European Journal of Orthodontics 28 (2006) 217–220
doi:10.1093/ejo/cji117
Advance Access publication 4 May 2006

Initial changes in pulpal microvasculature during orthodontic tooth movement: a stereological study

Milton Santamaria Jr*, Débora Milagres**, Adriana Sasso Stuani***, Maria Bernadete Sasso Stuani*** and Antônio Carlos de Oliveira Ruellas*
*Department of Orthodontics and Odontopediatric, Federal University of Rio de Janeiro, **Institute of Biology, State University of Rio de Janeiro, and ***Department of Infant Clinic, Preventive and Public Health, University of São Paulo, Ribeirão Preto, Brazil

SUMMARY Any alteration in blood flow or vascular pressure caused by a trauma may damage the pulp tissue. The aim of this study was to evaluate the vascular changes during the initial period of tooth movement. These alterations were assessed in coronal molar pulp tissue of 20 male Wistar rats, 90 days of age, submitted to mesial inclination movement by a closed coil spring, placed from the right maxillary first molar to the maxillary incisors. The animals were divided into three experimental groups of 6, 24, and 72 hours of 0.4 N force application, with five animals in each group, and a control group of five animals without tooth movement. The volume density of blood vessels (*Vv*) of the coronal pulp tissue in the experimental groups was calculated by stereology and compared with the control group.

The results demonstrated a significant increase in *Vv* at 6 hours of 10.2 per cent compared with 7.2 per cent for the control group ($P \leq 0.05$). At 24 and 72 hours, *Vv* was reduced, with values close to those observed for the control group ($P > 0.05$). These results demonstrate the high capacity of adaptation of the pulp tissue to an aggression, provided the biological limits of tolerance of the pulp are respected.

Introduction

The parameters most commonly employed in investigations of tissue response to orthodontic forces comprise measurements of pulpal vasculature and blood flow changes. In a laser Doppler flowmetric study, McDonald and Pitt Ford (1994) found that human pulpal blood decreased briefly (for approximately 32 minutes) when continuous light tipping forces were applied to a maxillary canine. This decrease was followed by a longer period of increased blood flow, which lasted approximately 48 hours. Similarly, Guevara *et al.* (1977) showed an initial decrease in blood flow using *in vivo* microscopy in rats. Kvinnsland *et al.* (1989), on the other hand, demonstrated a substantial increase in blood flow in the dental pulp of mesially tipped rat molars, using fluorescent microspheres. Mostafa *et al.* (1991) reported the presence of congested and dilated blood vessels and oedema of pulpal tissue in their histological study carried out on orthodontically extruded teeth. Anstendig and Kronman (1972), also utilizing histological techniques, observed fewer blood vessels after application of orthodontic forces. A histomorphometric study on rats by Nixon *et al.* (1993) contradicted some previous results, reporting a significant vascular change with an increase in the number of functional pulpal vessels. This result was supported by the study of Derringer *et al.* (1996), which showed an increase in angiogenic growth factors in the pulp of orthodontically moved teeth.

The aim of this study was therefore to quantify the vascular volume density (*V*v) in the initial periods of induced tooth movement in rats to verify the vascular reaction and the capacity of adaptation of the pulp tissue during initial force application.

Materials and method

The study design was submitted to and approved by the Institutional Review Board of the Dental School of the University of São Paulo.

Twenty male Wistar rats, 90 days of age, with an average weight of 300 g obtained from the Dental School, University of São Paulo, were used in this study. The animals were acclimatized for 1 week in plastic cages with a standard 12-hour light–dark cycle. They were fed a diet of soft laboratory food to minimize any discomfort after insertion of the orthodontic appliance and to minimize the risk of appliance displacement. The rats were randomly divided into three equal study groups ($n = 5$) and submitted to application of force for 6, 24, and 72 hours. A control group ($n = 5$) that received no appliances was included for comparison.

Each rat was anaesthetized with an intraperitoneal injection of 2.5 per cent tribromoethanol, 0.25 g/kg of body weight. Orthodontic force was applied by a 5-mm-long Nitinol closed coil spring (Morelli code 35.20.064, Sorocaba, São Paulo, Brazil) between the right maxillary first molar and incisors (Figure 1). The spring was fixed in place via 0.008-inch steel ligature wires surrounding the molar and incisor. Due to the lack of undercuts in the incisor area, a cervical groove was prepared on the tooth, in which

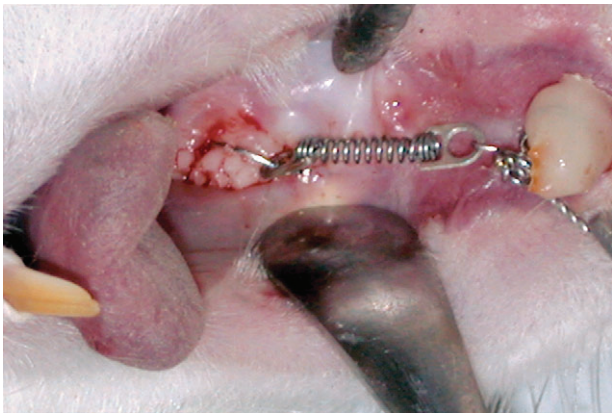

Figure 1 Closed coil spring between the right maxillary first molar and maxillary incisors of the animal. A force magnitude of 0.4 N was applied for mesial inclination of the molar.

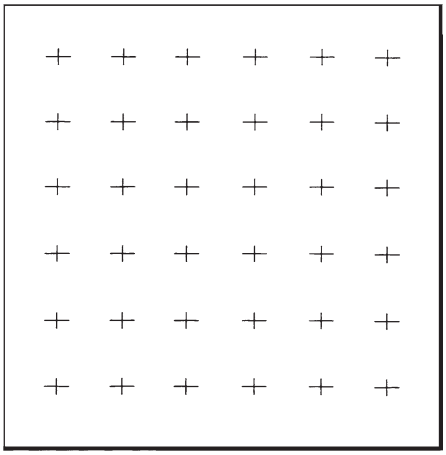

Figure 2 Test area, 36-point grid employed for calculation of the volume density of blood vessels in the pulp tissue (Mandarim-de-Lacerda, 2003).

the ligature wire was seated and secured with light-cured resin (Z100; 3M, Sumaré, São Paulo, Brazil). Each spring was placed according to the method of Heller and Nanda (1979) and the produced force was 0.4 N (Stuani, 2003).

At the end of the experimental periods, the animals were killed with a transcardial perfusion of 4 per cent paraformaldehyde the maxillae were removed and placed in the same fixative for 48 hours. Subsequently, the fixed tissues were decalcified in 10 per cent EDTA solution for 6–8 weeks. After completion of decalcification, the blocks of tissue containing the right first molars and surrounding alveolar bone were dehydrated in graded alcohol and embedded in paraffin. The specimens were orientated in the paraffin blocks so that mesio-distal sections parallel to the long axis of the teeth could be obtained. Serial sections (6 µm thick) were cut and mounted on polylysin-coated glass slides (Rana *et al.*, 2001). The sections were then stained with haematoxylin and eosin, mounted, and examined by light microscopy.

Blood vessel measurements

Stereology was utilized for blood vessel measurements. This study required a known frame (test area) to obtain the information from the slices. A transparent test system (test area with 36 points) was employed (Figure 2), which was superimposed on an image of the coronal pulp tissue over a monitor screen in a video microscopic system [DMRBE microscope (Leica Microsystems, Wetzlar, Germany) and a Triniton video monitor (Sony Electronics Inc., Montvale, New Jersey, USA)]. Thus, the points coincident with the structures of interest, the blood vessels, were counted and divided by the total number of points of the grid ($Vv = PP/PT$). Stereology requires this information for estimation of Vv. The area of pulp blood vessels was measured in ×400 fields from the centre of the coronal tissue, characterized by the presence of wider vessels (Figure 3). Forty total slices per group were used and

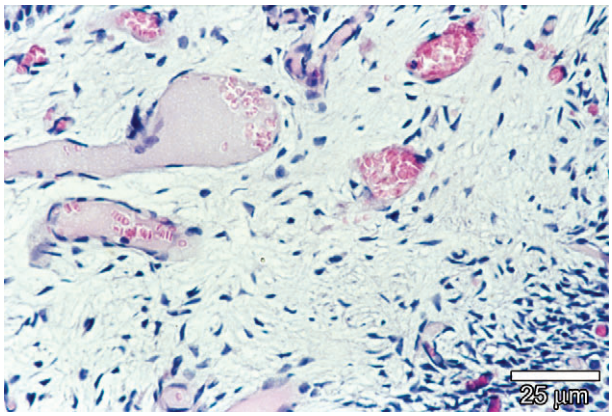

Figure 3 Coronal pulp tissue of an animal in the control group stained with haematoxylin and eosin, employed for calculation of the volume density of blood vessels. Magnification ×400.

one test area was applied randomly on each slice (at the centre of the coronal pulp). Vv was recorded as the percentage ($Vv = PP/PT$) of volume vessels in relation to the total pulp test area in the measured tissue (Mandarim-de-Lacerda, 2003).

Statistical analysis

Data are presented as the means and standard errors of the mean. The Mann–Whitney signed rank test was used to compare the results.

Results

The Vv observed in the coronal pulp tissue of orthodontically moved teeth was higher compared with the control group (Figure 4).

The pulp tissue in the control group displayed a mean Vv of 7.2 per cent. At 6 hours, the Vv was significantly increased to 10.2 per cent ($P \leq 0.05$), but after this period of tooth

movement, it reduced to 8.3 and 8.1 per cent at 24 and 72 hours, respectively, close to the values found for the pulp tissue in the control animals ($P > 0.05$).

Table 1 shows the values for *V*v and statistical comparison of the periods of tooth movement in relation to the control animals.

Discussion

Tooth movement can cause stimulation of blood vessels (Derringer and Linden, 2003), and activation of the vascular system is the key factor (Rygh *et al.*, 1986). The magnitude of force does not need to be excessive; even small forces of short duration of around 4 hours may be adequate to evoke cellular responses (Roberts and Ferguson, 1989). Age is a limiting factor in humans, with delayed cellular response and different cell populations and vascularization in adults (Hamersky *et al.*, 1980).

The present study was conducted on rat molar teeth for analysis of the vascular alterations occurring during the first hours of tooth movement. These teeth were selected because of the complete apical foramen and physiology similar to that of the human teeth, which are important for evaluation of vascular changes taking place in periods of tooth movement.

Because of the large variation in the number of vessels in the different areas of the pulp tissue, the standardized area for quantification was the centre of the coronal pulp. Moreover, this area contains the vessels coming from the root and is the first region at the upper portion of the pulp to present vascular changes secondary to an aggression (Seltzer and Bender, 1984).

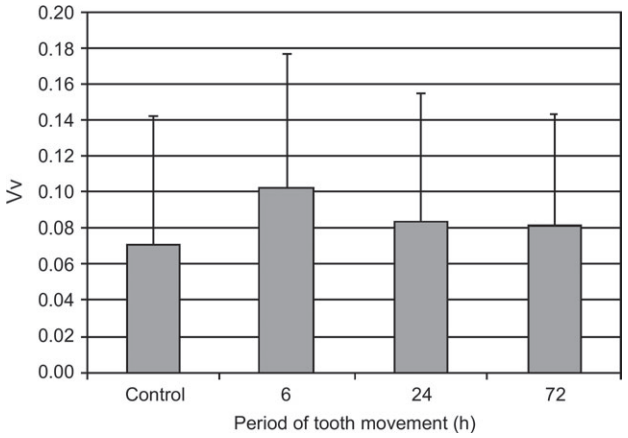

Figure 4 Means and standard deviations of volume density of blood vessels (*V*v) in the different periods of force application.

Table 1 Means and standard deviations of the volume density of blood vessels (*V*v) and statistical comparison of the periods of tooth movement in relation to the control group (Mann–Whitney test).

Period	Control	6 h	24 h	72 h
*V*v (%)	7.2 ± 7.1	10.2 ± 7.5**	8.3 ± 7.1*	8.1 ± 6.2*

*Not statistically significant in relation to the control group ($P > 0.05$).
**Statistically significant in relation to the control group ($P \leq 0.05$).

A significant increase in pulp tissue *V*v was observed after 6 hours of tooth movement, increasing from 7.2 to 10.2 per cent when compared with the control animals ($P \leq 0.05$). This increase in *V*v was expected, since a previous laser Doppler flowmetric study in humans reported an increase in blood flow during tooth movement (McDonald and Pitt Ford, 1994). The increase in *V*v may be assigned to an initial inflammatory process triggered by the force applied on the tooth, leading to vaso-dilatation.

Evaluation of the subsequent periods of tooth movement showed a decrease in *V*v to 8.3 and 8.1 per cent for the 24- and 72-hour groups, respectively, revealing the tendency to return to the values of the control animals. These results were not statistically significant compared with the control animals ($P > 0.05$). Thus, it might be assumed that the increase in *V*v in the first 6 hours was probably triggered by the vaso-dilatation normally occurring in the initial stages of inflammatory processes and tissue repair. If there had been maintenance or increase in *V*v in the subsequent periods of force application of 24 and 72 hours, the process might have been assigned to vascular congestion secondary to a mechanical obstruction, which would impair the venous return. The return of *V*v to normal levels demonstrates the capacity of adaptation of the pulp tissue and the absence of vascular congestion during the period of force application.

The mechanical obstruction of blood flow is related to the status of the pulp tissue, which is surrounded by inflexible walls and receives blood supply primarily from the apical foramen (Cohen and Burns, 1987). Therefore, the patient's age, presence of accessory root canals reaching the pulp tissue, and the type of movement may influence the observed tissue reaction. In the present study, a force of moderate intensity was used (Nixon *et al.*, 1993) with a mesial inclination movement of the molar. Vertical movements of intrusion or extrusion tend to further impair the pulp blood flow (Mostafa *et al.*, 1991).

The present study assessed the vascular changes occurring in the first hours of tooth movement; the increase in *V*v may not therefore be assigned to the process of angiogenesis; Derringer and Linden (2003) identified the human growth factors regulating angiogenesis after 2 weeks of force application.

The increase in pulp tissue *V*v in the present histomorphometric evaluation and the increase in blood flow observed in the physiological study of McDonald and Pitt Ford (1994) are the outcome of the inflammatory process triggered by tooth movement, in which blood supply and inflammatory cells reach the area in an attempt to achieve tissue repair. Such process is biologically variable

and may be more or less intense depending on the amplitude of the load, the duration, type of movement applied, and patient's age (Neville *et al.*, 1998).

Conclusion

At the first 6 hours of force application, the induced tooth movement yielded an increase in *V*v, when compared with the control group ($P \leq 0.05$).

The pulp tissue demonstrated an excellent capacity of adaptation, with a reduction of *V*v after 24 and 72 hours of force application, and a return to similar values in the control group ($P > 0.05$).

Address for correspondence

Milton Santamaria Jr
R. Professor Lourenço Roselino 630
Lagoinha
Ribeirão Preto–São Paulo
Brazil CEP 14095-170
E-mail: santamariajr@mailcity.com

Acknowledgments

The authors wish to thank Morelli, at Sorocaba, São Paulo, Brazil, for supplying the coil springs. This work was supported by Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References

Anstendig H S, Kronman J H 1972 A histological study of pulpal reaction to orthodontic tooth movement in dogs. Angle Orthodontist 42: 50–55

Cohen S, Burns R C 1987 Pathways of the pulp. C V Mosby, St Louis

Derringer K A, Jaggers D C, Linden R W A 1996 Angiogenesis in human dental pulp following orthodontic tooth movement. Journal of Dental Research 75: 1761–1766

Derringer K A, Linden R W A 2003 Angiogenic growth factors released in human dental pulp following orthodontic force. Archives of Oral Biology 48: 285–291

Guevara M J, McClugage S G, Clark J S 1977 Response of the pulpal microvascular system to intrusive orthodontic forces. Journal of Dental Research 56 (abstract 243)

Hamersky P A, Weimer A D, Taintor J F 1980 The effect of orthodontic force application on the pulpal tissue respiration rate in the human premolar. American Journal of Orthodontics 77: 368–378

Heller I J, Nanda R 1979 Effect of metabolic alterations of periodontal fibers on orthodontic tooth movement. American Journal of Orthodontics 75: 239–258

Kvinnsland S, Heyeraas K, Ofjord E S 1989 Effect of experimental tooth movement on periodontal and pulpal blood flow. European Journal of Orthodontics 11: 200–205

Mandarim-de-Lacerda C A 2003 Stereological tools in biomedical research. Anais da Academia Brasileira de Ciências 75: 469–486

McDonald F, Pitt Ford T R 1994 Blood flow changes in permanent maxillary canines during retraction. European Journal of Orthodontics 16: 1–9

Mostafa Y, Iskander K G, El-Mangoury N H 1991 Iatrogenic pulpal reactions to orthodontic extrusion. American Journal of Orthodontics and Dentofacial Orthopedics 99: 30–34.

Neville B W, Damm D D, Allen C M, Bouquot J E 1998 Patologia oral & maxilofacial. Editora Guanabara Koogan, Rio de Janeiro

Nixon C E, Saviano J A, King G J, Keeling S D 1993 Histomorphometric study of dental pulp during orthodontic tooth movement. Journal of Endodontics 19: 13–16

Rana M W, Pothisiri V, Killiany D M, Xu X M 2001 Detection of apoptosis during orthodontic tooth movement in rats. American Journal of Orthodontics and Dentofacial Orthopedics 119: 516–521

Roberts W E, Ferguson D 1989 Cell kinetics of the periodontal ligament. In: Norton L A, Burstone C J (eds) The biology of tooth movement. CRC Press, London, Chapter 4, pp. 55–69

Rygh P, Bowling K, Hovlansdal L, Williams S 1986 Activation of the vascular system: a main mediator of periodontal fiber remodeling in orthodontic tooth movement. American Journal of Orthodontics 89: 453–468

Seltzer S, Bender I B 1984 The dental pulp: biologic considerations in dental procedures. Lippincott Company, Philadelphia

Stuani A S 2003. Aspectos Histológicos do periodonto de dentes submetidos à força ortodôntica (lado de pressão). Thesis. Universidade Federal do Rio de Janeiro