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Ultrastructure of cementum and periodontal ligament after continuous intrusion in humans: a transmission electron microscopy study

R. M. Faltin*,**,***, K. Faltin**,***, F. G. Sander*** and V. E. Arana-Chavez*

*Laboratory of Mineralized Tissue Biology, Department of Histology and Embryology, Institute of Biomedical Sciences, University of São Paulo, and **Department of Orthodontics, School of Dentistry, University Paulista—UNIP, São Paulo, S.P., Brazil and ***Department of Orthodontics, School of Dentistry, University of Ulm, Germany

SUMMARY An ultrastructural study of the cementum and periodontal ligament (PDL) changes after continuous intrusion with two different and controlled forces in humans was carried out.

Twelve first upper premolars, at stage 10 of Nolla, orthodontically indicated for extraction from six patients (mean age 15.3) were used. They were divided into three experimental groups, distributed intra-individually as follows: control (not moved), continuously intruded for 4 weeks with 50 or 100 cN force, utilizing a precise biomechanical model with nickel titanium super-elastic wires (NiTi–SE), which were developed and calibrated individually. The teeth were extracted, fixed, decalcified, and conventionally processed for examination in a Jeol 100 CX II transmission electron microscope.

Evident signs of degeneration of cell structures, vascular components, and extracellular matrix (EM) of cementum and PDL were observed in all the intruded teeth, with more severe changes towards an apical direction and in proportion to the magnitude of force applied. Resorptive areas and an irregular root surface of the intruded teeth were noticed, according to the same pattern described above. Concomitant, areas of repair were also revealed in the cementum and PDL although the magnitude of forces remained the same throughout the experimental period. Thus, a reduction of continuous force magnitude should be considered to preserve the integrity of tissues.

Introduction

Basic research has been considered extremely important for the evaluation of clinical procedures in dentistry. This is of importance to appraise biomechanical methods and their biological consequence in the orthodontic and facial orthopaedic field (Schwarz, 1932; Oppenheim, 1944; Reitan, 1974). Therefore, histological studies are essential in order to clarify and guide adequate treatment procedures by moving teeth with minimum damage of the involved structures (Oppenheim, 1944; Storey and Smith, 1952; Ten Cate, 1976). Most studies have been conducted in animal models, which present several differences in the anatomical and histological pattern in comparison with humans (Reitan and Kvam, 1971; Bosshardt and Schroeder, 1996), as well as in the precision of the utilized mechanics (Barber and Sims, 1981; Lundgren *et al*., 1996).

Numerous factors, including sexual dimorphism, influence orthodontic tooth movement (Storey, 1963). However, their individual role in this complex process is still controversial (Rygh *et al*., 1986; Linge and Linge, 1991; Owman-Moll *et al*., 1996a). Factors related to mechanics such as duration, magnitude and type of force, which can be relatively influenced, remain unclear (Van Leeuwen and Maltha, 1995; Owman-Moll *et al*., 1996b; Pilon *et al*., 1996). On the other hand, individual reaction pattern (patient's biology) is

undoubtedly an important aspect which could not be overlooked (Reitan, 1974; Bosshardt and Schroeder, 1996; Lundgren *et al*., 1996).

The development of nickel titanium superelastic wires (NiTi–SE) has made possible the application of continuous forces for a determined period without a need for reactivation, thereby promoting efficient clinical results (Sander, 1990; Sander and Wichelhaus, 1995). However, histological studies concerning the consequences of this type of force in tooth movement have not previously been carried out.

The present study is part of a series of histological experiments (Faltin *et al*., 1998) to clarify the tissue reactions in the human periodontium, and their correlation with defined and controlled mechanics. In this investigation, an ultrastructural study using transmission electron microscopy to analyse aspects of tooth movement biology after continuous intrusion with varying force regimes was carried out.

Subjects and methods

Twelve first upper premolars, at stage 10 of Nolla (Nolla, 1960), orthodontically indicated for extraction from six patients (two females and four males) with a mean age of 15.3 years (14.1–16.7 years) were studied. All patients were fully informed about the procedures and their written consent was obtained. The study was authorized by the Ethical Committee of the University Paulista, Brazil.

The teeth were divided into three experimental groups: (1) non-moved control teeth (no force application, $n = 2$); (2) continuous force application of 50 cN for 4 weeks ($n = 5$); (3) continuous force application of 100 cN for 4 weeks ($n = 5$).

These experimental groups were distributed intra-individually as follows:

Patient 1 had a non-moved control tooth on one side (upper right first premolar), and a continuous force of 50 cN was applied on the other side (upper left first premolar) for 4 weeks. *Patient 2* had a non-moved control tooth on one side (upper right first premolar) and a continuous force of 100 cN was applied on the other side (upper left first premolar) for 4 weeks. *Patients 3–6* had a continuous force of 50 cN applied on one side (upper right first premolar) for 4 weeks, and a continuous force of 100 cN on the other side (upper left first premolar) for 4 weeks.

Biomechanics

A precise biomechanical model with special NiTi–SE-stainless steel springs, designed and individually tested, which developed constant forces because of their intrinsic mechanical properties was utilized. The two different regimes of force were adjusted by thermal treatment of the NiTi-portion (Sander, 1990). This model has previously been described (Faltin *et al*., 1998).

Procedure for transmission electron microscopy

After conventional extraction with forceps under local anaesthesia, the teeth were fixed in 2 per cent glutaraldehyde + 2.5 per cent formaldehyde (freshly prepared from paraformaldehyde) in 0.1 M sodium cacodylate buffer, pH 7.4 (Arana-Chavez *et al*., 1995) for 6 hours at room temperature with agitation and left at 4°C overnight. After washing for 1 hour in the same buffer, they were decalcified in an aqueous solution of 10 per cent EDTA containing 1 per cent formaldehyde for approximately 50 hours using a Pelco 3440 (Ted Pella Inc., Redding, CA, USA) laboratory microwave oven operating at medium setting with temperature programmed to a maximum of 37°C. After decalcification, the roots were transversely divided into six equal parts/heights (from cervical to the apical third) and subsequently trimmed longitudinally in four equal parts. They were washed in the same buffer and post-fixed in cacodylate buffered 1 per cent osmium tetroxide for 2 hours. All specimens were dehydrated in graded ethanol and acetone after which they were carefully orientated and embedded in Spurr resin (Electron Microscopy Sciences, Fort Washington, USA) for 72 hours at 70°C. Toluidine blue stained 1–2-μm thick sections were examined with a light microscope and selected regions trimmed for ultrathin sectioning. Ultrathin sections approximately

70–80-nm thick were cut using a Diatome diamond knife (Diatome Ltd, Bienne, Switzerland) in a Leica Ultracut R ultramicrotome (Leica Inc, Buffalo, USA), and collected onto copper grids. Specimens stained with lead citrate/uranyl acetate were examined and photographed in a Jeol 100 CX II (Jeol Ltd, Tokyo, Japan) transmission electron microscope operating at 80 kV.

Results

Teeth not intruded (control)

Cellular areas of cementum covering the root dentine of the control teeth exhibited numerous cementocytes with their cellular body within lacunae and their processes penetrating into canaliculae. The cementum surface was uniform and juxtaposed to a typical non-mineralized cementum layer. Parallel collagen fibrils forming conspicuous bundles were observed in these areas. The non-mineralized cementum was often penetrated by cellular processes of the adjacent cementoblasts, which appeared flattened with their long axis parallel to the surface of the cementum (Figure 1a). However, in areas of acellular cementum, at the cervical third, rounded profiles of cementoblasts were occasionally observed (Figure 1b).

The adjacent PDL showed typical fibroblasts surrounded by an extracellular matrix (ECM) mainly consisting of collagen fibrils. The blood vessels of the PDL appeared with a continuous wall formed by flattened endothelial cells (Figure 1c).

Teeth intruded with 50 cN

In this experimental group, clear alterations in the structural pattern of the cellular and extracellular components of the PDL and cementum were observed (Figure 2a). These alterations occurred intensively in the apical, moderately in the medium and rarely in the cervical thirds. The cell alterations (fibroblasts, cementoblasts, and cementocytes) consisted of different stages of degeneration characterized by unstructured nuclear chromatin with picnotic nucleus, electron opaque, also revealing partial segmentation (Figure 2a,b). The cytoplasm showed increased electron opacity, evidenced by numerous microfilaments, disarrangement and decrease of organelles, as well as excessive dilation of the rough endoplasmic reticulum (RER) cisterns. Loss of the cytoplasmic limit (plasma membrane) and cell fragmentation were also observed (Figure 2a). The EM of the PDL showed alteration, including loss of its fibrillar nature, a granular and amorphous aspect that was characteristic of the hyalinization process (Figure 2a).

A loss of cementoid was observed due to degeneration of cementoblasts and non-mineralized ECM. These areas showed a non-homogeneous electron-opaque mineralized cementum, exhibiting an irregular surface (Figure 2a). The boundaries of the cementocyte lacunae near the PDL showed irregular contour and loss of the limiting lamina. The space between the cementocyte cell body and the mineralized surfaces of the lacunae was reduced, presenting rare cementoid without fibrillar aspect (Figure 2b).

In the area of hyalinized PDL near the cementum, mono- and multi-nucleated macrophage-like cells were observed. They contained several phagosomes, lysosomes, digestive vacuoles, and a large number of well-developed RER. In addition, some clast-like cells with large dimensions and cytoplasm containing lysosomes and vacuoles, although without ruffled borders and with clear zones were observed. These cell types were normally near to the hyalinized zone, participating in the process of removing necrotic elements (not illustrated). In these regions lymphocytes and granulocytes were rarely observed.

These alterations were more evident in the apical third and discreet in the medium third. The blood vessels were frequently narrowed with approximation of their walls and alteration of the lumen shape. In addition, the endothelial cells appeared bulky (Figure 3a,b). Loss of the endothelium continuity with consequent extravasation of blood cells was noticed. The presence of erythrocytes at the ECM of the PDL revealed typical images of oedema in the PDL

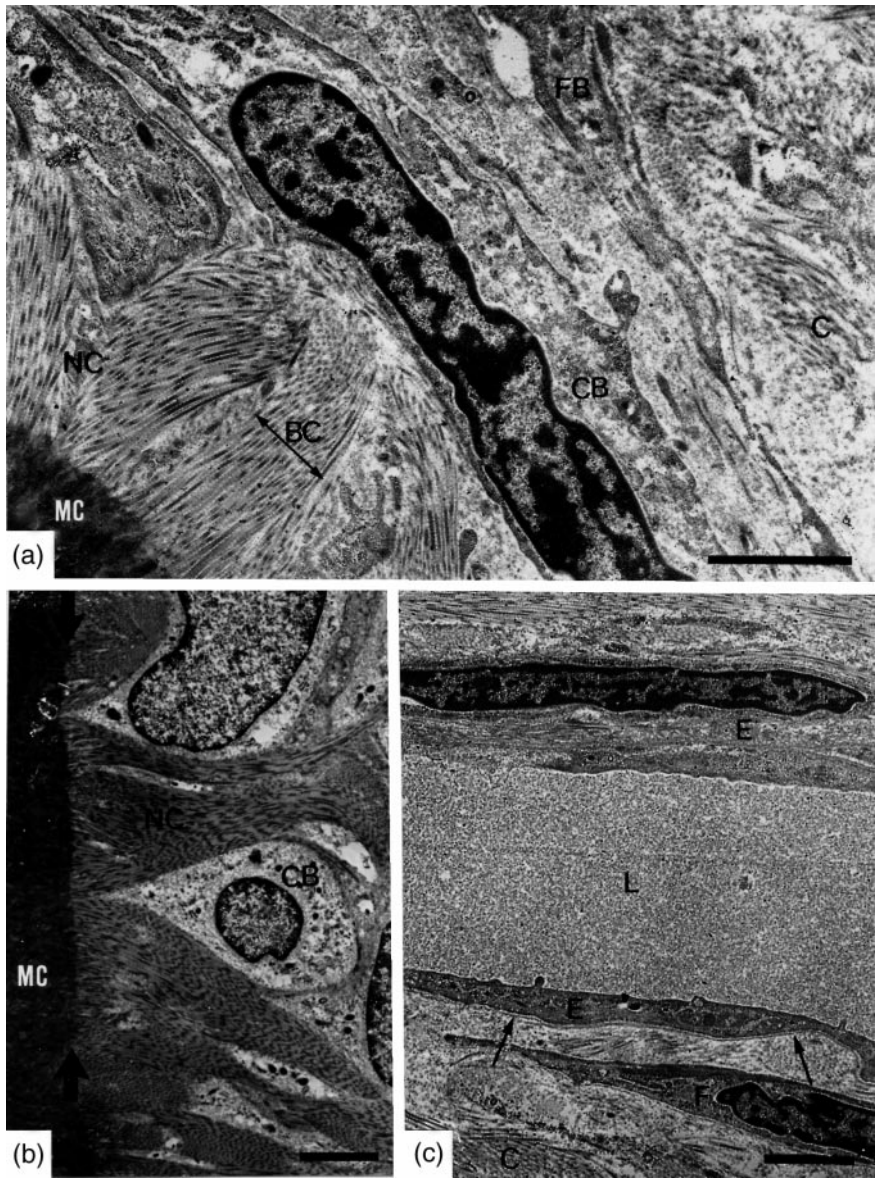

Figure 1 (a–c) *Teeth not intruded (control):* electron micrographs showing, (a, apical third), a portion of cementum (MC) adjacent to an evident layer of non-mineralized cementum (NC) constituted by conspicuous bundles of collagen fibrils (BC). Non-mineralized cementum is lined by typical resting cementoblasts (CB). On the right side of the micrograph, a portion of a fibroblast (FB) and numerous collagen fibrils (C) of periodontal ligament (PDL) are identified. Bar, 2 μm. (b, cervical third), cementum (MC) appears with a uniform surface (arrows), which is adjacent to a thin layer of non-mineralized cementum (NC). Some portions or processes of cementoblasts (CB) are seen penetrating the non-mineralized cementum. Bar, 2 μm. (c, medial third), part of a blood vessel is observed in the PDL. Endothelial cells (E) are adjacent to a continuous basal lamina (arrowheads). L, lumen of the blood vessel; F, fibroblast; C, collagen fibrils. Bar, 3 μm.

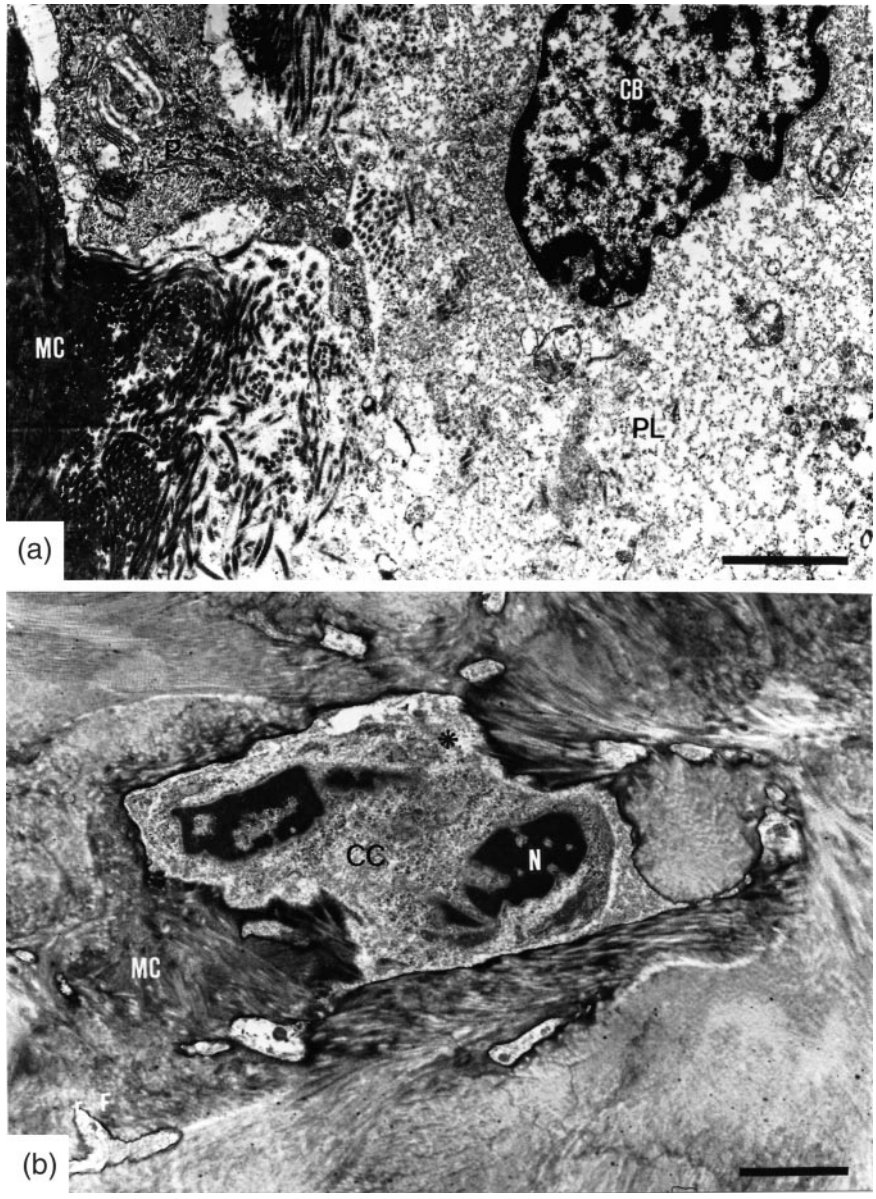

Figure 2 Teeth intruded with 50 cN (apical third): electron micrographs showing, (a) a portion of a degenerating cementoblast-like cell (CB) adjacent to the extracellular matrix of the periodontal ligament of granular aspect (PL). A cellular process of another cementoblast (P) is observed in close proximity with the cementum (MC). Non-mineralized cementum is absent in this region. Bar, 2 μm. (b) An area of cementum (MC) shows a cementocyte (CC) with its picnotic and segmented nucleus (N). Cementocyte has not defined the boundaries (asterisk) and a thin layer of non-mineralized cementum between the cell and its lacuna does not exist. Bar, 2 μm.

(Figure 3a). On the other hand, several sprouting capillaries with bulky endothelial cells, some of them in the mitosis process, were observed. These capillaries adjacent to the hyalinized zone, were evidence of angiogenesis and revascularization of the PDL (Figure 3b).

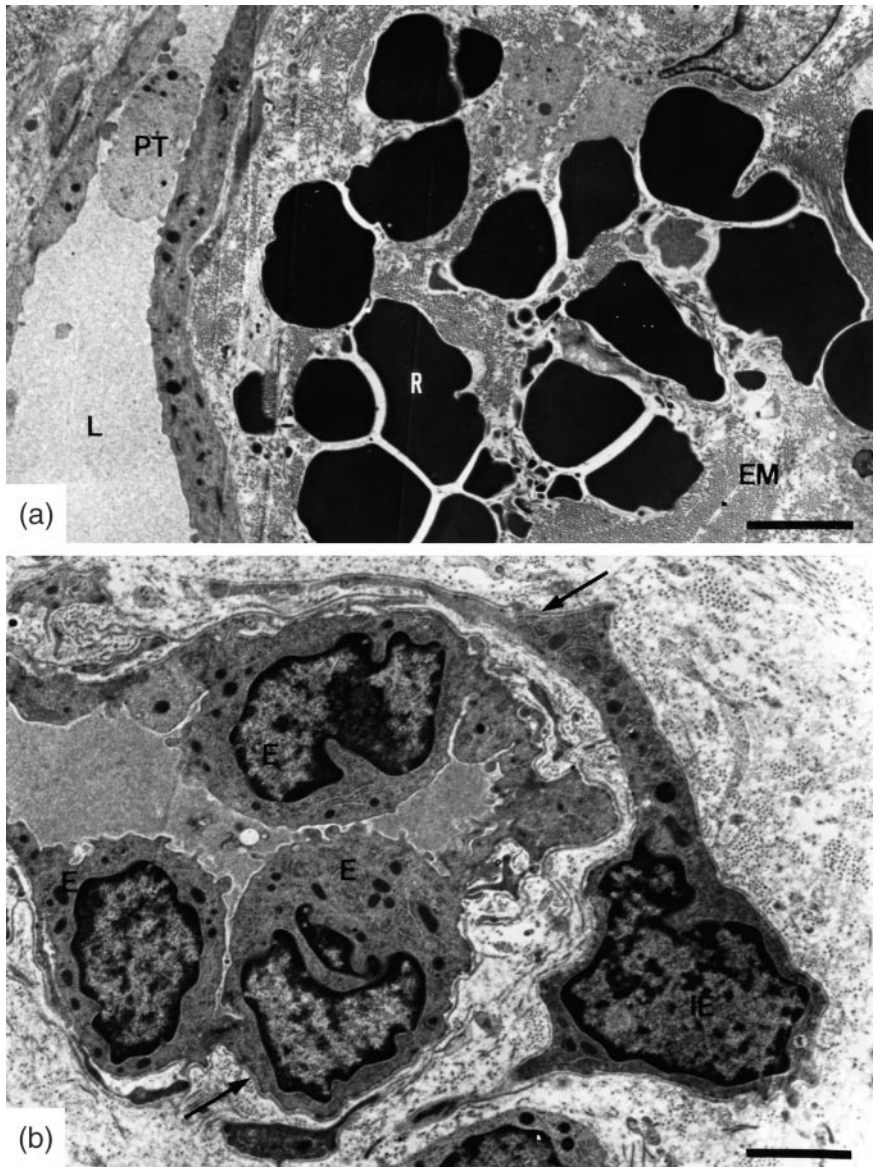

Figure 3 Teeth intruded with 50 cN (apical third): electron micrographs showing (a) a region of extravasation of blood cells in the periodontal ligament. Numerous red blood cells (R) are seen interspersed with the extracellular matrix (EM). In the lumen (L) of the adjacent blood vessel, a portion of a platelet (PT) is observed. Bar, 4 μm. (b) Another blood vessel with three round endothelial cells (E) is seen adjacent to a cell (IE) that seems to be engulfed by the two processes. This cell is also surrounded by a lamina basal (arrows). Bar, 2 μm.

Near to the cementum, rounded cells with their nucleus containing loose chromatin and obvious nucleolus, as well as voluminous cytoplasm with well-developed RER cisterns, secretion granules and mitochondria were observed. These cementoblast characteristics revealed an active

process of production and secretion of collagen matrix that corresponded to the intrinsic fibres of the cementum (Figure 4a). The observation of Sharpey's fibres and an organized and newly-synthetized non-mineralized cementum in these areas provided further evidence of the process of repair in the cementum (Figure 4a).

Fibroblasts of the PDL were also observed at different stages of degeneration as previously described for the cementoblasts. Outside the

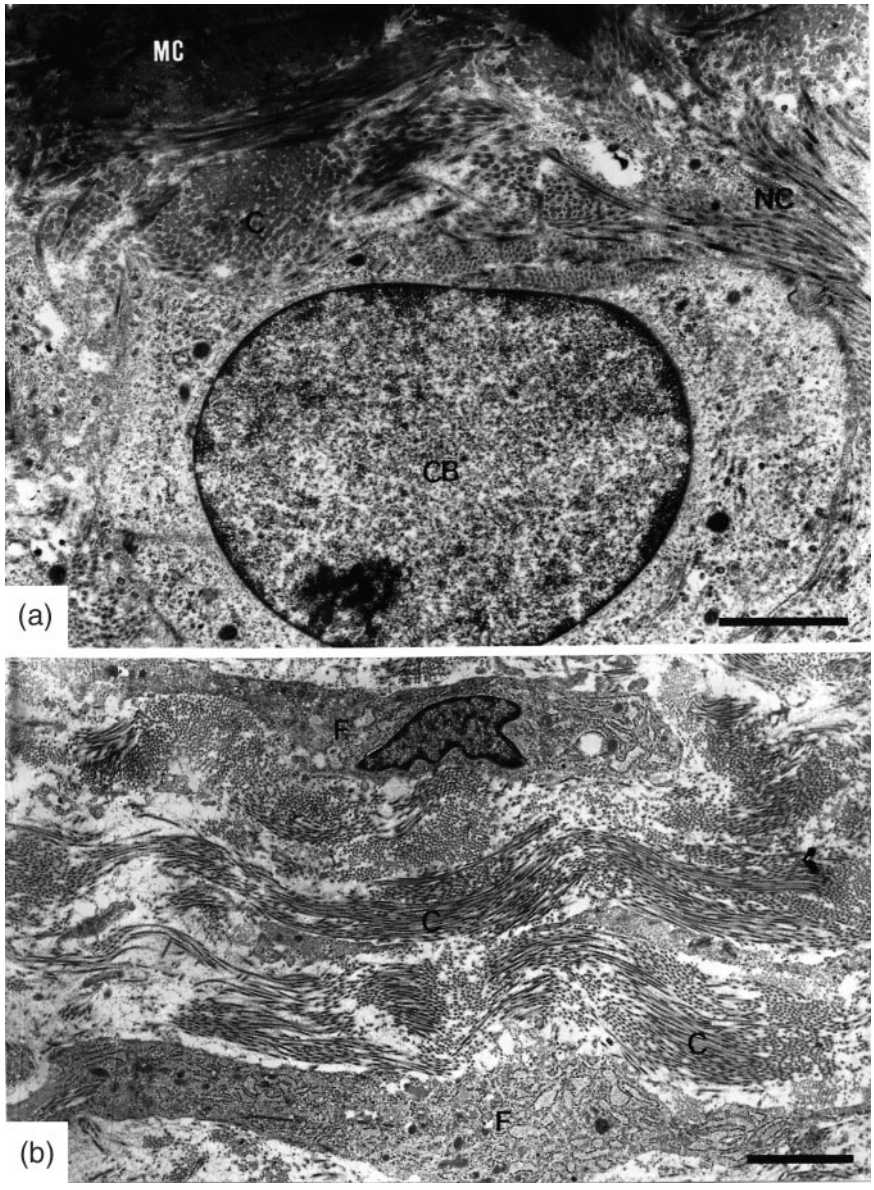

Figure 4 Teeth intruded with 50 cN: electron micrographs showing: (a, apical third), a cementoblast (CB) adjacent to a layer of non-mineralized cement (NC) constituted by typical collagen fibrils (C). In the upper region of the micrograph, there is a portion of cementum (MC). Bar, 2 μm. (b, medial third) A portion of periodontal ligament in which portions of active fibroblasts (F) are seen. Collagen fibrils (C) in longitudinal or cross sections constitute the extracellular matrix. Bar, 3 μm.

hyalinized zones, active fibroblasts were observed with their normal characteristics, suggesting the concomitant repair of the involved PDL. In these regions, the ECM presented a typical fibrillar aspect with the collagen fibrils organized in fibres surrounding the cellular components (Figure 4b).

Teeth intruded with 100 cN

The cellular and extracellular components of the cementum and PDL in the teeth intruded with a force of 100 cN exhibited a more intense alteration of the structural pattern in comparison with teeth intruded with 50 cN. Thus, they were clearly more extensive in the apical, numerous in the medium and rare in the cervical thirds. In these regions, cementoblasts and cementocytes showed different stages of degeneration, such as a nucleus with disarranged chromatin and enhanced electron opacity, which also revealed partial segmentation. The cytoplasm showed extreme electron opacity, an increased number of microfilaments, disarrangement and decrease of organelles, and excessive dilation of the RER cisterns. Cells exhibited loss of their cytoplasmic limit (plasma membrane) and, in some cases, cell fragmentation. A disturbed non-mineralized cementum with its ECM showing an amorphous and granular aspect was also observed in this group (Figure 5a,b). The surface of the cementum appeared fairly irregular with the presence of numerous concavities, which represent the resorption lacunae (Figure 5b). Near to these resorbed cementum surfaces, mono- and multi-nucleated macrophage-like cells were identified. These cells presented a cytoplasm with numerous phagosomes containing rests of cells, dense bodies, and matrix components as collagen fibrils and lysosomes containing material with diverse electron opacity, a great number of digestive vacuoles and developed RER. Multi-nucleated clast-like cells with increased dimensions, and cytoplasm containing lysosomes and vacuoles were observed. However, the majority of these clast-like cells did not exhibit a ruffled border and clear zones (Figure 6a,b,c). Lymphocytes and granulocytes were rarely identified in this group.

Rounded cells with their nucleus containing loose chromatin and obvious nucleolus, as well as with their voluminous cytoplasm containing numerous RER cisterns, secretion granules, and mitochondria were observed adjacent to the cementum. These characteristics revealed active cementoblasts in the repair process of the collagenous matrix, corresponding to the intrinsic fibres of the cementum (Figure 7). These cementoblasts were also observed at different stages of differentiation establishing intercellular junctions and membrane inter-digitations between them (not illustrated). The observation of Sharpey's fibres and a newly-synthesized non-mineralized cementum in these areas characterized even more the process of repair of the cementum (Figures 5b and 7).

Degenerating fibroblasts of the PDL were also observed. The onset of the degeneration process seemed to limit the areas of altered ECM. From the hyalinized zones, active fibroblasts with their normal characteristics were evidence of repair of the involved PDL (not illustrated).

Although many resorption concavities, especially in the apical third root, were deeper and more extensive, they were restricted to the cementum. Thus, root dentine was not affected by the resorptive process.

Discussion

These results reveal that human teeth intruded for 4 weeks with continuous forces show areas of resorbed cementum, and numerous cells and matrix elements in various degrees of degeneration in both cementum and PDL. However, even with the maintenance of the orthodontic stimuli, conspicuous areas of repair were also observed. The extension and severity of these areas, which were greater in the apical third of the root, were dependent on the magnitude of the applied force.

Methodological considerations

In the present study, precise mechanics with the application of true continuous forces were used. These mechanics with super-elastic wires have shown efficient clinical results in tooth

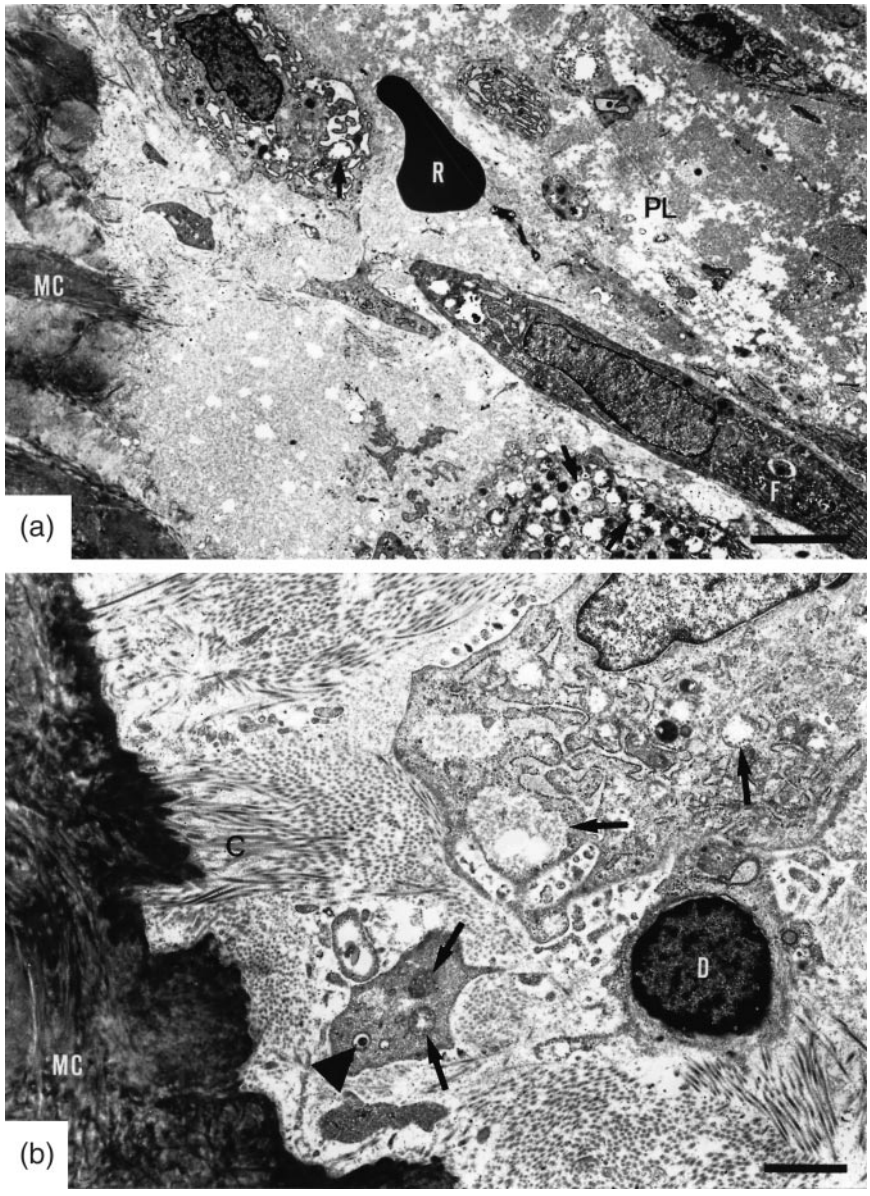

Figure 5 Teeth intruded with 100 cN (apical third): electron micrographs showing two regions of the cementum/periodontal ligament interface with severe signs of alteration. (a) Two cells, which exhibit numerous vacuoles (arrows), are adjacent to the irregular surface of cementum (MC). An elongated fibroblast (F) and a red blood cell appear between these cells. The remaining periodontal ligament (PL) exhibits a granular aspect with portions of degenerating cells. Bar, 4 μm. (b) Mineralizing collagen fibrils (C) are seen in continuity with the irregular surface of cementum (MC). Adjacent cells present numerous vacuoles (arrows), which contain phagocytosed extracellular matrix and lysosomes. In these cells, however, it is possible to observe some rough endoplasmic reticulum cisternae and, in addition, a degenerating cell (D). Arrowheads, membrane-bounded collagen fibril within a cytoplasmatic process. Bar, 2 μm.

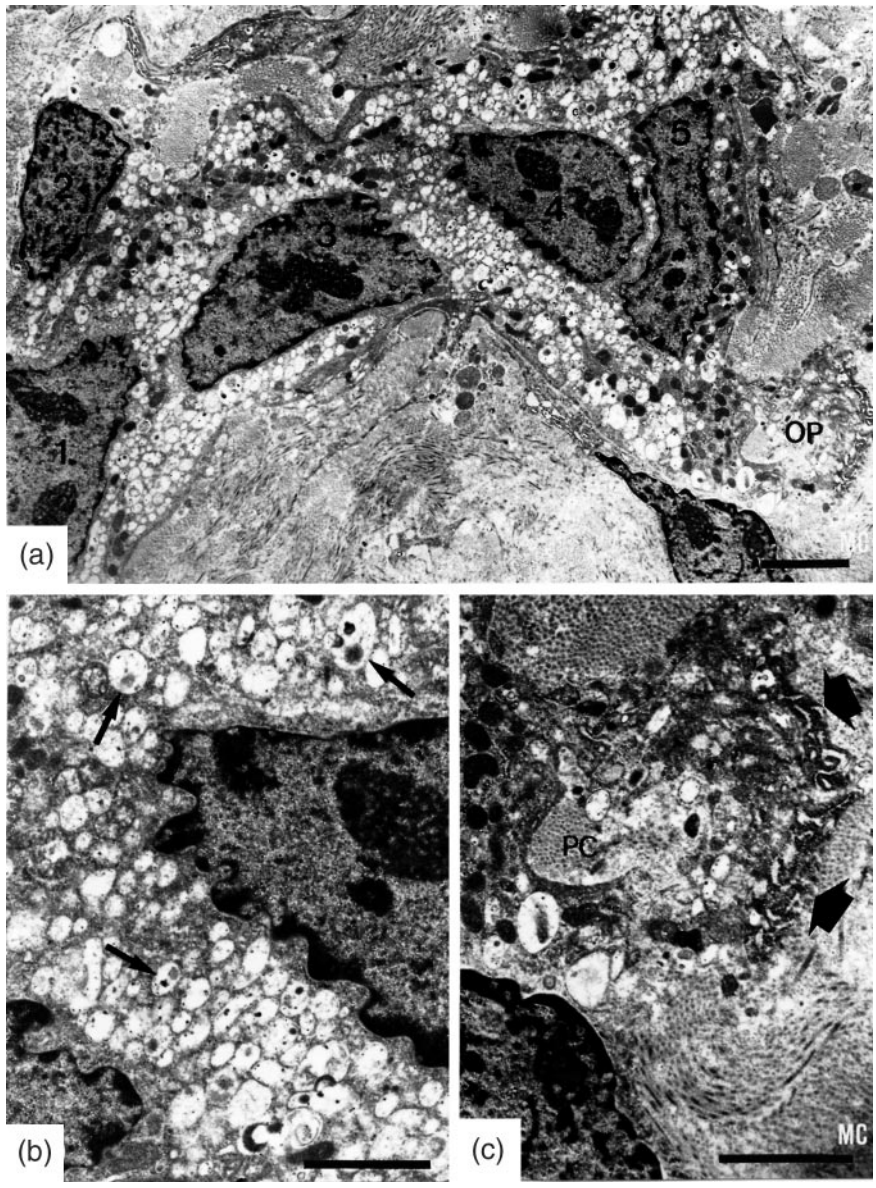

Figure 6 Teeth intruded with 100 cN (apical third): electron micrographs of (a), an odontoclast-like cell with a cellular process (OP) near to the cementum (MC). Five nuclei (1–5) and their cytoplasms replete of vacuoles are observed in the cell. Bar, 4 μm. (b) A high magnification view of a region of the cytoplasm shows the granular content of vacuoles (arrows). Bar, 2 μm. (c) The process containing phagocytosed collagen fibrils (PC) and other degraded matrix constituents is observed. Note the convoluted plasma membrane (arrowheads) of the process in its surface adjacent to the cementum (MC). Bar, 2 μm.

movement (Sander and Wichelhaus, 1995). Concerning the magnitude of force applied in this study, the smaller value of 50 cN, is close to the level of the ideal forces previously proposed (Schwarz, 1932; Storey and Smith, 1952; Lee, 1965, Bench *et al.*, 1978). However, this ideal level was estimated with the use of intermittent forces.

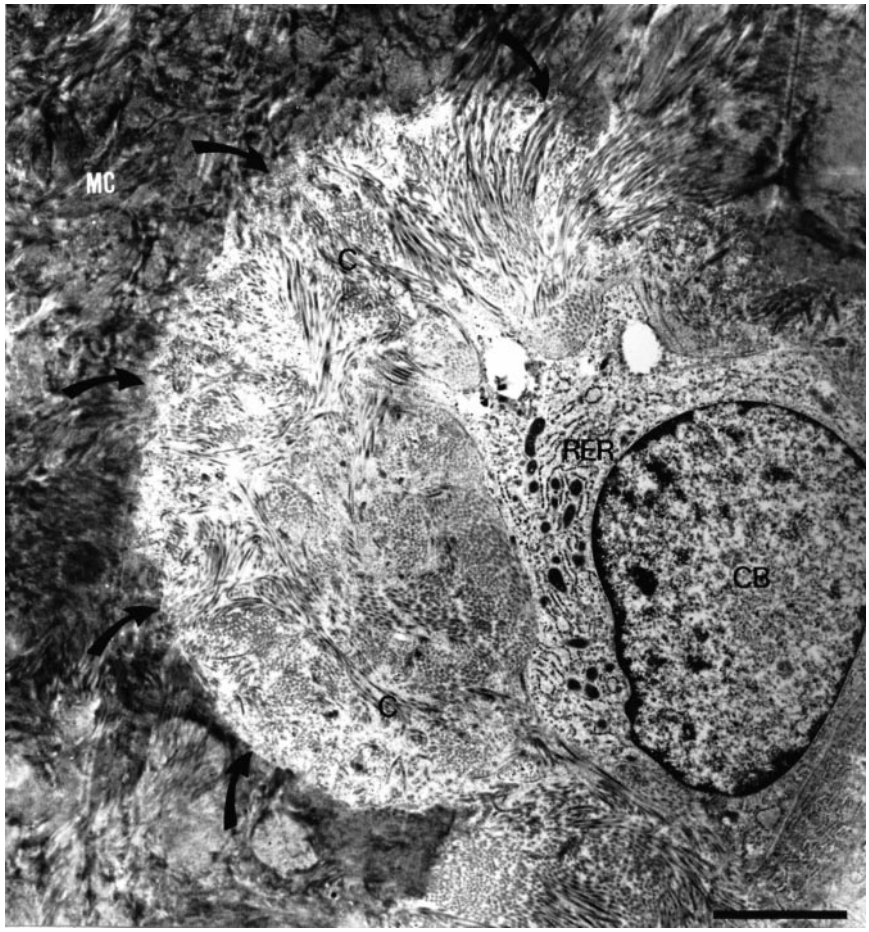

Figure 7 Teeth intruded with 100 cN (apical third): electron micrograph showing a previously resorbed cementum lacuna (arrows) with an adjacent cementoblast (CB), which exhibits profiles of rough endoplasmic reticulum (RER). Newly secreted collagen fibrils (C), some of them anchored to cementum (MC), are filling the lacuna. Bar, 3 μm.

Several authors have reported that individual variations are the main cause for the different predisposition to root resorption (Reitan, 1974; Bosshardt and Schroeder, 1996; Lundgren *et al.*, 1996; Owman-Moll *et al.*, 1996b; Pilon *et al.*, 1996). Thus employment of intra-individual studies should be considered, since they minimize these individual factors and variations.

The human model allows accurate application, control, and quantification of the mechanics during tooth movement, when compared with animal models. At the same time, obvious differences regarding morphological and functional aspects exist between the various models (Reitan and Kvam, 1971; Bosshardt and Schroeder, 1996).

Vascular alterations

Several alterations in the blood vessels of the PDL were observed in both experimental groups. The main change in vascular structures was the breakdown of endothelium continuity, with consequent haemorrhage and oedema near to the hyalinized zones, as observed by various

authors (Rygh, 1977; Göz and Rahn, 1992; Vandevska-Radunovic *et al*., 1994). Vascular alterations during tooth movement seem to be decisive in the modulation of tissue reactions, whereas oedema directly affects the arrangement and structure of the EM of the PDL (Khouw and Goldfaber, 1970). Close to hyalinized areas, numerous erythrocytes were observed, but not lymphocytes or granulocytes.

The vascular alterations that occurred more intensely in the intruded teeth with 100 cN and which were more frequently observed in the root apical third, are indicative of severe damage to the vascular bed at those overloaded areas. However, vascular repair was also noticed at adjacent regions and numerous capillary loops suggest capillary budding, i.e. new formation of blood vessels (Ghadially *et al*., 1982).

Hyalinization

Numerous and severe signs of degeneration in fibroblasts, cementoblasts, and cementocytes were observed more frequently in the apical third of the experimental teeth. Since the apical third is the zone of main pressure in intrusion movement, it is conceivable that mechanical stress may be responsible for the vascular flow alteration that triggers cellular degeneration. In addition, perhaps as a consequence of these cellular alterations, the ECM of the PDL showed a typical picture of hyalinization (Ghadially *et al*., 1982). Thus, collagen fibrils and bundles were often very disarranged, whereas the inter-fibrillar components exhibited a granular and amorphous appearance.

The ECM identified in the experimental teeth suggests intense collagen degradation. However, intracellular fibrils (phagocytosis) were rarely observed. Extensive areas with an absence or dispersion of collagen fibrils seems to be the consequence of extracellular degradation and less of macrophages or fibroblast activity, supporting the statements of Brudvik and Rygh (1995b), and Ten Cate and Anderson (1986). It seems reasonable to assume, according to the present findings, that the pressure zones present an increased metabolism of collagen.

Root resorption and removal of degenerated tissues

The control group (normal human premolars), did not shows signs of root resorption as previously observed by scanning electron microscopy (Faltin *et al*., 1998). These findings are contrary to other studies (Brezniak and Wasserstein, 1993). This contradiction may be explained by the fact that young teeth that were not orthodontically moved or submitted to occlusal trauma or function were examined.

Although one of the force magnitudes (50 cN) applied in this study may be considered ideal or close to ideal, all moved teeth showed some degree of root resorption. The teeth intruded with 50 cN exhibited resorptive and degenerative aspects that were less intense and extensive than those intruded with forces of a higher magnitude (100 cN). This confirms the previous findings by scanning electron microscopy (Faltin *et al*., 1998) and demonstrates that the severity of resorption is clearly dependent on the magnitude of the force applied. However, in studies of mesial movement in human premolars, non-significant alterations in the amount of root resorption were found, even with an increase in the magnitude of the force applied (Owman-Moll *et al*., 1996b). Van Leeuwen and Maltha (1995) also observed this independence of factors during the initial phase of tooth movement.

In addition to the severity of resorption after intrusion with higher forces, differences between the root thirds were discerned: the cementum surfaces of the apical third showed more extensive areas of resorption than those of the middle third; in the cervical third, resorption lacunae were rarely detected. The severe resorption of cementum in the apical third may be due to fewer Sharpey's fibres (less barrier), greater blood supply (clast cells), higher metabolism in the adjacent PDL, and its structure similar to alveolar bone, as previously suggested (Rygh, 1977; Lindskog and Hammarström, 1980). On the other hand, in the cervical third, the presence of a greater number of Sharpey's fibres may represent a barrier against the resorption process, in addition to the characteristics of the

intrusive movement, which tend not to overload this root third.

Mono- and, predominantly, multi-nucleated macrophage-like cells close to the resorption lacunae of the cementum were observed. These cellular types were also identified in great numbers surrounding the adjacent hyalinized zone (Brudvik and Rygh, 1993a,b). These findings characterize the removal of necrotic tissue.

Despite numerous and extensive areas of root resorption, typical multi-nucleated clast cells (odontoclasts) were rarely identified. These observations are contrary to the findings of Reitan (1974) and Rygh (1977), which revealed typical odontoclasts in the central areas of resorption (Brudvik and Rygh, 1993a). The cell type commonly observed in this experiment, adjacent to the periphery of the resorbed cementum, were multinucleated with numerous vacuoles and lysosomes, but without ruffled borders and clear zones, suggesting characteristics of giant multinucleated cells. There is a possibility that these cells may, however, be odontoclasts in a deactivation phase in which they lose their membrane differentiation, ruffled border, and lateral cytoplasmic projections (clear zone). These results could also support the idea of a cyclic clastic activity (Lindskog *et al*., 1987; McKee and Nanci, 1996). Even if these cells correspond to giant cells, it is possible that they can also participate in the cementum resorption.

In general, the ultrastructural results of the present study suggest that an orthodontic tooth movement without tissue damage, such as root resorption, is difficult.

Tissue repair

According to several authors (Reitan, 1974; Barber and Sims, 1981; Brudvik and Rygh, 1995a,b) tissue repair occurs mainly when mechanical stress is removed or reduced. However, in the present study, for the teeth intruded for 4 weeks, in which the magnitude of the forces remained constant, signs of repair were observed. These were noted in the examined tissues, cementum and PDL. This process occurred first in the periphery of the hyalinized zones, as well as close to the areas and lacunae of the resorbed cementum. The presence of numerous cells in diverse stages of differentiation, such as cementoblasts with similar characteristics to those during the cementogenesis process were observed. In addition, the great proliferation and differentiation of fibroblasts (Brudvik and Rygh, 1995a,b; Lindskog *et al*., 1987) suggest intense repair activity in the PDL.

A newly-synthesized fibrillar ECM of the cementum, was deposited on the resorption lacunae, characterizing a cementum of intrinsic fibres (Bosshardt and Schroeder, 1994). In the irregular surface of resorbed cementum, an electron-opaque cement line was observed to which collagen fibrils appeared anchored. These findings are indicative of remineralization and fibrillar re-attachment in the cementum (Kurihara and Enlow, 1980; Embery *et al*., 1987; Bosshardt and Schroeder, 1996). This distinct appearance suggests that this surface may possess a different composition.

Even with some repair, the tissues observed in the investigation were extremely different from those structural and physiological characteristics observed in the control groups.

Final considerations

Although continuous and light forces were proposed as the most appropriate physiological stimuli for moving teeth (Schwarz, 1932; Oppenheim, 1944; Reitan, 1974), this was reconsidered (Brudvik and Rygh, 1995a; Van Leeuwen and Maltha, 1995; Owman-Moll *et al*., 1996a). The reason for this is that only intermittent forces would permit intervals with an absence of mechanical stress and, consequently, allow the repair of the affected tissues (Rygh, 1977; Kurol *et al*., 1996). However, in this investigation, signs of repair concomitant with tissue damage by continuous mechanical stress were observed.

Based on the results obtained in the present intra-individual study and previous findings (Faltin *et al*., 1998), it seems that the ideal level of continuous forces should be lower than that recommended previously for intermittent forces. In general, a reduction of force magnitude is proposed.

Conclusions

This ultrastructural investigation regarding orthodontic tooth movement with application of continuous intrusive forces in humans shows that:

(1) the mineralized surface of the cementum becomes more resorbed in the apical part and is dependent on the magnitude of force applied;
(2) vascular, cellular, and extracellular components of the cementum and PDL become altered, compromising the structural and physiological characteristics of these tissues, more intensely in the apical third of the roots, and also dependent on the magnitude of applied force;
(3) concomitant, areas of tissue repair were identified in the cementum and PDL, even with the maintenance of the level of the mechanical stress.

Address for correspondence

Professor Victor E. Arana-Chavez
Laboratory of Mineralized Tissue Biology
Department of Histology and Embryology
Institute of Biomedical Sciences
University of São Paulo
05508–900 São Paulo, S.P.
Brazil

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