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ORIGINAL ARTICLE

Validation of a new experimental model of extrusive luxation on maxillary molars of rats: a histological study

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Abstract

Background/aim The aim of this study was to test and validate a new model of extrusive luxation trauma on maxillary first molars of rats.

Material and methods Forty adult male rats (*Rattus norvegicus albinus*, Wistar; weight = 230–250 g), 45 days old, were divided into eight groups ($n = 5$): control groups, in which animals were not subjected to any procedure and waited 1 day (GC1D) or 3 days (GC3D) for euthanasia, and experimental groups, in which animals were subjected to forces of 1100cN, 1300cN, or 1500cN and waited 1 or 3 days for euthanasia (GT1100/1D, GT1100/3D, GT1300/1D, GT1300/3D, GT1500/1D, GT1500/3D). In animals of the experimental groups, trauma was produced by an extrusive force in maxillary first right molars. Four-micrometer serial cuts stained with hematoxylin and eosin (HE) were made. Descriptive microscopic analysis of first upper right molar and semi-quantitative analysis (scores 1 to 4) of intensity of acute and chronic inflammation and vascular changes in the periodontal ligament and active and inactive external root resorption were conducted. The distribution of scores in the groups was compared using the Freeman-Halton extension of Fisher's exact test. The significance level was 5%.

Results It was observed that vascular disorders (bleeding) on the periodontal ligament became more evident with increasing extrusive force.

Conclusions This new method was capable of generating histological changes, proving its secure application in this research area. The 1500cN force produced more damage on the periodontal ligament.

Clinical relevance The validation of a new experimental method can produce more reliable evidence in further research.

Keywords Tooth injuries · Dental trauma · Models · Animal · Validation studies

Introduction

The treatment protocols related to traumatic dental injuries are still considered contradictory in many cases [1]. The low incidence of some types of dental traumatism, such as the extrusive luxation, and the need for multidisciplinary treatment, makes it difficult to conduct studies that produce reliable evidence [1–3]. In addition, the emergency characteristics related to traumatic dental injuries preclude the conduct of clinical trials in humans for ethical reasons [1].

Extrusive luxation is defined as the partial displacement of the tooth out of the alveolus [4] and represents a severe lesion to the periodontal ligament and pulp [5]. The repair process related to this kind of dental traumatism is scarce in the literature. Animal models are one of the methods used in the pursuit of understanding the sequelae and treatment measures of dental trauma [6], as these models occupy the base of the pyramid of scientific evidence [7]. Studies related to traumatic dental injuries conducted experimentally in rats have helped the understanding of the histogenesis of traumatic changes in the development of tooth germ [8], the biological events involved in dental re-implantation [9], as well as events occurring after the luxation of immature teeth [10].

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A challenge for researches is the use of an experimental method which produces effects related to the experimental procedure made, reducing the bias inherent to scientific method. In this aspect, the validation of new methods is essential as a mean of strengthen the results. In the dental trauma studies, it is not different, and concern has been expressed by researchers to validate their working methods [6, 11–13].

Thus, the objective of this research was to test, evaluate, and validate a new method of dental trauma simulation, extrusive luxation type, on maxillary first molars of rats.

Material and methods

This research was performed in accordance with the criteria established in the ARRIVE guidelines [14]. The research protocol was approved by the Ethics Committee on Animal Use of the Dental School, "Julio de Mesquita Filho" São Paulo State University, Araçatuba, São Paulo, Brazil (protocol no. 2014-00815). All experimental procedures were made between February and June of 2015.

Forty young adult male rats (*Rattus norvegicus albinus*, Wistar), aged 45 days and weighing 230–250 g at the beginning of the procedures, obtained from São Paulo State University's bioterium, were used. The animals were kept in plastic cages under controlled climate conditions with a light cycle of 12/12 h and constant temperature and were fed with solid feed (Ração Ativada Produtor®; Anderson & Clayton S.A., Abbott Laboratories, São Paulo, SP, Brazil) and water ad libitum. The cages were sanitized every other day, and no postsurgical analgesic was administered to avoid interferences in the inflammatory process [15] related to the repair of the structures that are the object of this study.

All procedures were performed under general anesthesia. The animals received an intramuscular injection of xylazine hydrochloride (Dopaser; Caleir S.A., Barcelona, Spain; 0.03 ml per 100 g body weight) and ketamine hydrochloride (VETASET; Fort Dodge Animal Health, Iowa, USA; 0.07 ml per 100 g body weight). The animals were randomly divided into eight groups ($n = 5$) as described in Table 1. For that, the animals received a random number between 1 and 40. Then, a random sequence was generated on Excel 2010 for Windows program, and the animals were allocated for each group following that sequence.

Conducting the extrusive luxation trauma

Extrusive luxation trauma was induced experimentally in the right first maxillary molars of the animals. The rats were placed in an operating table in the supine position. Their legs were trapped to restrict movement, the heads were kept still, and their mouths remained open during the procedures [11].

To perform the extrusive luxation, the following protocol was used (Fig. 1):

Table 1 Group division

Groups	Procedures
Group control, 1 day (GC1D)	No procedure Euthanasia after 1 day
Group control, 3 days (GC3D)	No procedure Euthanasia after 3 days
Group trauma, 1100cN, 1 day (GT1100/1D)	Trauma of EL with 1100cN Euthanasia after 1 day
Group trauma, 1300cN, 1 day (GT1300/1D)	Trauma of EL with 1300cN Euthanasia after 1 day
Group trauma, 1500cN, 1 day (GT1500/1D)	Trauma of EL with 1500cN Euthanasia after 1 day
Group trauma, 1100cN, 3 days (GT1100/3D)	Trauma of EL with 1100cN Euthanasia after 3 days
Group trauma, 1300cN, 3 days (GT1300/3D)	Trauma of EL with 1300cN Euthanasia after 3 days
Group trauma, 1500cN, 3 days (GT1500/3D)	Trauma of EL with 1500cN Euthanasia after 3 days

1. A 0.010-in. ligature wire (Morelli®; Sorocaba, São Paulo, Brazil) was inserted, with the aid of a clinical Perry model clamp (Golgran; São Caetano do Sul, São Paulo, Brazil), from the buccal to lingual direction between the first and second maxillary right molars (Fig. 1a).
2. The two ends of the wire were placed on the mesial side of the first maxillary molar and twisted in the form of a pig tail, with the aid of a Mathieu 17.0-cm needle holder (Quinelato; Rio Claro, São Paulo, Brazil), until the wire fit and remained close to the animal's tooth (Fig. 1b).
3. A dental mirror handle (Golgran; São Caetano do Sul, São Paulo, Brazil) was positioned between the two remaining ends of the wire, and a new twist was performed (Fig. 1c), to create a ring in which the tensiometer's active end was adapted (model 75.02.006; Morelli, Sorocaba, São Paulo, Brazil) and placed (Fig. 1d).
4. The tensiometer was positioned on an adjustable support device as described by Pereira et al. [11], but with a traction angle of 60° to the vertical plane of the biarticulated arm (Fig. 1e).
5. Forces of 1100cN, 1300cN, or 1500cN were applied for 5 s (Fig. 1f), and the device was removed.

After the end of experimental period for each group, the animals were euthanized by an overdose of anesthetic (sodium pentobarbital, 150 mg/kg) and then decapitated. The maxilla was fixed in buffered 10% formalin for 48 h.

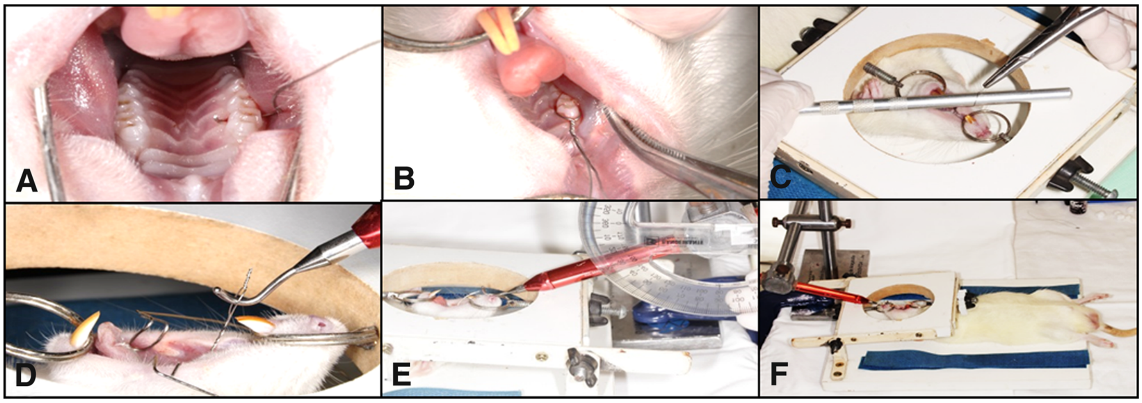


Fig. 1 **a**–**f** Method of trauma application

The right hemi-maxilla of each animal was decalcified in 10% Plank-Rychlo solution for 8 days, embedded in paraffin, and cut into 4-μm serial cuts (sections) in the longitudinal direction of the root. A histological slide with three sections of each specimen was created and stained with hematoxylin and eosin (HE).

Inclusion criteria for the selection of histological section

The selected sections for analysis were chosen by the following inclusion criteria: (a) microscopic visualization of the entire longitudinal extent of the buccal mesial root and buccal distal root of the first upper right molar, (b) the presence of open apical foramen, and (c) the presence of interradicular septum (Fig. 2a, b). Of the three sections presented in each slide, the one that mostly suited the inclusion criteria was evaluated. Thus, one section per slide, per specimen was evaluated.

Assessment of histological sections

For descriptive and semi-quantitative histological analysis, a light microscope (Leica Microsystems, Wetzlar GmbH, Germany) was used. To obtain the photomicrographs, with magnifications of × 200 and × 400, a digital camera (JVC; Victor Company of Japan, Ltd., Japan) attached to the microscope was used. The images were captured using the Leica QWin Standard v2.4 software (Leica Microsystems Imaging Solutions, Ltd., Cambridge, UK) for further analysis.

The specific areas of the study for descriptive analysis were the following: (1) the periodontium of the mesial and distal sides of buccal mesial and buccal distal roots in the cervical, middle, and apical thirds; (2) the periodontium of the furcation region; (3) the mesial bone crest; (4) the interradicular septum; and (5) the interdental septum between the first and second upper right molars (Fig. 3). The histopathological events that were investigated were external root resorption, areas of hyalinization, inflammatory infiltrate, the presence of giant multinucleate cells, and the presence of bleeding. Each event was evaluated as follows: −, absence; +, occasional presence; ++, moderate presence; and +++, intense presence.

Semi-quantitative analysis

For the quantification of histological events, scores 1 to 4 were assigned for the different events listed below. Specific areas of this analysis were (1) the distal root, mesial side, and cervical and middle thirds; (2) the mesial root, distal side, and cervical and middle thirds; (3) mesial, middle, and distal furcation; and (4) mesial side of the mesial buccal root (adjacent region mesial bone crest).

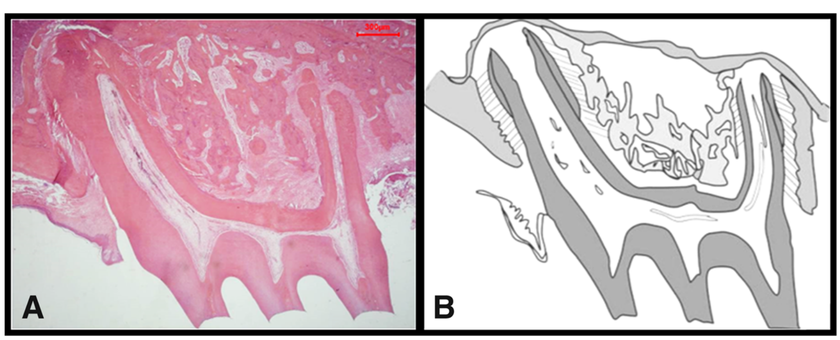


Fig. 2 Representation of a typical histological cut. **a** Histological cut of the first upper right molar of a rat (× 4 magnification). **b** Representation of the upper right first molar of a rat

The events investigated and their respective scores were as follows:

1. Intensity of acute inflammatory process (presence of polymorphonuclear cells) in the periodontal ligament [16]

(a) Absence or occasional presence of inflammatory cells
(b) Small number of inflammatory cells, up to 10 cells per field with a magnification of × 400
(c) Moderate number of inflammatory cells, from 11 to 50 cells per field with a magnification of × 400
(d) Large number of inflammatory cells, above 50 inflammatory cells per field with a × 400 magnification

2. Intensity of chronic inflammation (multinucleated giant cells) in the periodontal ligament [16]

(a) Absence or occasional presence of inflammatory cells
(b) Small number of inflammatory cells, up to 10 cells per field with a magnification of × 400
(c) Moderate number of inflammatory cells, from 11 to 50 cells per field with a magnification of × 400
(d) Large number of inflammatory cells, above 50 inflammatory cells per field with a × 400 magnification

3. Vascular changes in the periodontal ligament (Fig. 4)

(a) Absence or occasional presence of blood vessels
(b) Presence of hyperemic blood vessels
(c) Presence of light bleeding (some loose red blood cells in the ligament)
(d) Bleeding of the periodontal ligament (many free red blood cells in the periodontal ligament)

Fig. 3 Areas assessed. MBR mesial buccal root, DBR distal buccal root, MBC mesial bone crest, IRS interradicular septum, IDS interdental septum

4. Active and inactive external root resorption [16]

(a) Absence of resorption area
(b) Presence of inactive resorption area (absence of clastic cells)
(c) Presence of small areas of active resorption
(d) Presence of extensive areas of active resorption

The variables were summarized using relative frequency measurements. For statistical analysis, the SPSS software features (version 17.0) were used. The distribution of scores between the experimental groups and control group and that between the experimental groups at different times were compared using the Freeman-Halton extension of Fisher's exact test. The significance level was 5%.

Results

Descriptive analysis

Control groups

Histological characteristics of control groups GC1D and GC3D were not different. The periodontal ligament had normal characteristics and was rich in fibroblasts. The collagen fibers were arranged obliquely in relation to the root surface in the mesial and distal sides of both roots, horizontally in the furcation region, and disorganized in the root apex region. There were no areas of hyalinization or presence of inflammatory infiltrate and multinucleated giant cells in any of the animals. The root surfaces were completely intact. The interradicular septum had a normal appearance. The bone crest appeared normal. The dental cementum is continuous up to the apex.

GT1100/1D

The periodontal ligament had light bleeding, particularly in the furcation region in all animals, with no inflammatory infiltrates. Areas of hyalinization or external root resorption were not found in any of the specimens. Rare multinucleated giant cells were observed on the surface of the mesial bone crest, interradicular septum, and interdental septum. The dental cementum had a normal appearance.

GT1300/1D

The periodontal ligament had moderate bleeding, particularly in the furcation area of all animals. There were no inflammatory infiltrates. Areas of hyalinization or external root resorption were not found in any of the specimens. Rare

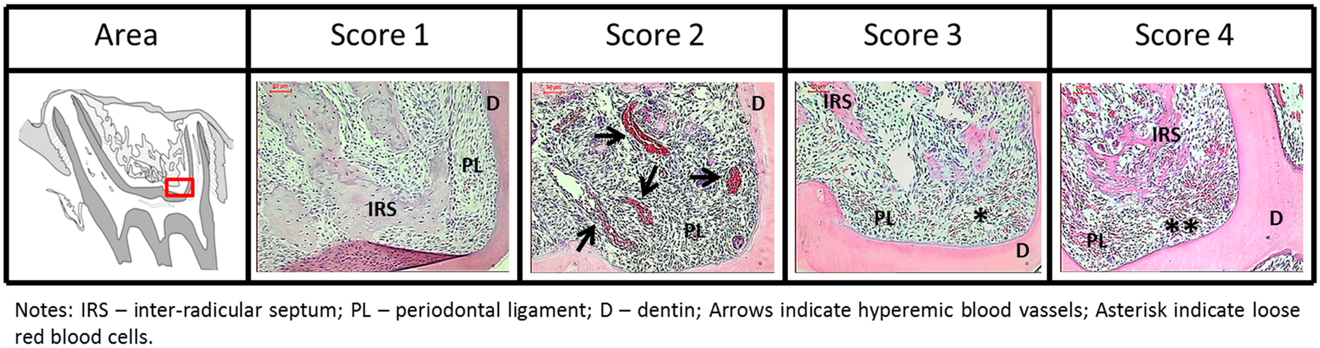


Fig. 4 Example of semi-quantitative scoring of vascular changes. Score 1, absence or occasional presence of blood vessels; score 2, presence of hyperemic blood vessels; score 3, presence of light bleeding (some loose red blood cells in the ligament); score 4, bleeding of the periodontal ligament (many free red blood cells in the periodontal ligament). IRS interradicular septum, PL periodontal ligament, D dentin. Arrows indicate hyperemic blood vessels. Asterisks indicate loose red blood cells. Area of observation - cervical third, mesial side of buccal distal root

multinucleated giant cells were observed on the surface of the mesial bone crest, interradicular septum, and interdental septum.

GT1500/1D

The periodontal ligament had severe areas of bleeding, particularly in the furcation region in all animals. There were no acute inflammatory infiltrates in the periodontal ligament. Areas of hyalinization were not found in any of the specimens. Two animals showed external root resorption, but only in the cervical third of the mesial side of the mesial buccal root. Rare multinucleated giant cells were observed on the surface of the mesial bone crest and interradicular septum.

GT1100/3D

The periodontal ligament had normal characteristics, except for rare free red blood cells in the PL. There were no acute inflammatory infiltrates in the periodontal ligament. Areas of hyalinization or external root resorption were not found in any of the specimens. Rare multinucleated giant cells were observed on the surface of the mesial bone crest, interradicular septum, and interdental septum.

GT1300/3D

The periodontal ligament had a few congested blood vessels, particularly in the distal furcation region. There were no acute inflammatory infiltrates in the periodontal ligament. Areas of hyalinization or external root resorption were not found in any of the specimens. Rare multinucleated giant cells were observed on the surface of mesial bone crest, interradicular septum, and interdental septum.

GT1500/3D

The periodontal ligament had many congested blood vessels, particularly in the furcation region. There were no acute inflammatory infiltrates in the periodontal ligament. Areas of hyalinization or external root resorption were not found in any of the specimens. Rare multinucleated giant cells were observed on the surface of the mesial bone crest, interradicular septum, and interdental septum.

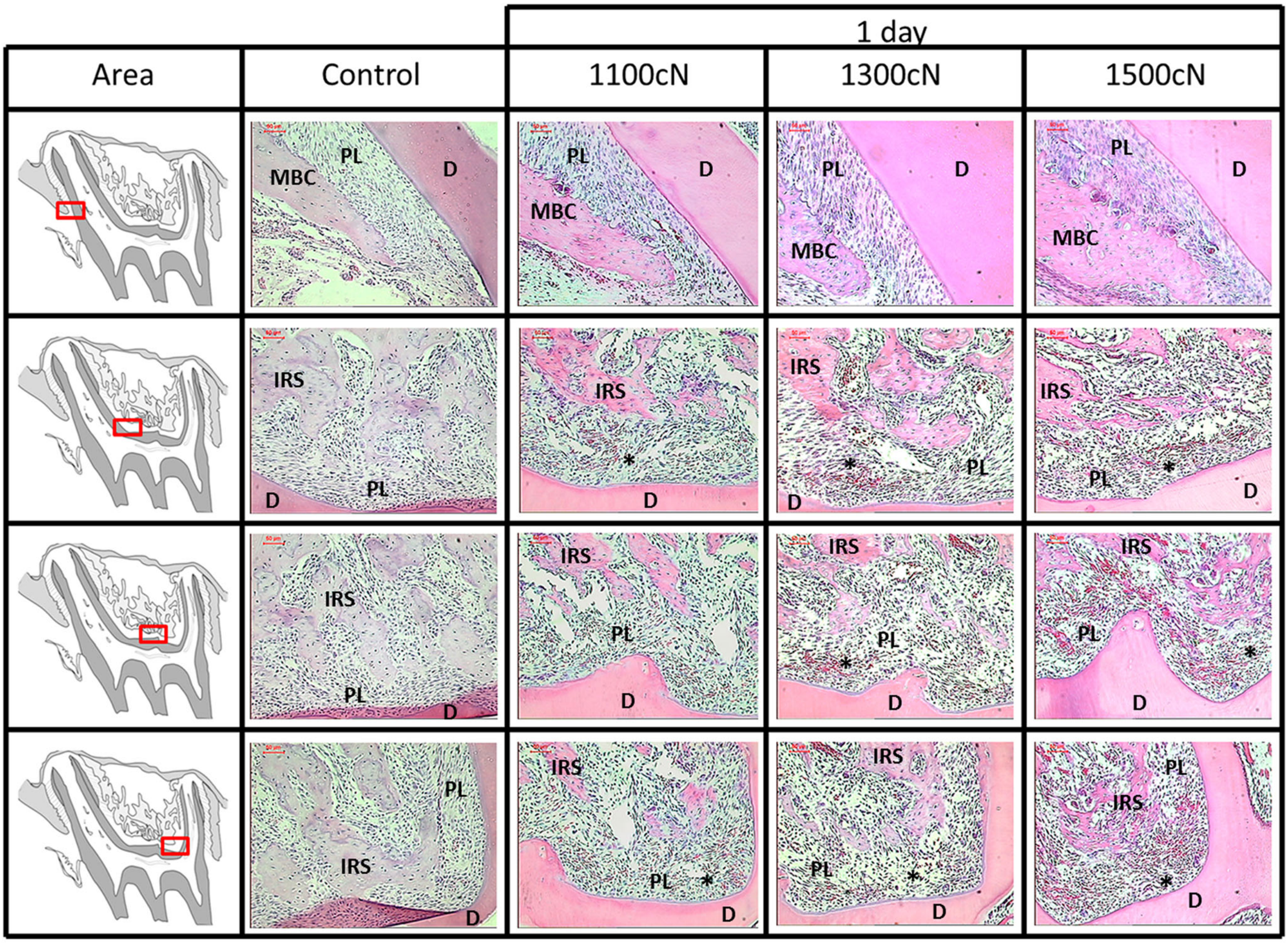
The results are shown in Table 2 and Figs. 4 and 5.

Table 2 Summary of the descriptive analysis

Groups	Bleeding	Congested vessels	Chronic inflammatory infiltrate	Acute inflammatory infiltrate	Areas of active resorption in IS
GC1D/3D	−	−	+	−	−
GT1100/1D	+	−	+	−	−
GT1300/1D	++	−	+	−	−
GT1500/1D	+++	−	+	−	−
GT1100/3D	+	−	+	−	−
GT1300/3D	+	+	+	−	−
GT1500/3D	+	+++	+	−	−

−, absence; + occasional presence; ++ moderate presence; +++ intense presence

IS interradicular septum



Notes: MBC – mesial bone crest; IRS – inter-radicular septum, PL – periodontal ligament; D – dentin; Arrows indicate hyperemic blood vassels; Asterisk indicate loose red blood cells

Fig. 5 Summary of findings for day 1. It can be observed that the periodontal ligament had more severe bleeding areas with the increase on magnitude of extrusive force applied. MBC mesial bone crest, IRS interradicular septum, PL periodontal ligament, D dentin. Asterisks indicate loose red blood cells. The red rectangles represent the area of observation

Semi-quantitative analysis

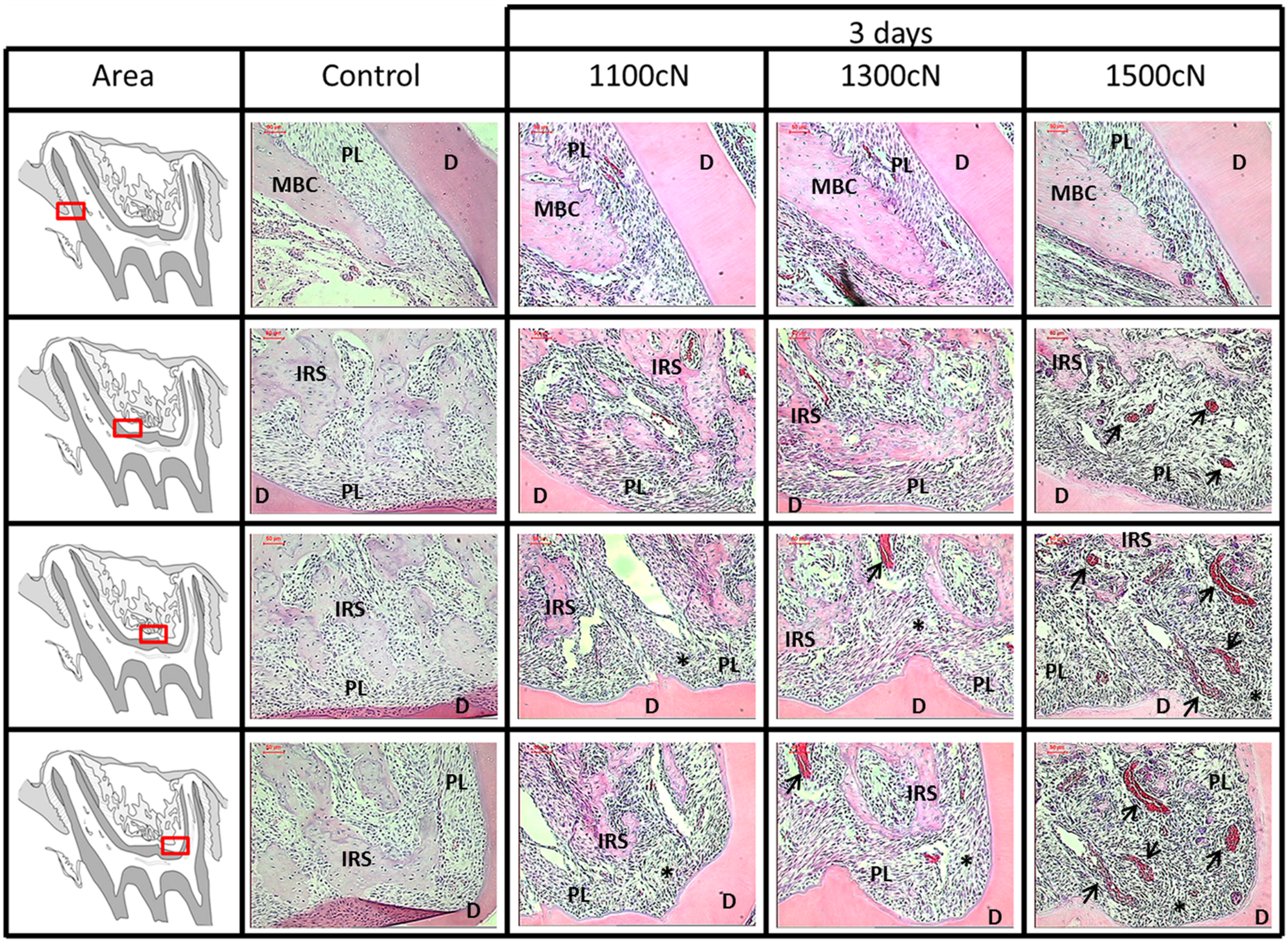
On the evaluation of acute and chronic inflammatory process in the periodontal ligament and reabsorption of the interradicular septum, score 1 was found on 100% of samples at 1 and 3 days (Fig. 6).

Table 3 describes the distribution of scores for the vascular changes in the evaluated anatomic regions in absolute frequency and relative frequency. It is shown that vascular alterations in 1500cN force groups, especially on furcation area, reach scores 3 and 4, the most intense ones. These results are confirmed by the statistical analysis presented in Table 4, suggesting that the vascular alterations occur more intensely on the first day in 1300cN and 1500cN groups and decrease on the third day, except on middle furcation ($p = 0.04$) and distal furcation ($p = 0.04$) in the 1500cN group.

The vascular changes did not appear to have a significant reduction ($p = 0.09$) in the 1500cN group in the mesial region of the furcation between days 1 and 3 (Table 4). Vascular changes in the 1500cN group are also significant in the cervical region of the mesial side of the distal root and in the distal side of the mesial root ($p < 0.01$); however, a significant reduction in these characteristics after the period of 3 days ($p < 0.01$) was noted.

Discussion

An experimental method for studies of dental trauma in rat molars was presented and evaluated. This method was effective for this kind of study because it allowed extrusive luxation simulation generating changes that can be



Notes: MBC – mesial bone crest; IRS – inter-radicular septum, PL – periodontal ligament; D – dentin; Arrows indicate hyperemic blood vassels; Asterisk indicate loose red blood cells

Fig. 6 Summary of findings for day 3. It can be observed that the periodontal ligament had more hyperemic blood vessels on the group where a higher magnitude of extrusive force was applied. MBC mesial bone crest, IRS interradicular septum, PL periodontal ligament, D dentin. Arrows indicate hyperemic blood vessels. Asterisks indicate loose red blood cells. The red rectangles represent the area of observation

observed microscopically and that were directly related to the procedure made.

In humans, extrusive luxation represents a severe lesion to the periodontal ligament and pulp [5]. A human molar is approximately 50 times larger than a rat molar [17]. Thus, the forces used in this study (1.100cN, 1.300cN, and 1.500cN) are comparable to 55.000cN, 65.000cN, and 75.000cN on a human molar, which represents the forces of approximately 550, 650, and 750 kg, respectively. Those forces were capable to generate such stress that results on bleeding of the periodontal ligament that are compatible to extrusive luxation traumatism [4].

The most striking result in response to the induced dental trauma was vascular changes, specifically bleeding in the periodontal ligament when 1500cN force was applied on day 1 (GT1500/1D). The standardization of angulation and the forces used generated homogeneous microscopic changes and were compatible with the induced dental traumatism throughout the studied sample.

The tensiometer used to perform the dental trauma allowed for the standardization of force in its magnitude. The biarticulated arm, the same used by Pereira et al. [11], allowed for the standardization of the applied force direction (angle). The positive and differential features of this method were generated in uniformity in the biological responses that was found in every sample.

In the study of Birkedal-Hansen [18] that used surgical levers to traumatize rats' molars resulted in variability of inflammatory responses, possibly due to the lack of standardization of the magnitude and applied angle force to the rats' tooth.

In 1990, Miyashin et al. [6] developed a method to standardize the variables of duration, magnitude, and direction of force in the upper molars of rats with incomplete root formation. However, this method did not determine the forces

Table 3 Distribution of scores for the vascular changes in the evaluated anatomic regions

Areas	1 day				3 days			
	Control	1100cN	1300cN	1500cN	Control	1100cN	1300cN	1500cN
Mesial root, mesial side								
Cervical third (score 1)	5 (100%)	5 (100%)	4 (100%)	5 (100%)	5 (100%)	5 (100%)	5 (100%)	4 (100%)
Middle third (score 1)	5 (100%)	4 (100%)	4 (100%)	4 (100%)	5 (100%)	5 (100%)	4 (100%)	4 (100%)
Mesial root, distal side								
Cervical third								
Score 1	5 (100%)	2 (40%)	2 (50%)	0 (0)	5 (100%)	5 (100%)	5 (100%)	4 (100%)
Score 3	0 (0)	3 (60%)	2 (50%)	3 (60%)	0 (0)	0 (0)	0 (0)	0 (0)
Score 4	0 (0)	0 (0)	0 (0)	2 (40%)	0 (0)	0 (0)	0 (0)	0 (0)
Middle third								
Score 1	5 (100%)	3 (60%)	2 (50%)	5 (100%)	5 (100%)	5 (100%)	5 (100%)	4 (100%)
Score 3	0 (0)	2 (40%)	2 (50%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Mesial furcation								
Score 1	5 (100%)	4 (80%)	0 (0)	1 (20%)	5 (100%)	5 (100%)	5 (100%)	2 (50%)
Score 2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (50%)
Score 3	0 (0)	1 (20%)	4 (100%)	2 (40%)	0 (0)	0 (0)	0 (0)	0 (0)
Score 4	0 (0)	0 (0)	0 (0)	2 (40%)	0 (0)	0 (0)	0 (0)	0 (0)
Middle furcation								
Score 1	5 (100%)	0 (0)	0 (0)	0 (0)	5 (100%)	5 (100%)	5 (100%)	1 (25%)
Score 2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (75%)
Score 3	0 (0)	4 (80%)	4 (100%)	2 (40%)	0 (0)	0 (0)	0 (0)	0 (0)
Score 4	0 (0)	1 (20%)	0 (0)	3 (60%)	0 (0)	0 (0)	0 (0)	0 (0)
Distal furcation								
Score 1	5 (100%)	0 (0)	0 (0)	0 (0)	5 (100%)	5 (100%)	5 (100%)	1 (25%)
Score 2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Score 3	0 (0)	4 (80%)	4 (100%)	1 (20%)	0 (0)	0 (0)	0 (0)	
Score 4	0 (0)	1 (20%)	0 (0)	4 (80%)	0 (0)	0 (0)	0 (0)	0 (0)
Distal root, mesial side								
Cervical third								
Score 1	5 (100%)	5 (100%)	1 (25%)	0 (0)	5 (100%)	5 (100%)	5 (100%)	3 (75%)
Score 2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (25%)
Score 3	0 (0)	0 (0)	3 (75%)	3 (60%)	0 (0)	0 (0)	0 (0)	0 (0)
Score 4	0 (0)	0 (0)	0 (0)	2 (40%)	0 (0)	0 (0)	0 (0)	0 (0)
Middle third								
Score 1	5 (100%)	5 (100%)	4 (100%)	4 (100%)	5 (100%)	5 (100%)	4 (100%)	4 (100%)

corresponding to different root formations [13]. In 1998, Shibue et al. [13] showed an extrusive force method based on the degree of root formation, but the proposed method is complex and used materials of difficult access.

In 2010, Pereira et al. [11] suggested a subluxation method with a standardization of the force used. This method, however, uses an intrusive force and does not standardize the angle of force application.

The reliability of an experimental method is related to its ability to allow reproducibility of all characteristics of the tested procedures with similar biological results. In this aspect, the method presented here is shown to be efficient and effective.

The extrusive luxation method proposed produced a tooth displacement in the occlusal mesial direction, featured a controlled tilting movement (rotation), and was capable of forming compression and stretching areas on the periodontal ligament that were very similar. This resulted in similar histological responses in every sample (Fig. 7).

The initial phase of the inflammatory process is a vascular-exudative phenomenon which develops between days 1 and 3 in humans and culminates in its resolution or chronification

Table 4 Statistical analysis of vascular changes in between experimental groups (1100cN, 1300cN, or 1500cN) and control group and between experimental groups at different times

Areas	1 day			3 days			1 versus 3 days		
	1100cN	1300cN	1500cN	1100cN	1300cN	1500cN	1100cN	1300cN	1500cN
Mesial root, mesial side									
Cervical third (score 1)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Middle third (score 1)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mesial root, distal side									
Cervical third	0.16	0.16	< 0.01*	1.00	1.00	1.00	0.16	0.16	< 0.01*
Middle third	0.44	0.16	1.00	1.00	1.00	1.00	0.44	0.16	1.00
Mesial furcation	0.49	< 0.01*	0.04*	1.00	1.00	0.16	0.49	< 0.01*	0.09
Middle furcation	< 0.01*	< 0.01*	< 0.01*	1.00	1.00	0.04*	< 0.01*	< 0.01*	< 0.01*
Distal furcation	< 0.01*	< 0.01*	< 0.01*	1.00	1.00	0.04*	< 0.01*	< 0.01*	0.04*
Distal root, mesial side									
Cervical third	1.00	0.04*	< 0.01*	1.00	1.00	0.44	1.00	0.04*	< 0.01*
Middle third (score 1)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

*$p < 0.05$, statistically significant differences (Freeman-Halton extension of Fisher's exact test)

[19]. To a great extent and as its main effect, the application of force causes bleeding.

The bleeding was the most striking biological response related to the model analyzed and is consistent with hemostasis of the repair process phase [4]. It was more exuberant in the periodontal ligament region adjacent to the distal furcation area, which is the region where the periodontal ligament has undergone great compression. This characteristic was even more exuberant as greater force was applied (Figs. 6 and 7).

Between the study limitations, it can be cited the impossibility of evaluation of some areas on histological cuts, decreasing the number of animals evaluated per group. Because the responses were very similar, it was decided not substitute or increase the number of animals. All efforts were made to attend the principles of animal use in scientific research.

The proposed method is simple and standardizes the angle and intensity of the applied force, producing similar biological responses across the sample without crown, root, or alveolar bone fractures. The validation of this trauma model allows new research to be conducted with the assurance that all the animals were exposed to the same type of trauma.

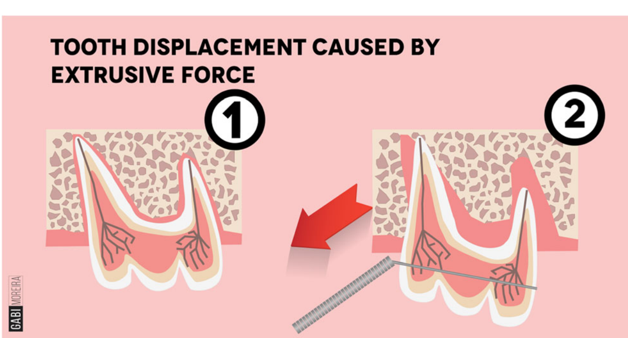


Fig. 7 Tooth displacement caused by extrusive force

Conclusion

The method tested was efficient for simulating the dental trauma of extrusive luxation, and the force magnitude of 1500cN was the most effective on producing biological damage.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent For this type of study, formal consent is not required.

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