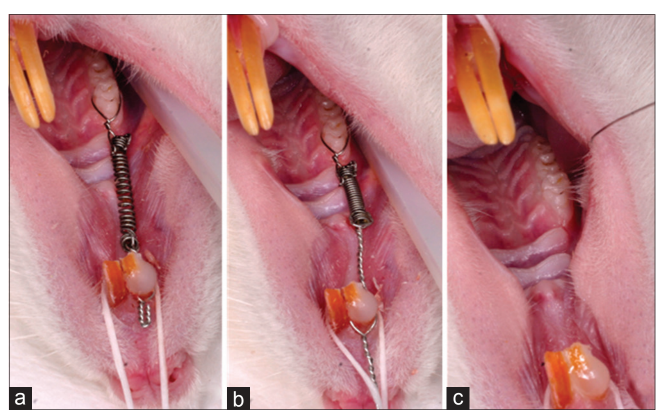

Figure 1: Orthodontic tooth movement device of the upper right molar anchored to the upper right incisor. Activation (a), deactivation (b), and removal of the spring (c) generating continuous, continuous interrupted, and intermittent forces, respectively

Resorption areas were quantified as a percentage of the total area of the roots.

Data were interpreted in a factorial scheme of completely random design by analysis of variance. Homogeneity of variances was established by Bartlett's test, and normality of the residues was established by the Shapiro–Wilks test. When there were significant differences, Tukey's test was applied to the means to detect the difference. A significance level of 5% was adopted.

For the purpose of method error analysis, 36 sections were randomly selected, and the two roots were once again measured after a 1-week interval. The intraexaminer systematic error was calculated by the Wilcoxon Signed Rank Test. The casual error was determined using the error calculation proposed by Dahlberg.[23]

Results

Compared to the other groups, the CF group showed the greatest OTM on day 5, and the CIF group showed the greatest OTM on days 7 and 9. However, only the IF group significantly differed among the groups [Table 1].

The compression side of the IB root demonstrated evident hyaline areas with a significant difference on day 5 versus days 7 and 9. The CF group achieved the largest hyaline area (mean 23.58% on day 5), with a significant difference compared to the CIF and IF groups [mean 5% on day 5, Table 1 and Figures 2-4].

The IB root showed modest resorption on the compression side. Resorption increased gradually until day 9 and reached 3% in the CF group, 1.39% in the CIF group, and 0.30% in the IF group, but with no statistically significant difference among the groups [Table 1 and Figures 2-4].

Discussion

In this study, the amount of OTM was measured to compare microscopic alterations at different time periods, with the goal of revealing the effects of different types of force on the teeth and supporting tissues. The ankylosis of the right upper incisor of each animal was performed to obtain a stable anchorage to support the molar tooth movement and to promote the three types of force. The incisors of the rats have a continuous eruption of around 0.4 mm/day. This promotes a change in the direction of the force to incisal, as well as another traction to the molar, making difficult to obtain the three types of force. Therefore, ankylosis of the incisors was previously induced by extraction, removal of the PDL, cutting of the dental papilla, removal of the pulp, filling of the root canal with calcium hydroxide and dental reimplantation.[16,17] The direction of force must be controlled during OTM, to prevent dubious results from being obtained. In this study, the incisors induced before ankylosis showed no movement due to loss of anchoring, the installed spring, or even continuous eruption, demonstrating that the direction of force was well-maintained.

Tooth movement and hyaline areas

OTM was analyzed quantitatively by a method used by other authors.[21,22] No significant difference between the CF and CIF groups was found after 5, 7, or 9 days of OTM. In the CF group, there was substantial initial movement until day 5, followed by stagnation of movement until day 7 and a return to movement until day 9. Several authors[2,12,13,15] have described a plateau

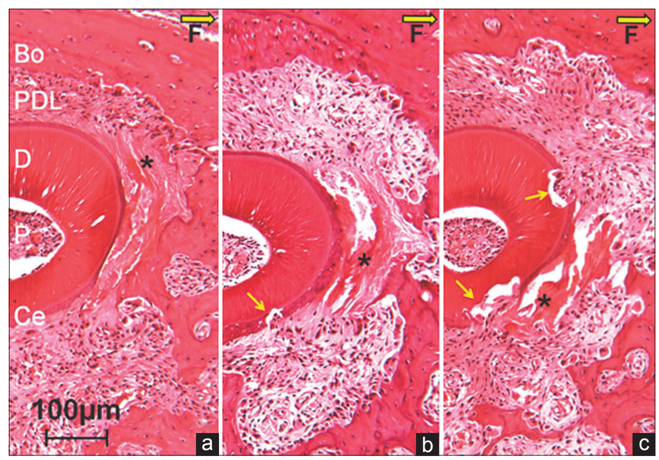

Figure 2: Sequence of the compression side of the intermediate root in the continuous force group on day 5 (a), day 7 (b), and day 9 (c). Increase in the thickness of the PDL and resorption of the hyaline areas (*) was observed from day 5–9. Indirect bone resorption on the periphery of the hyaline areas was observed until day 9. On days 7 and 9, root resorption with the formation of Howship's lacunae (arrows) was evident. P – Pulp; D – Dentin; Ce – Cement; PDL – Periodontal Ligament; Bo – Bone; F – Force, (H and E, ×40)

Table 1: Mean values and standard deviations (mm) of the amounts of tooth movement. Average percentages of hyaline areas and root resorption in compression side of the intermediate buccal root, groups and periods.

Group	5 days				7 days				9 days				Movement	Hyaline	Resorption
	Movement		Hyaline	Resorption	Movement		Hyaline	Resorption	Movement		Hyaline	Resorption			
	Mean	SD			Mean	SD			Mean	SD					
CF	0.26	0.09	23.58%	0.25%	0.24	0.13	7.19%	1.23%	0.32	0.15	2.25%	3.02%	a	a	a
CIF	0.19	0.03	5.9%	0%	0.30	0.11	0.42%	0.76%	0.39	0.41	0.40%	1.39%	a	b	a
IF	0.11	0.08	5.5%	0%	0.24	0.10	1.36%	0.60%	0.13	0.08	1.45%	0.30%	b	b	a
	A		A	A	A		B	A	A		B	A			

Groups with the same letter have no significant difference between them. Capital letter refers to the comparison between the time and lowercase between groups

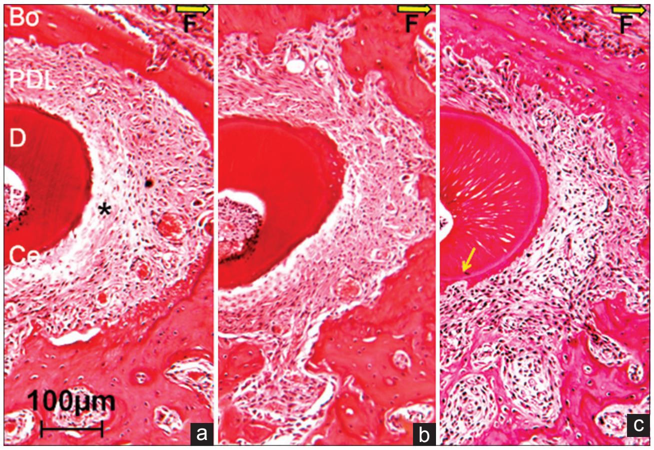

Figure 3: Sequence of the compression side of the intermediate root in the continuous interrupted force group on day 5 (a), day 7 (b), and day 9 (c). Root resorption with formation of Howship's lacunae (arrow) appears on day 9. Hyaline area (*); P – Pulp; D – Dentin; Ce – Cement; PDL – Periodontal Ligament; Bo – Bone; F – Force, (H and E, ×40)

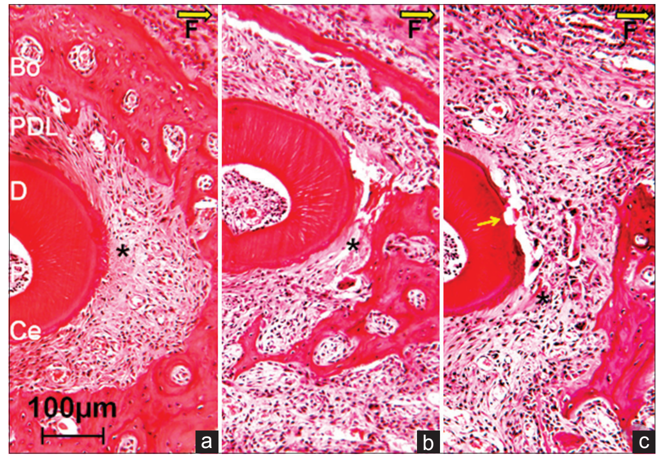

Figure 4: Sequence of the compression side of the intermediate root in the interrupted force group on day 5 (a), day 7 (b), and day 9 (c). The PDL appears compressed on day 7. Small hyaline areas (*) are present by day 9. Root resorption with formation of Howship's lacunae (arrow). P – Pulp; D – Dentin; Ce – Cement; PDL – Periodontal Ligament; Bo – Bone; F – Force, (H and E, ×40)

phase, or interruption of movement, after the initial phase, which has been attributed to the formation and subsequent elimination of hyaline areas. This initial phase is described as tooth movement due to compression of the PDL, bone flexing, and tooth extrusion.[24] Although without statistical significance, movement in the CIF group exceeded that in the CF group on days 7 and 9. This finding could be explained by the rest period (in which force was interrupted), which would allow tissue repair and could favor hyaline elimination because the amount of active force is unable to promote tooth movement.[2,12,13,15]

The hyaline area is a homogeneous region of the PDL that is free of nuclei or cells,[15] due to cell migration or necrosis. As a result, the area contains only extracellular matrix, which changes and assumes a glassy or hyaline aspect. A study[25] examining transverse sections of the roots of immobile rat molars reported that the PDL thickness of the mesiobuccal (MB) root was almost twice that of the IB root. These findings suggest that the application of intense force that completely compresses the PDL of the IB root would compress the PDL of the MB root to only half of its thickness. The greater PDL thickness in the MB root leads to better force dissipation; the stress is more concentrated in the thinner PDL of the IB root, with a greater potential for hyaline formation and root resorption.[25] Thus, these results contribute to evaluate the IB root.

The amount of force in the IB root was high in all groups, especially on day 5, as evidenced by the greater presence of hyaline areas. In subsequent periods, the hyaline areas were gradually eliminated from the periphery, allowing the larger movement seen on day 9. After 5 days of OTM with CF, the effects are similar to but more intense than, those occurring in 3 days, especially in the hyaline areas.[26] This information corroborates the initial evaluation of this experiment, from day 5 of OTM. Significantly greater formation of hyaline areas was observed in all periods in the CF group [Figures 2-4]. Teeth were moved by continuous interrupted, and IFs showed hyaline areas that were nearly five times smaller compared to those that received CF. Nakano *et al*.[27] report the amount of tooth movement decrease with the application of an excessive magnitude force. This is due to the greater formation of hyaline areas, similar to what occurred in this present study in the CF group that used CF. The tooth movement decreases until the hyaline areas were removed. Hayashi *et al*.[28] established that the amount and duration of the force are important factors in stimulating osteoclast recruitment to the PDL. They found that the amount of initial tooth movement was similar for continuous, intermittent, and interrupted forces. In the present study, the amount of tooth movement was significantly less for the IF group in all periods, except on day 7 when the springs were active. With the removal of the force, the amount of movement declined markedly for the IF group.

Root resorption

Root resorption, caused by excessive stress or tension that generates hypoxia or anoxia in the cells or as a consequence of dentoalveolar trauma,[4,5] begins after the death of the root-lining cementoblast layer. Clasts begin the resorption process in the periphery of the hyaline areas.[12,14] In this study, root resorption was noticed at the sides of these areas from the fifth day of OTM in the CF groups [Figure 2].

Some authors have stated that CFs produce greater amounts of resorption,[12,14] and that the magnitude of the force does not influence the process of root resorption.[29] In 2003, Weiland[30] stated that the possibility of root resorption is 140% greater with superelastic wires than with stainless steel wires. The superelastic wire promotes a CF, similar to the CF group in the present study. On the other hand, the stainless steel wire produces a CIF similar to the CIF group. Therefore, the results of the present study, as well as Weiland,[30] show that the CIF provides a tooth movement similar to that of CF, but with more satisfactory biological effects. These findings corroborate with Isola *et al*.,[9] that not only the intensity of the force but also its duration has great influence on the dental movement.

The initiation and maintenance of high stress on the PDL produces hyaline areas. CF, by producing a greater amount of hyaline areas, has a greater potential for the development of root resorption than continuous interrupted and IFs. The root resorption was greatest in the CF group (although not statistically significant), it seems evident that the greatest potential for deleterious effects was presented by the CF compared to the other forces.

Usually, professionals want to get a fast tooth movement, and for this, apply intense and uninterrupted forces. For this, they use wires and springs with alloys that release force continuously between activations throughout the treatment.

The application of the forces must be of a careful way in any phase of the orthodontic treatment, or even in any type of dental movement. The presence of previous root resorption or bone loss reduces the area of orthodontic force dissipation, changes the biomechanical control, and potentiates the concentration of stress in the periodontium. In regions with a thin bony crest, it may increase the potential for gingival retraction, dehiscence or bone fenestration during tooth movement, especially when loss of this biomechanical control or incorrect application of a force occurs. Farahamnd *et al*.[31] concluded that the thickness of the buccal bone crest of the maxillary incisors and canines is around 0.6 mm. Thus, it is fundamental to control forces applied to the tooth movement, especially in the retraction of the incisors, preferentially applying interrupted forces, which allow better repair of the tissues.

Even in cases where biomechanical control is not crucial, as in the traction of impacted teeth, the use of interrupted forces should be the choice. Approximately 90% of impacted teeth receive orthodontic traction, while about 10% erupt spontaneously.[32] Apical or cervical root resorption in the included teeth is a common sequel seen in the traction teeth. Probably caused by increased stress in the periodontium. Thus, it would be prudent to use wires or springs with reduced limits of elasticity and shape memory, which dissipate CIFs. The use of superelastic alloys with high limits of elasticity and shape memory should be intercalated with the deactivation of the springs or replacement of the wires to interrupt the force.

The present work demonstrates through histological cuts that the continuous application of force produces more hyaline areas and greater risk of root resorption. On the other hand, when a repair time is allowed for tissues by force interruption, the biological response is better. There is less formation of hyaline areas, less chances of root resorption, the repair process is better, as well as tooth movement.

Conclusions

Based on the experimental study and literature review, it can be concluded that: The use of CIFs produce fewer hyaline areas, facilitating their elimination, PDL repair as well as tooth movement. The CFs produce more hyaline areas with greater potential for the development of root resorption than interrupted and IFs. The IFs produce hyaline areas similar to interrupted forces, however, permit a recurrence of the movement.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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