Dental Traumatology

*Dental Traumatology* 2010; **26**: 37–42; doi: 10.1111/j.1600-9657.2009.00837.x

# Histological evaluation of experimentally induced subluxation in rat molars and its implications on the management of orthodontic treatment

**Alex Luiz Pozzobon Pereira[1], Marcos Rogério de Mendonça[1], Celso Koogi Sonoda[2], Osmar Aparecido Cuoghi[1], Wilson Roberto Poi[2]**

[1]Discipline of Orthodontics, Department of Pediatric and Community Dentistry, Dental School of Araçatuba, São Paulo State University (UNESP), Araçatuba, SP, Brazil; [2]Discipline of Integrated Clinics, Department of Surgery and Integrated Clinics, Dental School of Araçatuba, São Paulo State University (UNESP), Araçatuba, SP, Brazil

Correspondence to: Marcos Rogério de Mendonça, Departamento de Odontologia Infantil e Social, Disciplina de Ortodontia, Faculdade de Odontologia de Araçatuba/ UNESP, Rua José Bonifácio 1193, CEP 16015-050, Araçatuba, SP, Brazil
Tel.: +55 18 3636 3236
e-mail: marcosrm@foa.unsp.br

Accepted 22 August, 2009

**Abstract** – The purpose of this study was to evaluate the histological alterations occurred in the periradicular region of rat molars after intentional subluxation using an experimental method to induce dentoalveolar trauma. Eighteen adult male Wistar rats (*Rattus norvegicus albinus*) were selected for the study. The dentoalveolar trauma was experimentally induced by the application of an occlusogingival force on the occlusal surface of the maxillary right first molar using a tensiometer secured on a fully articulated support with adjustable steel shafts. The animals were assigned to six groups ($n = 3$), according to the intensity of the force applied to induce trauma: Group I (GI, control) – no force application; Groups II–VI (GII–GVI) – the animals were subjected to 600, 700, 800, 900 and 1000 cN force, respectively. After experimental induction of trauma, the animals were sacrificed by anesthetic overdose and the right maxillas were removed and processed for histological analysis under light microscopy. In the animals of GII, GIII and GIV, the histological alterations were similar to those described for GI. GVI (1000 cN) presented the most severe alterations, with the occurrence of buccal bone plate fracture, alveolar fracture and root fracture, which are not present in mild traumatic injuries like subluxation. The 900 cN force (GV) was capable to produce clinical and histological alterations in the gingival and periodontal tissues compatible with those observed in subluxation.

Dentoalveolar trauma is a subject of great interest in dental practice and has been considered an important public health problem not only because of its relatively high prevalence but also because its functional and esthetic sequelae may have a substantial impact on the patient's life. High levels of interpersonal violence, traffic accidents and a greater engagement of children in sports activities have contributed significantly to raise the prevalence of traumatic tooth injuries in the last years (1, 2).

Increased overjet frequently associated with Class II, division 1 malocclusion (3) and inadequate lip coverage of maxillary incisors are considered as significant risk factors for dental trauma (4). According to some authors (5, 6), early preventive orthodontic treatment is recommended for patients with these characteristics not only to correct the malocclusion but also to reduce the risk of trauma and fracture of the maxillary incisors.

According to Caldas and Burgos (1), most cases of dental trauma occur in patients aged 6–15 years, which is the age group that most often seek orthodontic care. Orthodontic movement of traumatized teeth or restart of orthodontic treatment interrupted due to trauma has increased in daily clinical practice. However, the lack of studies does not allow establishing safe treatment protocols elaborated on the basis on research data. Great part of the literature referring to dental trauma and orthodontic treatment comprises clinical trials with different methodologies, anecdotal case and reports, retrospective review articles with small patient samples an even empirical clinical experiences (3, 7–16), which makes it difficult to establish realistic comparisons or obtain reliable outcomes that can rule the clinical management of orthodontic patients who have sustained dental trauma. Orthodontists need scientifically based evidence that substantiate the orthodontic movement in traumatized teeth with minimal sequelae at the end of the treatment.

This study evaluated the histological alterations occurred in the periradicular region of rat molars after

© 2010 The Authors. Journal compilation © 2010 John Wiley & Sons A/S

intentional subluxation using an experimental method to induce dentoalveolar trauma.

## Material and method

The research project was independently reviewed and approved by the Animal Research Ethics Committee of the Dental School of Araçatuba, São Paulo State University (UNESP, Brazil). All guidelines regarding the care of animal research subjects were strictly followed.

Eighteen young adult male Wistar rats (*Rattus norvegicus albinus*) aged 3–4 months and weighing between 250 and 350 g were selected for the study. The animals were housed in plastic cages under climate-controlled conditions (12 h light/12 h dark; thermostatically regulated room temperature) and were fed a standard solid chow (Ração Ativada Produtor; Anderson & Clayton S.A. Indústria e Comércio, São Paulo, SP, Brazil) and water *ad libitum*.

All experimental procedures were performed under anesthesia. The animals received an intramuscular injection of ketamine hydrochloride (Vetaset®; Fort Dodge Animal Health, Overland Park, KS, USA; 0.07 ml 100 $g^{-1}$ body weight) and xylazine hydrochloride (Dopaser®; Caleir S.A., Barcelona, Spain; 0.03 ml 100 $g^{-1}$ body weight). The dentoalveolar trauma was experimentally induced by the application of an occlusogingival acute force on the occlusal surface of the maxillary right first molar. After anesthesia, the animals were placed on a surgical table lying on their backs and had their forefeet and hindfeet secured with VELCRO®-type fasteners. During the experimental method to induce dentoalveolar trauma, the mouth was kept opened with a holding device and two rings positioned in the maxillary incisors maintained the head immobilized. The animals were assigned to six groups ($n = 3$), according to the intensity of the experimentally induced dentoalveolar trauma. In Group I (GI, control), the animals were not subjected to dentoalveolar trauma. In Groups II–VI (GII–GVI), the animals were subjected to 600, 700, 800, 900 and 1 000 cN force, respectively.

The device proposed in the present study consists basically of two parts: a tensiometer (Morelli, Sorocaba, SP, Brazil – code 72.02.006) and a support with adjustable galvanized steel shafts (Fig. 1a). One of the tensiometer ends was modeled to a round shape with acrylic resin (Orto-Class; Clássico, São Paulo, SP, Brazil) (Fig. 1b). In this way, when the opposite end of the tensiometer was stretched and then released, the acrylic-adapted end transmitted an impact corresponding to the force on the graduated scale of the tensiometer. In order to standardize the direction of the applied force and the position of the acrylic-adapted end on the tooth to be subjected to trauma, the tensiometer was secured on a fully articulated galvanized steel support. This support consists of a 30-cm vertical shaft fixed on a rectangular base measuring $15 \times 7.5$ cm, a 30-cm horizontal shaft connected to the vertical shaft by a 6.0-cm threaded intermediate connector, and another threaded wrist-shaped connector that is attached to one of the horizontal shaft's ends responsible for the vertical adjustment and fixation of the tensiometer. The threaded connectors provide a precise positioning of the tensiometer, whereas the intermediate connector allows for adjusting the horizontal shaft in the antero-posterior direction as well as its height and a 360° rotation around the vertical shaft. The wrist-shaped connector fastens the tensiometer and drives it vertically around the horizontal shaft, allowing for a correct

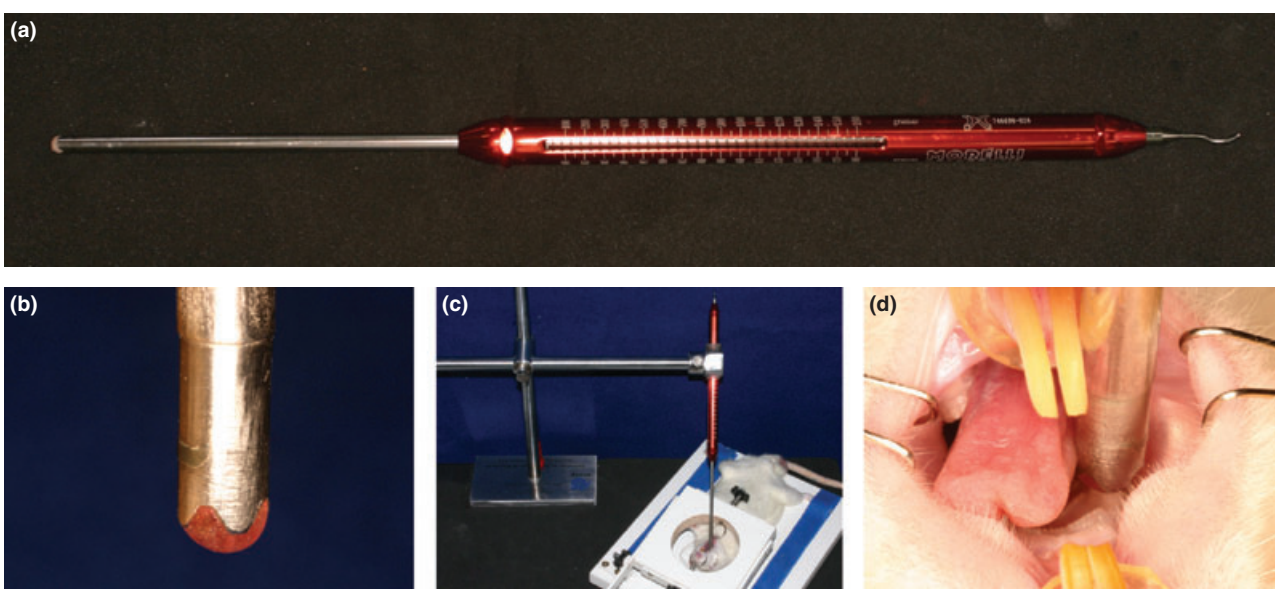


*Fig. 1.* Description of the device used for experimental induction of dentoalveolar trauma. (a) Tensiometer used in the study with capacity to measure forces up to 1 600 g. (b) Active end of the tensiometer modeled with acrylic resin to a rounded shape. (c) Tensiometer adjusted on the rat molar. (d) Round-shaped end of the tensiometer adjusted on the mesiobuccal crest of the rat maxillary right first molar.

© 2010 The Authors. Journal compilation © 2010 John Wiley & Sons A/S

adaptation of the tensiometer end over the tooth (Fig. 1c).

Before experimental induction of the dentoalveolar trauma, the rectangular base of the support was firmly fixed by two straps on a workbench in order to avoid any displacement of the device during the experiment. Next, the horizontal shaft and the tensiometer were adjusted by their connectors, providing a precise positioning of the acrylic-adapted tensiometer end on the occlusal surface of the rat maxillary right first molar (Fig. 1d). Different forces were applied (600–1000 cN force), according to the experimental groups.

The animals were sacrificed by anesthetic overdose immediately after experimental induction of trauma. The right maxillas were removed, fixed in 10% buffered formalin for 24 h and decalcified in acid 10% EDTA solution for 5 weeks. After decalcification, the specimens were embedded in paraffin and serial histological sections were cut from the mesial root of the maxillary right first molar (including the surrounding tissues) in a buccolingual direction following the long axis of the tooth. For histological analysis, sections from the middle portion of the root were collected, stained with hematoxylin, and eosin and observed under light microscopy.

## Results

The histological analysis comprised the coronal portion and the mesial root of the maxillary right first molar. Dentin, cementum, alveolar bone tissue, periodontal ligament (PDL) and the junctional epithelium of the gingival papilla were examined in these regions (Table 1).

In GI, the junctional epithelium was juxtaposed to the cemental surface at the cervical root third height. Underneath this region, the connective tissue was rich in fibroblasts and collagen fibers, which were attached to the root surface and to the gingival tissue (Fig. 2a). The pulp tissue was well vascularized, rich in cell components and with great amount extracellular matrix (Fig. 2b). There were a large number of fibroblasts and the collagen fibers did not have a well defined organization. The root dentin walls presented a parallel disposition and the pulp tissue in this region exhibited large blood vessels disposed parallel to the root canal walls (Fig. 2c). The odontoblasts close to the dentin surface were arranged in columns. Close to these cells, there was a predentin layer, which was continuous in all analyzed areas (Fig. 2c). The PDL was rich in collagen fibers and fibroblasts. The collagen fiber bundles were arranged horizontally in the cervical region, while in the middle and apical thirds they presented an oblique arrangement (Fig. 2d). In the periapical and furcal regions, these fibers presented an irregular arrangement. Close to the root apex, the dentin was covered by a thin layer of primary cementum which, in turn, was covered by a thin layer of secondary cementum (Fig. 2d). The alveolar walls were rich in osteoblasts and osteocytes.

In the animals of GII, GIII and GIV, the histological alterations were similar to those described for GI.

The most significant alterations were observed in the mesial root of the animals subjected to 900 and 1000 cN alveolar trauma (GV and GVI).

In GV, the coronal and root pulps exhibited the same characteristics observed in GI. In two specimens, the junctional epithelium of the marginal gingiva located in the mesial surface of the mesial root was detached from the cemental surface (Fig. 3a). Contiguous to the epithelial tissue, some specimens presented small gaps in the area of gingival connective attachment. In the same region, this connective tissue exhibited edema and disorganized collagen fibers, without reaching the PDL space. Areas of disrupted collagen fibers and blood vessels were also observed (Fig. 3b).

*Table 1.* Clinical and histological alterations observed in the specimens

| | | Clinical alterations | | | | Histological alterations | | | |
|---|---|---|---|---|---|---|---|---|---|
| Groups | Animals | M | B | CF | BBPF | RPDL | REIGS | ABF | RF |
| GI (no force) | Rat 1 | | | | | | | | |
| | Rat 2 | | | | | | | | |
| | Rat 3 | | | | | | | | |
| GII (600 cN) | Rat 4 | | | | | | | | |
| | Rat 5 | | | | | | | | |
| | Rat 6 | | | | | | | | |
| GIII (700 cN) | Rat 7 | | | | | | | | |
| | Rat 8 | | | | | | | | |
| | Rat 9 | | | | | | | | |
| GIV (800 cN) | Rat 10 | | | | | | | | |
| | Rat 11 | | | | | | | | |
| | Rat 12 | | | | | | | | |
| GV (900 cN) | Rat 13 | * | | | | * | * | | |
| | Rat 14 | * | | | | | | | |
| | Rat 15 | * | | | | | * | | |
| GVI (1000 cN) | Rat 16 | * | * | | * | * | * | | |
| | Rat 17 | * | * | * | * | | * | * | * |
| | Rat 18 | * | * | | * | | * | * | |

Asterisk indicates presence of the histological event. M, mobility; B, bleeding; CF, crown fracture; BBPF, buccal bone plate fracture; RPDL, rupture of the PDL; REIGS, rupture of the epithelial insertion of the gingival sulcus; ABF, alveolar bone fracture; RF, root fracture.

© 2010 The Authors. Journal compilation © 2010 John Wiley & Sons A/S

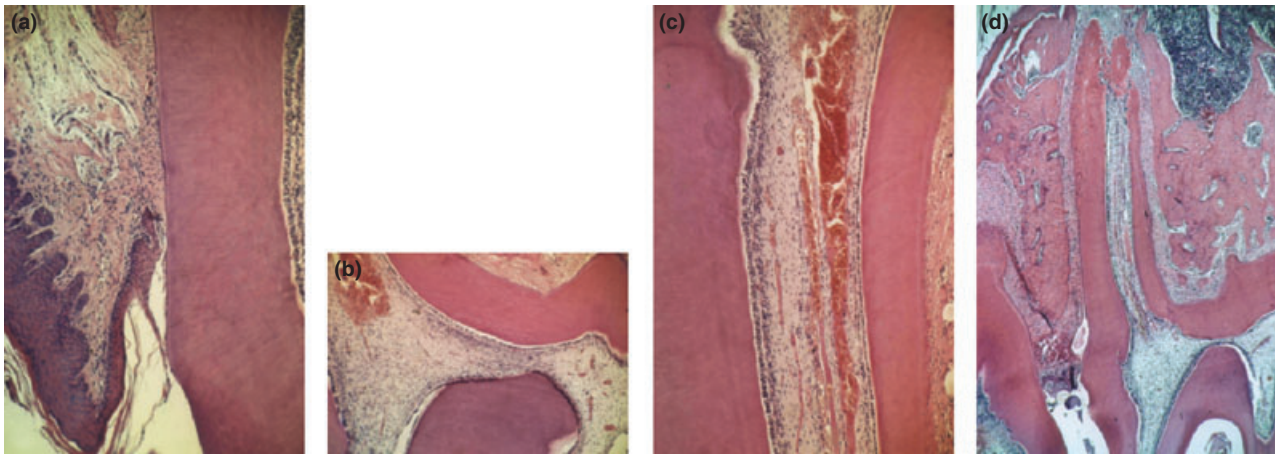


*Fig. 2.* Group I (Control – no force application). (a) Mesial root of the rat maxillary right first molar with preserved junctional epithelium and PDL. HE. Original magnification ×63. (b) Coronal pulp rich in blood vessels and cell components. HE. Original magnification ×100. (c) Dilated blood vessels in the pulp tissue of the middle root third. HE. Original magnification ×63. (d) Mesial root of the rat maxillary right first molar with primary cementum covering the dentin of the pulp region. HE. Original magnification ×25.

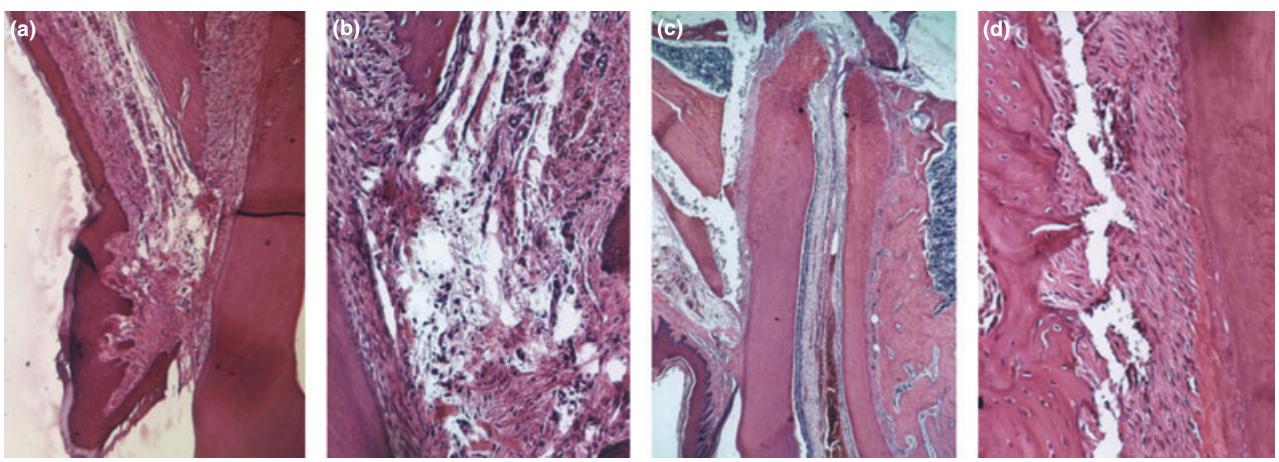


*Fig. 3.* Group V (900 cN): (a) Mesial surface of the mesial root with disrupted sulcular epithelium. HE. Original magnification ×25. (b) Mesial wall of the mesial root with fragmented collagen fibers and blood vessels. HE. Original magnification ×100. Group VI (1000 cN): (c) Mesial root of the rat maxillary right first molar with alveolar bone crest fracture and rupture of the PDL along its entire extension. HE. Original magnification ×63. (d) Rupture of the PDL with gap formation. HE. Original magnification ×63.

The well-vascularized PDL presented a large number of fibroblasts and collagen fiber bundles. In the furcal and periapical regions the fibers presented a ill-defined organization, whereas in the middle and apical root thirds they had an oblique arrangement.

GVI (1000 cN) presented the most severe clinical and histological alterations, with the occurrence of buccal bone plate fracture, alveolar fracture and root fracture, which are not present in mild traumatic injuries like subluxation. In one specimen, the presence of a gap on PDL insertion, extending along the entire root extension was observed only in the mesial surface of the mesial root (Fig. 3c). The fiber separation line was irregular in this area, and fragments of PDL fibers and cells were observed (Fig. 3d). In one of the specimens, this separation line was close to the alveolar bone wall and in another specimen it was present in the middle third of the fibers. Blood clot formation was observed in the periapical region of one specimen. No alteration was found in the furcal region and the remaining PDL areas.

The alveolar bone crest was fractured in its most coronal portion, close to the marginal gingiva (Fig. 3c). In another specimen, this fracture also involved the apical third of the alveolar bone wall.

## Discussion

The goal of the experimental method to induce dentoalveolar trauma present in this study is to reproduce as much as possible clinical and histological alterations similar to those occurring in subluxation. The advantage of this method is the standardization of the intensity and direction of the applied force, which allows simulating the trauma with the same characteristics.

© 2010 The Authors. Journal compilation © 2010 John Wiley & Sons A/S

Turley et al. (15) performed an experiment in which the four first premolars of three dogs were subjected to different amounts of experimental traumatic intrusion. The results revealed that the teeth subjected to less severe injury responded more satisfactorily to the orthodontic repositioning and dental re-eruption. Although the purpose of that study was to examine two common management techniques for the repositioning of traumatically intruded teeth, it did not produce reliable outcomes for the study of orthodontic movement of traumatized teeth because the experimentally induced trauma was not standardized.

The tensiometer used in the present study has a graduated scale that served as a parameter for standardization of the forces applied to the teeth during the course of the experiment and was determinant for the choice of this device to induce experimental dentoalveolar trauma. The ease of purchasing, low cost and ease of handling also contributed for choosing this device. This tensiometer is also commercialized under different brand names with the same purpose of standardizing and measuring force application.

The cylindrical small-sized tensiometer ends allowed for a ready access and adaptation to the occlusal surface of the rat maxillary first molar. Animal models using rats have been proposed for the study of orthodontic movement (17). The adaptation of the tensiometer end that evaluated load under pressure was made with acrylic resin, which is a widely used dental material and commonly available in dental offices. Acrylic resin can be modeled to different shapes and adapted to the dental surfaces of other animals or teeth of different sizes.

In the tensiometer, the force that caused experimental dentoalveloar trauma was generated by spring compression, in a similar manner to that produced by a device recently used by Gomes et al. (18) to induce intrusive luxation in dog's incisors. However, Gomes et al. (18) used forces of greater intensity, producing a more severe trauma (intrusive luxation), and expressed the forces in joules (J). The tensiometer used for experimental induction of trauma records the forces in grams. According to Isaacson et al. (19), grams are mass units and are thus not adequate to express force. Forces are expressed in Newton (N), and the conversion factors are 1 g = 0.00981 N and 1 N = 101.937 g. According to these conversion factors, a value of 600 g recorded in the tensiometer corresponds to approximately 600 cN, more precisely 588.6 cN.

Among the different levels of intensity of force applied to the teeth, the specimens of GII, GIII and GIV presented similar histological alterations to those of GI, with maintenance of PDL integrity and no alveolar bone crest fracture. On the other hand, the specimens of GV and GVI presented more significant clinical and histological alterations. Clinically, mobility was observed in both groups, being more severe in GVI. All specimens of this group also presented gingival bleeding, probably due to the rupture of the PDL fibers and epithelial insertion in this region. Only one specimen of GVI presented crown fracture. All specimens of GVI and only two specimens of GV presented rupture of the epithelial insertion of the gingival sulcus. One specimen of GV and one specimen of GVI exhibited rupture of the PDL along its entire extension forming a gap. On the other hand, alveolar bone crest fracture was observed in two specimens of GVI, while root fracture occurred in only one specimen of this group.

Although GV did not present the most severe clinical and histological alterations, the force applied to the specimens of this group (900 cN) was sufficient to cause PDL trauma. Comparing the results of GV to the literature (20, 21), the clinical and histological alterations are similar to those of subluxation. Histologically, there is minimal damage to the gingival and periodontal tissues and the neurovascular supply to the pulp is usually not affected by the traumatic injury. Clinically, mobility is observed, but without tooth displacement or bleeding within the gingival sulcus because the PDL fibers are preserved. These features are consistent with the findings of the present study.

In the present study, the experimental dentoalveolar trauma induced by the application of an occlusogingival (axial) acute force on the occlusal surface of the maxillary right first molar produced clinical and histological alterations that are more common in traumas caused by frontal impacts, such as concussion and subluxation. In intrusive luxations, resulting from axial impacts, the histological alterations are usually more severe and localized in the apical region (18). The clinical and histological alterations observed in present study may due to the fact that the experimental trauma was induced by an acute force that, though axial, was of mild intensity.

Malmgren et al. (9) have stated that teeth sustaining mild or moderate dentoalveolar trauma with PDL preservation can be treated orthodontically after 4–5 months with similar prognosis to that of non-traumatized teeth. According to Kindelan et al. (22), the recommended observation period prior to orthodontic tooth movement depends on the severity of the injury, and this period is necessary for the PDL to heal. In dental traumas causing minor periodontal injuries (concussion and subluxation), an initial observation period of 3 months should be allowed prior to the institution of orthodontic forces for the injured teeth. The same observation period is recommended when these types of trauma occur during orthodontic treatment, and a rest period is necessary if root resorption becomes apparent during treatment. A recent review of the guidelines for the diagnosis and management of traumatic dental injuries states that radiographic abnormalities are usually not found in concussion/subluxation, but indicates clinical and radiographic follow up 4, 6–8 weeks and 1 year after trauma. On the other hand, in more severe cases of trauma with poor prognosis of the traumatized teeth, the orthodontic treatment may be intended to align the arches and maintain space for a future premolar transplant, adhesive bridge or implant (22).

The results of the present study suggest that the severity of the damage to the teeth and periodontal structures in mild to moderate traumatic injuries, such as concussion and subluxation. Although the periodontal tissues of protection and support are not affected, clinical and radiographic follow up as recommended by

© 2010 The Authors. Journal compilation © 2010 John Wiley & Sons A/S

Flores et al. (21) is necessary for monitoring the health of the traumatized teeth and surrounding tissues.

Further studies using greater force intensities should be performed to correlate clinical and histological alterations resulting from more severe traumatic injuries and investigate their clinical implications.

In conclusion, the method described in this study produced clinical and histological alterations similar to those observed in traumatic dental injuries and it was proved viable for experimental induction of dentoalveolar trauma in rat molars. The 900 cN force (GV) was capable to produce clinical and histological alterations in the gingival and periodontal tissues compatible with those observed in subluxation.

## References


1. Caldas AF Jr, Burgos MEA. A retrospective study of traumatic dental injuries in a Brazilian dental trauma clinic. Dent Traumatol 2001;17:250–3.
2. Traebert J, Peres MA, Blank V, Boell RS, Pietruza JA. Prevalence of traumatic dental injury and associated factors among 12-year-old school children in Florianópolis, Brazil. Dent Traumatol 2003;19:15–9.
3. Järvinen S. Incisal overjet and traumatic injuries to upper permanent incisors: a retrospective study. Acta Odontol Scand 1978;36:359–62.
4. Bauss O, Röhling J, Schwestka-Polly R. Prevalence of traumatic injuries to the permanent incisors in candidates for orthodontic treatment. Dent Traumatol 2004;20:61–6.
5. Wieslander L. Intensive treatment of severe Class II malocclusions with a headgear-Herbst appliance in the early mixed dentition. Am J Orthod 1984;86:1–13.
6. Wieslander L. Long-term effect of treatment with the headgear-Herbst appliance in the early mixed dentition. Stability or relapse? Am J Orthod Dentofacial Orthop 1993;104:319–29.
7. Linge BO, Linge L. Apical root resorption in upper anterior teeth. Eur J Orthod 1983;5:173–83.
8. Reitan K, Rygh P. Biomechanical principles and reaction. In: Graber TM, Swain BF, editors. Orthodontics: current principles and techniques. St Louis, MO: Mosby; 1994. p. 96–192.
9. Malmgren O, Goldson L, Hill C, Orwin A, Petrini L, Lundberg M. Root resorption after orthodontic treatment of traumatized teeth. Am J Orthod 1982;82:487–91.
10. Hines FB Jr. A radiographic evaluation of the response of previously avulsed teeth and partially avulsed teeth to orthodontic movement. Am J Orthod 1979;75:1–19.
11. Gazit E, Sarnat H, Lieberman M. Timing of orthodontic tooth movement in a case with traumatized and avulsed anterior teeth. ASCD J Dent Child 1988;55:304–7.
12. Chaushu S, Shapira J, Heling I, Becker A. Emergency orthodontic treatment after the traumatic intrusive luxation of maxillary incisors. Am J Orthod Dentofacial Orthop 2004;126:162–72.
13. Brin I, Ben-Bassat Y, Heling I, Engelberg A. The influence of orthodontic treatment on previously traumatized permanent incisors. Eur J Orthod 1991;13:372–7.
14. Turley PK, Crawford LB, Carrington KW. Traumatically intruded teeth. Angle Orthod 1987;57:234–44.
15. Turley PK, Joiner MW, Hellstrom S. The effect of orthodontic extrusion on traumatically intruded teeth. Am J Orthod 1984;85:47–56.
16. Jacobs SG. The treatment of traumatized permanent anterior teeth: case report & literature review. Part I – Management of intruded incisors. Aust Orthod J 1995;13:213–8.
17. Ren Y, Maltha JC, Kuijers-Jagtman AM. The rats as a model for orthodontic tooth movement – a critical review and a proposed solution. Eur J Orthod 2004;26:483–90.
18. Gomes JC, Gomes CC, Bolognese AM. Clinical and histological alterations in the surrounding periodontium of dog's teeth submitted for an intrusive luxation. Dental Traumatol 2008;24:332–6.
19. Isaacson RJ, Lindauer SJ, Davidovitch M. The ground rules for arch wire design. Semin in Orthod 1995;1:3–11.
20. Andreasen JO, Andreasen FM. Essentials of traumatic injuries to the teeth. Copenhagen: Munksgaard; 1990. 168 pp.
21. Flores MT, Andersson L, Andreasen JO, Bakland LK, Malmgren B, Barnett F et al. Guidelines for the management of traumatic dental injuries. I. Fractures and luxations of permanent teeth. Dental Traumatol 2007;23:66–71.
22. Kindelan SA, Day PF, Kindelan JD, Spencer JR, Duggal MS. Dental trauma: an overview of its influence on the management of orthodontic treatment. Part 1. J Orthod 2008;35:68–78.


© 2010 The Authors. Journal compilation © 2010 John Wiley & Sons A/S

This document is a scanned copy of a printed document.  No warranty is given about the accuracy of the copy.
Users should refer to the original published version of the material.