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Orthodontic movement of traumatized teeth increase the velocity of tooth movement and cause external root resorption

O movimento ortodôntico de dentes traumatizados aumenta a velocidade do movimento dentário e causa reabsorção radicular externa

El movimiento ortodóncico de dientes traumatizados aumenta la velocidad del movimiento dental y provoca la reabsorción radicular externa

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Adriana Paula Batista da Costa Pasa[1]
ORCID: https://orcid.org/0009-0003-0325-9907
State University of Western Paraná, Brazil
E-mail: drica_odonto@hotmail.com
Anna Caroliny Detogni[2]
ORCID: https://orcid.org/0000-0003-1003-0097
State University of Western Paraná, Brazil
E-mail: annadetogni456@gmail.com
Dhienara Sgarbosa Tomin[2]
ORCID: https://orcid.org/0000-0002-6461-4488
State University of Western Paraná, Brazil
E-mail: dhienara.tomin@unioeste.br
Mauro Carlos Agner Busato[1]
ORCID: https://orcid.org/0000-0002-8379-9211
State University of Western Paraná, Brazil
E-mail: mauro.busato@unioeste.br
Elaine Manoela Porto[3]
ORCID: https://orcid.org/0000-0002-0538-2461
State University of Western Paraná, Brazil
E-mail: e_laineporto@yahoo.com.br
João Paulo de Arruda Amorim[3]
ORCID: https://orcid.org/0000-0003-2245-548X
State University of Western Paraná, Brazil
E-mail: amorimjpa@yahoo.com.br

Abstract
The success of tooth movement in treating traumatized teeth depends on the severity of the initial injury. The objective of this study was to evaluate the histological structure of periodontal tissues and root resorption during orthodontic movement of traumatized teeth. A total of 24 female Wistar rats were used and divided into four experimental groups: Group 1, 6 rats that were not subjected to any experimental procedure (control); Group 2, 6 rats that were subjected to induced tooth movement (ITM); Group 3, 6 rats that underwent dentoalveolar trauma (DT); and Group 4, 6 rats that were subjected to DT+ITM. At the end of the experimental period, the animals were euthanized, and the maxilla were removed and processed for histology. Has been observed that the animals in the DT+ITM group had a significantly higher tooth movement rate than the animals in the ITM group. The animals of CTL group had normal findings, while alterations were found in the remaining animals. In ITM group, disorganization of the periodontal ligament (PL) and vascular changes, in addition to moderate root resorption, were observed. The DT group also demonstrated these changes, in addition to presenting with giant cells in the furcation region. In DT+ITM group, in addition to the findings seen in the other experimental groups, also presented with PL disorganization, moderate vascular changes, more intense root resorption and hyaline areas. We conclude that of induced tooth movement associated with the extrusive subluxation trauma causes increases the rate of tooth movement and root resorption.
Keywords: Tooth movement; Dental trauma; Rats.

[1] Postgraduate Program in Dentistry, Biologics Science and Health Center. State University of Western Paraná - UNIOESTE, Brazil.
[2] Laboratory of Tissue Biology and Reproduction, Biologics Science and Health Center. State University of Western Paraná - UNIOESTE, Brazil.
[3] Laboratory of Tissue Biology and Reproduction and Postgraduate Program in Dentistry, Biologics Science and Health Center. State University of Western Parana - UNIOESTE, Brazil.

Resumo

O sucesso da movimentação dentária no tratamento de dentes traumatizados depende da gravidade da lesão inicial. O objetivo deste estudo foi avaliar a estrutura histológica dos tecidos periodontais e a reabsorção radicular durante a movimentação ortodôntica de dentes traumatizados. Foram utilizadas 24 ratas Wistar, divididas em quatro grupos experimentais: Grupo 1, 6 ratas que não foram submetidas a nenhum procedimento experimental (controle); Grupo 2, 6 ratas que foram submetidas à movimentação dentária induzida (ITM); Grupo 3, 6 ratas que foram submetidas a trauma dentoalveolar (DT); e Grupo 4, 6 ratas que foram submetidas a DT+ITM. Ao final do período experimental, os animais foram eutanasiados, e a maxila foi removida e processada para histologia. Foi observado que os animais do grupo DT+ITM apresentaram uma taxa de movimentação dentária significativamente maior do que os animais do grupo ITM. Os animais do grupo CTL apresentaram achados normais, enquanto alterações foram encontradas nos animais restantes. No grupo ITM, foram observadas desorganização do ligamento periodontal (LP) e alterações vasculares, além de reabsorção radicular moderada. O grupo DT também demonstrou essas alterações, além de apresentar células gigantes na região de furca. No grupo DT+ITM, além dos achados observados nos demais grupos experimentais, também apresentou desorganização do LP, alterações vasculares moderadas, reabsorção radicular mais intensa e áreas hialinas. Concluímos que a movimentação dentária induzida associada ao trauma de subluxação extrusiva causa aumento na taxa de movimentação dentária e reabsorção radicular.
Palavras-chave: Movimento dentário; Traumatismo dentário; Ratos.

Resumen

El éxito del movimiento dentario en el tratamiento de dientes traumatizados depende de la gravedad de la lesión inicial. El objetivo de este estudio fue evaluar la estructura histológica de los tejidos periodontales y la reabsorción radicular durante el movimiento ortodóncico de dientes traumatizados. Se utilizaron un total de 24 ratas Wistar hembras y se dividieron en cuatro grupos experimentales: Grupo 1, 6 ratas que no fueron sometidas a ningún procedimiento experimental (control); Grupo 2, 6 ratas que fueron sometidas a movimiento dentario inducido (ITM); Grupo 3, 6 ratas que fueron sometidas a trauma dentoalveolar (DT); y Grupo 4, 6 ratas que fueron sometidas a DT+ITM. Al final del período experimental, los animales fueron sacrificados y se les extrajo el maxilar y se procesó para histología. Se ha observado que los animales del grupo DT+ITM tuvieron una tasa de movimiento dentario significativamente mayor que los animales del grupo ITM. Los animales del grupo CTL tuvieron hallazgos normales, mientras que se encontraron alteraciones en los animales restantes. En el grupo ITM se observó desorganización del ligamento periodontal (PL) y cambios vasculares, además de reabsorción radicular moderada. El grupo DT también demostró estos cambios, además de presentar células gigantes en la región de furca. En el grupo DT+ITM, además de los hallazgos observados en los otros grupos experimentales, también se presentó desorganización del PL, cambios vasculares moderados, reabsorción radicular más intensa y áreas hialinas. Concluimos que el movimiento dentario inducido asociado al trauma de subluxación extrusiva provoca aumentos en la tasa de movimiento dentario y reabsorción radicular.
Palabras clave: Movimiento dentario; Traumatismo dental; Ratas.

1. Introduction

A great challenge in orthodontics is dentoalveolar trauma (DT), especially that involving the supporting periodontium, because the success of orthodontic treatment depends on the integrity of these structures (Andreasen *et al*., 2002; Busato *et al*., 2014). Studies have shown that the prevalence of DT is high in the population, especially among children and adolescents, and orthodontic treatment in these patients represents an important aspect of the field (Tondelli *et al*., 2010; Glendor, 2008). This shows the importance of defining procedures that orthodontists should adopt regarding the ideal time to start treatment in patients who have suffered DT, as well as knowledge of the complications that may occur during orthodontic treatment or in patients with a previous history of DT.

Andreasen & Andreasen (2001) classified the types of DT that involve the periodontal tissues according to their intensity as follows: concussive; subluxative; extrusive, lateral and intrusive; and avulsive. Among these, extrusive dislocation (EX), which can be defined as a partial displacement of the tooth out of its socket and may also be called partial avulsion, is clinically characterized by an elongated appearance, excessive mobility and negative response of the tooth to the sensitivity test and, radiographically, by an increase in the space of the periodontal ligament (Andreasen & Andreasen, 2001; DiAngelis *et al*., 2012). In addition, this type of dentoalveolar trauma is one of the most common and increases the risk of periodontal complications (Hermann *et al*., 2012; Gulinelli *et al*., 2008).

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Root resorption is one of the main sequelae studied with regard to DT and induced tooth movement (ITM), both alone and in combination. This sequela may occur by the activation of clastic cells, which reabsorb mineralized tissues via a mechanism similar to that of bone resorption, leading to loss of dental elements in extreme cases (Busato *et al*., 2014, Rothbarth *et al*., 2014; Kikuta *et al.*, 2015).

During orthodontic tooth movement, root resorption does not occur because cementoblasts do not have receptors for bone resorption mediators. However, a tooth that has suffered dental trauma may have caused damage to the cementoblast layer. Thus, the root surface is covered by neighboring osteoblasts, which assume the functions of cementoblasts and are called "cementoblast-like". If there is a large accumulation of bone resorption mediators in the periodontal ligament, the "cementoblast-like" osteoblasts function as an osteoremodeling unit, as they have receptors that capture the biochemical messages responsible for this function (Consolaro, 2005). This could occur in the use of ITM for treating traumatized teeth, increasing the risk of root resorption and even dental ankylosis (Kindelan *et al*., 2008). The objective of this study was to evaluate the histological structure of periodontal tissues and root resorption during the orthodontic movement of traumatized teeth.

2. Methodology

Experimental, laboratory research was carried out, qualitative (histological analyses) and quantitative in nature through the use of numerical variables and quantities (Pereira et al., 2018) and statistical analyzes were carried out on the numerical results (Shitsuka et al., 2014) to reach the discussions and conclusions of the study.

2.1 Animals

Twenty-four female *Wistar* rats (60 days old, weighing approximately 150 g) were acquired from the Unioeste Animal Farm. The animals were adapted and kept in the Sectorial Vivarium of the Laboratory of Physiology, Center for Biological and Health Sciences (CCBS), State University of West Paraná, Unioeste, Cascavel-PR, in collective polyethylene cages (43x30x15), housed individually or in pairs under controlled temperature conditions (22°C and 25°C), relative humidity of approximately 55% and a 12 hour light/dark cycle (lights on from 7:00 to 19:00). The animals received food and water *ad libitum.* The experimental procedures were in accordance with the Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation (COBEA) and were analyzed by the Committee on Ethics in Animal Experimentation (CEEA) of Unioeste.

2.2 Experimental groups

The animals were randomly divided into four experimental groups: Group 1, control (CTL), composed of 6 rats that were not subjected to any experimental procedure; Group 2, composed of 6 rats that were subjected to ITM for 7 days; Group 3, composed of 6 rats that underwent DT; and Group 4, composed of 6 rats that were subjected to both ITM and DT for a period of 7 days.

2.3 Sedation of animals

The experimental procedures (application of DT and installation of the ITM device) were performed under general anesthesia by applying ketamine hydrochloride-based anesthetic (DOPALEN, Sespo Indústria e Comércio, Paulínia-SP) at a dose of 75mg/kg and xylazine hydrochloride-based muscle relaxant (ANASEDAN, Sespo Indústria e Comércio, Paulínia-SP) at a dose of 15 mg/kg, both intraperitoneally.

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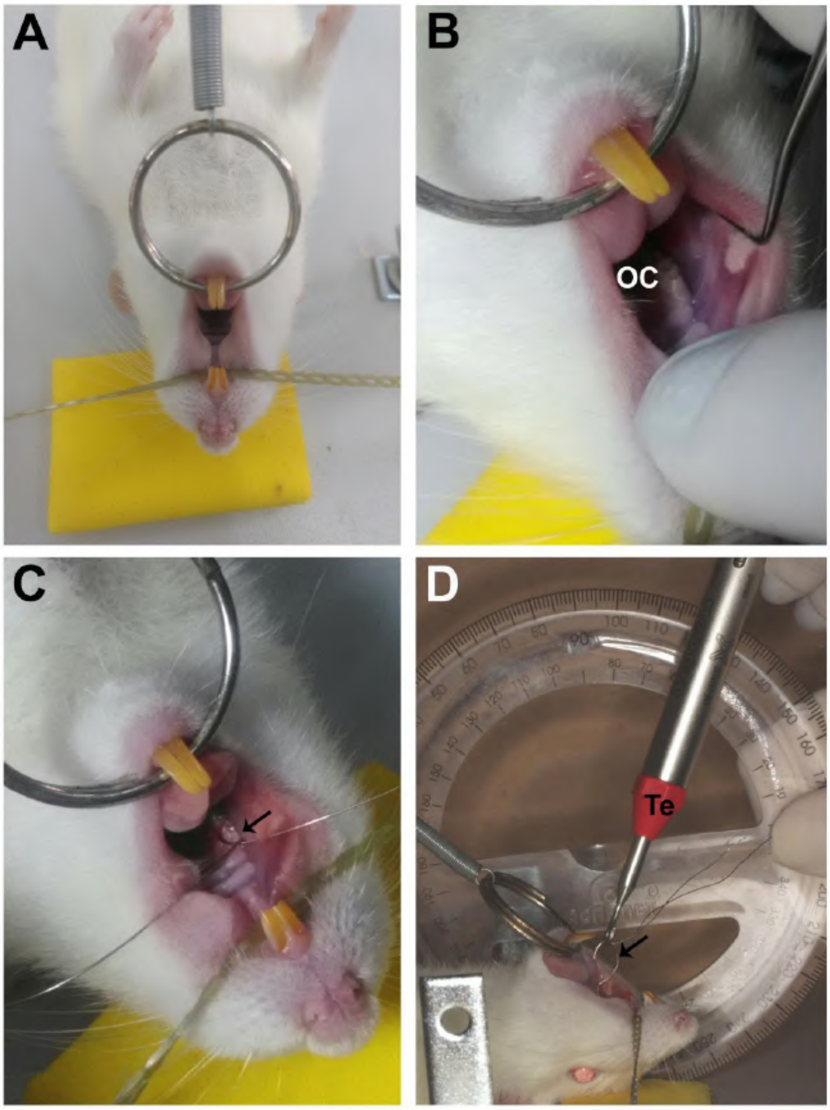
2.4 Application of extrusive subluxation trauma

At 75 days, extrusive subluxation (ES)-type dentoalveolar trauma was performed using the methodology proposed by Costa *et al*. (2018), Mezzaroba et al. (2022), Lucietto et al. (2024). The animals were fasted for 12 hours during the night before the procedure. On the following morning, five minutes before onset, a single dose of antibiotic prophylaxis with intramuscular ceftriaxone (50 mg/kg) (EMS, Brazil) and analgesia with subcutaneous sodium dipyrone (50 mg/kg) (EMS, Brazil) was administered. After anesthesia, asepsis of the region was performed with 1% povidone iodine (Riodeine®; Indústria Farmacêutica Rioquímica Ltda., São José do Rio Preto, SP, Brazil). The animals in Groups 3 and 4 were subjected to ED-type DT on the upper right first molar, all by the same operator, who inserted a 0.025 mm ligature wire. (Morelli®; Sorocaba, São Paulo, Brazil) in the palatal buccal direction between the maxillary first and second molars. The two ends of the wire were placed on the mesial surface of the maxillary first molar and twisted with the aid of a 17.0 cm Mathieu needle holder (Quinelato; Rio Claro, São Paulo, Brazil) for fixation of the wire around the tooth. A loop-shaped bend was made at the distal end of the inserted wire with the aid of a mirror handle to create a ring in which the active end of the tensiometer was placed. The tensiometer was positioned on the handle, and traction at an angle of 60° to the vertical plane was performed for 15 seconds at a force of 1500 cN. Then, the thread was removed (Figure 1).

Figure 1 - Photographs of the dentoalveolar trauma procedure. A. Positioning the animal in the supine position on the operating table; B. Opening of the animal's oral cavity (CO); C. Yellow wire inserted between the upper right first and second molars (arrow); D. Tensiometer (Te) positioned on the handle (arrow) and traction at a 60° angle.



Source: Lucietto et al. (2024).

2.5 Installation of the device for induced tooth movement (ITM)

At 90 days, an ITM device was installed in the animals in Groups 2 and 4. The device used in this study was similar to that proposed by Heller & Nanda (1979) and Pasa et al. (2024). The total period of ITM device placement was 7 days. This modified device consisted of a closed-section, nickel-titanium spring (Sentalloy®, GAC, NY, USA) with a force magnitude of 50 cN. The magnitude of the spring force was previously assessed using tensiometer (Zeusan Exporting Ltda. Campinas, São Paulo, Brazil). The animals were fasted for 12 hours during the night before the procedure. The following morning, five minutes before onset, a single dose of antibiotic prophylaxis with intramuscular ceftriaxone (50 mg/kg) (EMS, Brazil) and analgesia with subcutaneous sodium dipyrone (50 mg/kg) (EMS, Brazil) was administered. After anesthesia, asepsis of the oral region with 1% povidone iodine was performed; two segments of 0.25 mm thick ligature wire (Morelli, Sorocaba, SP, Brazil) were connected to each end of the spring, one going around the maxillary right first molar and the other around the maxillary right central incisor. To stabilize the ligature wire on the buccal surface of the incisor, a slot was made in the cervical region, and the wire was fixed with light-curing composite resin (Amelogen® Plus, Ultradent Products Inc., USA) to avoid displacement of the wire.

2.6 Euthanasia and collection of biological material

At the end of the experimental period (approximately 97 days), all animals were weighed and euthanized with an excessive dose of anesthetic and subsequent decapitation by guillotine. The right hemimaxillae were removed and fixed in 10% buffered formalin for 24 hours, washed in running water for 48 hours, subsequently decalcified in decalcifying acid solution (Allkimia®) for 19 hours and stored in 70% alcohol.

2.7 Quantitative analysis of tooth movement

Immediately after euthanasia, the amount of tooth movement was obtained as the difference between the distances from the mesial surface of the maxillary first molar to the distal surface of the maxillary third molar on the moved right side and the unmoved left side (Gameiro et al., 2008; Pasa et al., 2024). The measurements were obtained using a digital caliper (Mitutoyo, São Paulo, Brazil) with the aid of an ocular magnifying glass.

2.8 Laboratory processing

After decalcification, the pieces were dehydrated in an increasing series of alcohols, cleared in xylene and embedded in paraplast. For the histological analyses, serial 5 µm-thick sections were made in the longitudinal plane of the mesiobuccal and distobuccal roots of the maxillary right first molar, from mesial to distal, using a manual rotating microtome (Olympus 4060) equipped with a steel razor. The sections were deparaffinized with xylene, hydrated with distilled water and subjected to staining with hematoxylin-eosin (HE) for analysis.

A light microscope (Olympus BX61) was used for histological analysis. An Olympus DP71 digital camera with DP Controller 3.2.1.276 software was used to obtain the photomicrographs at 200x and 400x magnification.

2.9 Descriptive analysis of histological slides

The specific areas of the slides subjected to the descriptive analysis were 1) the periodontal ligament of the mesiobuccal and distobuccal roots on the mesial and distal surfaces, cervical, middle and apical thirds; 2) periodontium of the furcation region; 3) mesial bone crest; 4) interradicular septum; and 5) interdental septum between the maxillary right first molar and the maxillary right second molar. The histopathological items investigated were external root resorption, areas of hyalinization, acute inflammatory infiltrate, chronic inflammatory infiltrate, presence of multinucleated giant cells, presence of vascular changes, and organization of the periodontal ligament. Each item was evaluated as follows: absence, occasional presence, moderate presence and strong presence (Costa *et al*., 2018).

2.10 Histomorphometric analysis of external root resorption

For the quantitative analysis of external root resorption, the mesial surface of the distal root in its cervical and middle thirds was considered because this is the region most affected by compression of the periodontal ligament in both trauma from extrusive subluxation and induced tooth movement. The photomicrographs at 400X magnification were analyzed using Image-Pro Plus 6.0 software (Media Cybernetics, Rockville, MD, USA), where the total area of each resorption was quantified in square micrometers (μm^2). Each measurement was performed three times, and the mean value was calculated. When the root region presented with more than one area of root resorption, the areas were added together to obtain the total area of resorption per area/animal (Pasa *et al*., 2024).

2.11 Statistical analysis

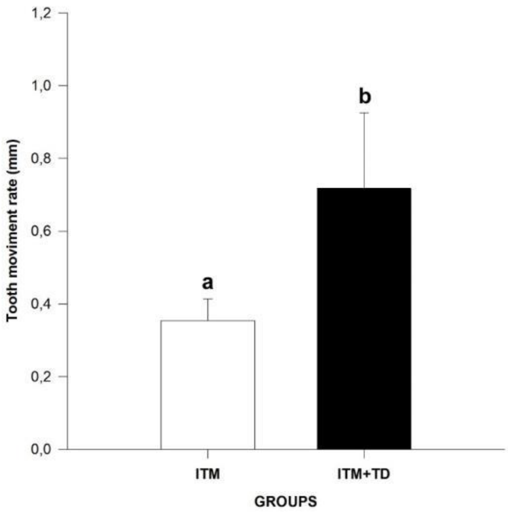
For data analysis, Student's t test or analysis of variance (ANOVA) with Tukey's a posteriori test was used, depending on the characteristics of the studied variable. Differences were considered statistically significant when $p<0.05$. SigmaPlot version 11.0 (Systat Software Inc., San Jose, CA, USA) was used to perform the statistical analyses and plot the graphs.

3. Results

3.1 Analysis of tooth movement

The animals in the ITM+DT group exhibited a significantly higher amount of tooth movement (0.71 mm) than the animals in the ITM group (0.35 mm) ($P < 0.05$) (Graph 1).

Graph 1 - Tooth movement in the experimental groups subjected to induced tooth movement (ITM). Data are expressed as the mean ± standard deviation. N = 6 animals/group. Student's t test. [a, b] indicates significant differences between groups ($P < 0.05$).



Source: Authors' files.

3.2 Descriptive analysis of the histological slides

Group 1 - CTL: The periodontal ligament (PL) was normal and rich in fibroblasts and collagen fibers. The surfaces of the roots were continuous throughout in most animals, with some occasions of root resorption. The interradicular septum and mesial bone crest were normal.

Group 2 - ITM: The PL was moderately disorganized near the interradicular septum region, and intense vascular changes were observed. Root resorption was moderately present in the cervical and middle thirds of the mesial and distal surfaces of the distobuccal root, in the cervical and middle thirds of the distal surface of the mesiobuccal root, and in the periodontium region of the furcation.

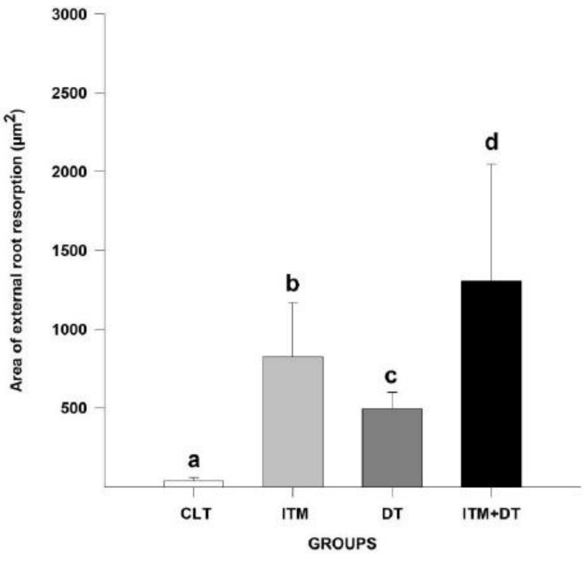
Group 3 - DT: The PL was occasionally disorganized, with vascular changes in the interradicular septum, in the cervical third of the mesial surface of the distobuccal root, and in the furcation region, which also presented with a moderate presence of giant cells. Root resorption was observed in the cervical and middle thirds of the distal surface of the mesiobuccal root, in the periodontium of the furcation, in the middle third of the mesial surface of the distobuccal root, and in the middle third of the distal surface of the distobuccal root.

Group 4 - ITM+DT: The PL was moderately disorganized in the periodontium of the furcation region and in the cervical third of the distal surface of the mesiobuccal root and occasionally disorganized in all thirds of the mesial surface of the distobuccal root. The interradicular septum was also disorganized. Vascular changes were moderate in the periodontium of the furcation, in the interradicular septum, in the middle third of the mesial surface of the distobuccal root, and in the cervical and apical thirds of the distal surface of the mesiobuccal root. Root resorption was strongly present in the furcation region, in the cervical third of the distal surface of the mesiobuccal root, and in the cervical and middle thirds of the mesial and distal surfaces of the distobuccal root. Hyaline areas were recorded on the mesial surface of the distobuccal root.

3.3 Histomorphometric analysis of root resorption

The animals in the ITM group had significantly greater areas of root resorption than the animals in the CTL and DT groups (*P*<0.001) (Graph 2). The TD group had a larger root resorption area than the CTL group and a smaller root resorption area than the ITM and ITM+DT groups (*P*<0.001). The ITM+TD group presented the largest amounts of root resorption areas among all experimental groups (*P*<0.001) (Graph 2).

Graph 2 - Area of external root resorption in the different experimental groups. Values are expressed as the mean ± standard deviation. N = 6 animals/group. Analysis of variance (one-way ANOVA) with Tukey's posttest. [a, b, c, d] indicate significant differences between groups (*P* <0.001).



Source: Authors' files.

4. Discussion

Dentoalveolar trauma is a subject of great interest in dental practice, especially in the field of orthodontics, and has been considered an important public health problem not only because of its relatively high prevalence, especially in young individuals, but also because its functional consequences and aesthetics can have a substantial impact on the patient's life (Pereira *et al.*, 2011).

Knowledge of the complications arising from tooth movement associated with dentoalveolar trauma is of great importance, as this could avoid possible sequelae resulting from this association. According to the results, the animals in Group 4 had significantly greater tooth movement (0.52 mm) than the animals in Group 2 (0.35 mm) because teeth subjected to dental trauma and orthodontic movement exhibit greater mobility, without displacement of the cavity, after trauma induction. This result depends on the stress state of the PL, which plays a critical role in tooth movement initiated by orthodontic treatment. The can be observed in the present study because the ES type of trauma used in this study was of low to moderate intensity, causing rupture of some fibers of the periodontal ligament, thus leading to mobility without causing tooth displacement (Pereira *et al.*, 2011; Toms & Eberhardt, 2003; Losso *et al*., 2011).

Regarding the histological changes observed in this study, Group 1 showed normal PL characteristics, unlike the experimental groups (Groups 2, 3 and 4), which showed different degrees of PL disorganization. This pattern of disorganization includes vascular changes, which were intense near the region of the interradicular septum in Group 2. Vascular changes were also observed in Group 3, in the regions of the interradicular septum and in the cervical third of the mesial surface of the distobuccal root; in Group 4, these changes were moderate in the regions of the periodontium of the furcation region and in the cervical third of the distal surface of the mesiobuccal root, and occasional disorganization of the PL was observed throughout the mesial surface of the distobuccal root. The presence of these vascular changes indicates that an inflammatory process had occurred in the periodontal tissue, which was in the process of reorganization, evidence of the repair of the periodontal ligament, which is an important biological response to the treatments performed (Panzarini *et al*., 2013). It should also be noted that the inflammatory infiltrate present, which results from vascular changes, is a response to the inflammatory process caused by trauma and the creation of the ITM flap in the periodontal ligament, specifically to inflammatory chemical mediators such as cytokines and neurotransmitters that will continue these processes until tissue repair is complete (Krishnan *et al.*, 2006).

In addition, in Group 3, giant cells were moderately present in the furcation region, which can be considered an indication of clastic activity. The presence of this event near the areas of root resorption indicates that inflammatory-type root resorption was found in these regions (Panzarini *et al.*, 2013).

With regard to root resorption, which was the main event analyzed in this study, it should be noted that this occurs mainly as a biological response to the application of orthodontic forces (Sella *et al.*, 2012). Thus, in the present study, we observed that Group 1 presented with continuous roots throughout their extension in most animals, as this group was not subjected to ES or ITM.

Regarding the experimental groups, root resorptions were moderately present in both Group 2 and Group 3. These resorptions resulted from ITM and ES-type DT, respectively, as a response to the application of forces on the dental elements (Sella *et al.*, 2012). The orthodontic forces applied to the tooth transformed into biological stress on the periodontal ligament, acting equally and simultaneously on the alveolar bone and on the cementum, promoting the partial destruction of the precementum lining layer. When this lining layer is damaged by a local physical agent, odontoclasts gain access to the mineralized tissues of the tooth and begin the resorption process together with macrophages (Rego *et al.*, 2004; Mezzaroba et al., 2022).

In Group 4, root resorption was strongly present in the furcation region, in the cervical third of the distal surface of the mesiobuccal root, and in the cervical and middle thirds of the mesial and distal surfaces of the distobuccal root. This is because during the posttraumatic repair period that occurs in teeth subjected to ED, the additional damage caused by orthodontic movement to the cementum accentuates the inflammatory stimuli, prolonging the destructive phase and increasing the risk of resorption. Thus, the type and intensity of trauma directly affect the response to orthodontic treatment, i.e., mild or moderate injuries have less obvious indicators of root resorption and respond better to orthodontic treatment than more severe trauma (Pereira *et al*., 2011; Mezzaroba et al., 2022). The distributions of resorption gaps, histologically, are directly related to the amount of force applied under the root surface, and the rate of development of these gaps is faster with increasing applied forces, although this relationship is not directly proportionate (Rego *et al.*, 2004).

The periodontal hyalinization observed in Group 4 (with the formation of a degenerated and acellular zone resulting from the compression of the periodontal ligament and its components, with consequent reduction of the nutritional support) precedes the root resorption process during the orthodontic treatment, which is linked to the extent and duration of the hyalinization area. Thus, the force applied to the root surface is directly related to the hyalinization process, which itself is directly proportional to root resorption (Rego *et al.*, 2004; Mezzaroba et al., 2022; Pasa et al., 2024).

5. Conclusion

We conclude that of induced tooth movement associated with the extrusive subluxation trauma causes increases the rate of tooth movement and root resorption. Furthermore, new studies may contribute to the emergence of new information about of dental trauma associated with orthodontic tooth movement, leading to more effective and personalized treatments.

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Competing Interests

Authors have declared that no competing interests exist.

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