The hyaline zone and associated root surface changes in experimental orthodontics in rats: a light and scanning electron microscope study

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SUMMARY Light- and scanning electron microscopic (SEM) examinations were used to study the hyaline zone and associated root resorption after orthodontic treatment of the upper first molars of rats. The orthodontic treatment consisted of a fixed buccal expansion appliance with an initial force of 250 mN. The animals were divided into nine experimental groups with orthodontic treatment and one control group without orthodontic treatment. Three groups were sacrificed immediately after 1, 3, and 7 days of treatment, and six groups after 7 days of treatment followed by 1, 2, 3, 4, 5, and 6 weeks without treatment.

A hyaline zone on the pressure side of the mesial root was identified at the light microscopic level of examination as well as the SEM examination as early as 1 day after the initiation of treatment. In the SEM it was found that on the roots of teeth studied in the first few days after initiation of treatment the hyaline tissue was so firmly attached to the root that it remained in place during the extraction and preparation procedures. After longer treatment periods these hyaline zones were lost at extraction or during the preparation for the SEM examination. The cementum surface under the hyaline zone had a smooth appearance suggesting that the surface had been modified by substances released from the hyaline zone. After 1 week of treatment, resorption in the cementum could be noticed. The resorption extended to the dentine as uncovered dentinal tubuli were found. Formation of reparative cementum started two weeks after treatment. Changes in the cementum surface as well as root resorption cavities could be seen for as long as 6 weeks after the cessation of orthodontic treatment.

Introduction

The biological response incident to orthodontic tooth movement involves tissue reactions in the periodontal ligament (PDL). Vascular injuries have been demonstrated in the compressed areas of the PDL which undergo hyalinization of the fibrous tissue within a few hours (Reitan, 1960; Rygh, 1972; Lilja et al., 1981). The hyaline zone is described in the literature as a cell-free area which evoke cellular reactions in the adjacent alveolar bone and root surface (Kvam, 1969; Rygh, 1974; Lilja et al., 1983). A limited hyalinized zone may be expected to persist from 2 to 4 weeks in a young patient (Reitan, 1951; Rygh, 1973). A variety of cells have been suggested to take place in its elimination, e.g. macrophages and leucocytes. It has been found that there is an association between root resorption and mechanical PDL injury (Nakane and Kameyama, 1987). According to Reitan (1960), the alveolar bone adjacent to the hyaline zone is removed by indirect resorption, i.e. by osteoclasts working from adjacent marrow spaces or on the surface of the alveolar bone. A corresponding resorption of the dental root occurs in the area bordering the hyaline zone (Oppenheim, 1936; Kvam, 1972; Reitan, 1974; Rygh, 1974, 1977; King and Fischlscheiger, 1982; Engström et al., 1988; Hellsing and Hammarström, 1991; Brudvik and Rygh 1993). However, the structure of the cementum surface under the hyaline zone, as well as the healing pattern of the resorption cavities after elimination of the hyaline zone are poorly known.
The aim of the present investigation was to examine in the scanning electron microscope (SEM) the influence of the hyaline zone on the cementum surface of rat molars, to follow the sequence of events from the initial changes via the active root resorption to the healing process and to correlate the root surface changes as they appeared in the SEM, with those observed in the same area in histological sections in the light microscope.

Materials and methods

Animals

Thirty adult, female Sprague-Dawley rats aged 3–5 months and averaging 250 g in weight were used as experimental animals. Twenty-seven animals were treated with a fixed orthodontic appliance with a treatment time from one to seven days and three rats were controls. The observation period after cessation of the orthodontic treatment lasted up to six weeks. The rats were given a powdered diet (Evos R3, Ewos AB, Södertälje, Sweden) and water ad libitum. They were kept in cages on net floors to prevent the litter normally used in the cages interfering with the orthodontic appliances. The weight of the animals was controlled before and after the experimental period.

Orthodontic appliances

A fixed buccal expansion appliance earlier described by Hellsing and Hammarström (1991) was used on the upper first molars (Fig. 1). The appliance comprised a 0.011-inch Australian light wire (Wilcock, Ortopro, Göteborg, Sweden) which was bent to fit between the molars and the curvature of the palate. A wire mesh was welded on the wire to cover the occlusal surfaces of the first molars and the arm lengths of the wire were 5.5 mm. The orthodontic appliance was constructed in such a way that it resulted in an equal bilateral tipping movement as well as a slight mesio-buccal rotation. When the experiment started the appliance was expanded 1 mm more than the distance between the mesio-palatal surfaces of the first molars and an initial buccal force of approximately 250 mN (25 g) was delivered to the upper first molars. A force measuring gauge earlier described by Hellsing and Hammarström (1991), was used to calibrate the expansion appliances.

Experimental protocol

The animals were anaesthesized with an i.p. injection of 0.3 ml Hypnorm vet (Pharmacia, Malmö, Sweden) per 1000 g body weight. The distance between the upper first molars was measured and the orthodontic appliance was adjusted to the desired distance. The individual orthodontic appliances were then, after activation, attached with bonding adhesive (Transbond, Unitek/3M, USA). The animals were divided into nine experimental and one control group with three rats in each group. The experimental periods for group 1, 2, and 3 were 1, 3, and 7 days, respectively, and the animals were killed after removal of the appliances. In groups 4, 5, 6, 7, 8, and 9 the rats were treated with a fixed appliance for 1 week and then killed after another 1–6 weeks without further treatment. The control rats did not receive any treatment. The maxillae of all animals were divided and one-half was prepared for light microscopic examination. The first molar of the other half was prepared for SEM examination.
Histological preparations

Immediately after the animals were killed, the heads were cut off and the maxillae divided mid-sagitally. One side was fixed in Histofix (Histolab., Göteborg, Sweden) for 5 days and then decalcified in 20 per cent formic acid for 3 weeks, dehydrated, embedded in paraffin, and sectioned in a bucco-lingual direction as parallel as possible to the long axis of the mesial root of the first molar. Sixty semi-serial sections with a distance of 4 μm were taken through the central portion of the root and stained with haematoxylin-eosin.

The other side of the maxilla was fixed in 5 per cent glutaraldehyde in cacodylate buffer for 5 days and stored in a refrigerator. The first molar was extracted, rinsed in distilled water and then kept in 10 per cent sodium hypochlorite (Dakin’s solution) for 10 minutes. The teeth were repeatedly rinsed in water, dried, and glued to aluminium stubs with conductive silver paint. The teeth were coated with gold (100 Å) and examined in a Jeol JSM-820 scanning electron microscope.

Results

The animals maintained their normal weight during the experimental period and did not seem to be disturbed by the treatment. The orthodontic appliance exerted an equal bilateral effect on the first molars which allowed comparison of the light microscope sections with the SEM micrographs of the mesial roots from each animal. The SEM micrographs of the control group showed that the acellular cementum surface denuded of organic tissue after immersion in Dakin’s solution had irregular depressions surrounded by thin ridges giving the surface a mosaic-like impression. On the mesial part of the root there were some distinct resorption cavities. They were usually small and covered by normal cementum.

1 day with appliance

The histological sections showed a few structureless areas with reduced numbers of fibroblasts on the pressure side of the PDL between the alveolar bone crest and root surface suggesting an initial stage in the development of a hyaline zone. When compared with the SEM micrograph, the cementum showed some smooth surface areas on the mesio-buccal side of the root approximately at the level of the alveolar crest on the histological sections.

3 days with appliance

In the light microscope on the pressure side of the PDL there were distinct cell-free areas indicating hyaline zones. There were resorption cavities on the alveolar bone crest. The corresponding area of the root showed an intensified uneven staining of the cementum surface (Fig. 2). Correspondingly on the SEM micrograph of the mesio-buccal side of the root, tissue remnants with an uneven surface previously described by Kvam (1972) as characteristic for the hyaline zone, could be seen attached to the root surface of the extracted tooth (Fig. 3a). Further treatment of the same tooth for another 10 min with 10 per cent Dakin’s solution eliminated these tissue remnants (Fig. 3b). After resputting with gold and re-examination in the SEM it was found that
Figure 3 (a) SEM micrograph of the pressure side of the mesial root of the upper first molar showing remnant of the hyaline zone (Hy) attached to the root surface (R) (3 days with appliance group). C, crown. Bar=500 μm. (b) Corresponding area of the same tooth as in (a) after removal of the hyaline zone. The smooth area indicated by arrow heads was previously covered by the hyaline zone. Bar=500 μm. (c) Enlargement of the framed area in (b) showing the mosaic-like surface of the acellular cementum (AC) and the smooth surface (SS) which was earlier covered by the hyaline zone. Bar=10 μm.
the cementum surface previously covered by the tissue remnants had lost the normal mosaic-like appearance and become very smooth (Fig. 3c).

1 week with appliance

In the histological section root resorption cavities could be seen in the root surface as well as in the alveolar bone. The SEM micrograph showed that small areas of the tissue remnants remained attached to the root surface. Neighbouring areas were smooth and there were small resorption cavities in the cementum.

1 week with appliance followed by 1 week without appliance

In the light microscope extensive resorption of the alveolar bone crest and corresponding root surface was seen. The resorption was situated coronally to the hyaline zone. There were multinucleated giant cells at the periphery of the cell-free hyaline zone and the root surface neighbouring the hyaline zone was intensely stained by haematoxylin (Fig. 4). In the SEM, root resorption cavities of varying sizes could be seen on the mesio-buccal side (Fig. 5A). Between the resorption cavities smooth root surface areas, as well as unaffected acellular cementum were noted. On the buccal side of the root, small resorption cavities were seen. The root surface in the resorption area showed numerous holes which were interpreted to be patent dentinal tubuli indicating that the resorption had penetrated some distance into the dentine (Fig. 5B). There were also areas with a distinct difference between the acellular cementum and the smooth surface earlier covered by the hyaline zone similar to that seen in the 3-day appliance group (Fig. 6).

1 week with appliance followed by 2–3 weeks without appliance

The root resorption cavities were reduced in depth and the cells in the PDL were evenly distributed in the histological sections. No distinct hyaline zones were registered. Some resorption areas were partially filled with a reparative cementum (Fig. 7). There were no tissue remnants attached to the root surface of the teeth after preparation for SEM. Smooth areas in the cementum surface could still be seen adjacent to the resorption, and the floor of the resorption cavities displayed a glossy, even structure (Fig. 8).

1 week with appliance followed by 4 weeks without appliance

No hyaline zones were visible in the light microscope. The reparative cementum showed intense staining at the surface. The SEM displayed a difference in level between the depressed surface of the resorption, the smooth areas affected by the hyaline zone and the unaffected acellular cementum.

1 week with appliance followed by 5 to 6 weeks without appliance

The SEM micrograph showed no remarkable changes from the previous group except for more shallow resorption cavities and larger areas of reparative cementum.
Figure 5  (A) SEM micrograph of the root surface after 1 week with appliance followed by 1 week without appliance. Numerous resorption cavities of varying sizes (open arrow heads), as well as a large resorption area can be seen on the mesio-buccal side. Smooth areas (arrow heads) as well as unaffected cementum surfaces could be observed on the root. Bar = 100 μm. (B) Enlargement of the area framed in (A) showing the surface of the resorption area. The numerous holes were interpreted to be patent dentinal tubuli. Bar = 10 μm.

Figure 6  SEM micrograph from the 1 week with appliance followed by 1 week without appliance group illustrating an uneven surface of the acellular cementum (AC) and the smooth surface (SS) earlier covered by the hyaline zone. Bar = 10 μm.

Discussion
The standardized technique with which the orthodontic appliances were produced made it possible to compare the results in the light microscope sections and the SEM micrographs both within each rat and also between all experimental animals in the present study. The development of hyaline zones, root resorptions, and the healing process could be followed in the
different groups. Because of the mesio-buccal rotation of the molar caused by the appliance the hyaline zone was located at the mesio-buccal surface of the mesial root.

Small resorption pits on the buccal side of the mesial root were found in the control teeth as well as in the experimental teeth indicating that resorption in this area is a normal process, and probably caused by intermittent damage of the periodontal ligament and the root surface at the edge of the alveolar bone crest. The occurrence of root resorption cavities in non-treated human teeth has previously been noted by Kvam (1972), and Harry and Sims (1982).

The mosaic- or cobblestone-like surface found on the unresorbed acellular cementum in all groups has earlier been described and suggested to be caused by dissolved Sharpey's fibres by Barber and Sims (1981), Lindskog (1982), and Goldie and King (1984).

Limited areas with hyalinized tissue were seen in the PDL as early as one day after application of the appliance. All teeth used for the SEM examination were treated with Dakin's solution for 10 min. Apparently this time was not sufficient to completely eliminate the organic remnants of the hyaline zone of the teeth up to 2 weeks after active treatment. However, these organic remnants were eliminated after a further 10 min in Dakin's solution. The initially persisting remnants on the root surface are in agreement with the light microscopic findings of a dense hyaline zone closely associated with the root surface. The cementum underlying the hyaline zone gave a smooth impression in the SEM suggesting that the outer surface had been degraded or changed, possibly due to enzymes released from the mechanically damaged PDL. This change in surface structure of the cementum under the hyaline zone has not previously been described. Since the teeth had been treated with Dakin's solution prior to the SEM examination it should be borne in mind that the 'surface' actually represents the mineralizing front of the cementum suggesting that the unmineralized cementoid had been degraded and the mineralized cementum exposed. In the periphery of the hyaline zone multinucleated cells were found. Possibly, these cells were attracted there by the exposed mineralized cementum. Degradation of the osteoid layer has been found to precede osteoclastic bone resorption (Chambers and Fuller, 1985).

This investigation, like other studies has shown that there is no root resorption immediately under a hyaline zone (Kvam, 1972; Reitan, 1974; Rygh, 1977). However, this study also demonstrated that there is a complex pattern on the root surface when a tooth is exposed to an orthodontic force. Smooth areas and resorption cavities alternated with normal cementum. A possible explanation could be that a varying thickness and density of the hyaline zone resulted in a different elimination time and the resorption cells may then gain access to the exposed surface at varying time intervals. Root resorption in our study could be seen as early as 7 days after active treatment in rats which was also found by Goldie and King (1984), and Brudvik and Rygh (1991).

The healing of the resorption cavities seemed to occur in all directions, i.e. the lacunae were reduced in width as well as in depth. In the SEM the surface of the reparative cementum had an even, glossy structure which in the histological sections showed an intense staining. Even after 6 weeks following removal of the appliance there was still a depression in the root surfaces. In the repaired resorption cavities no holes interpreted to be dentinal tubuli were found. This suggests that root resorption associated with the elimination of the hyaline zone is followed by formation of reparative cementum which prevents further communication between the pulpal and periodontal tissues. The reparative cementum observed in the study was acellular. This is in contrast to Langford and Sims (1982) who in a similar experiment in man observed cellular cementum in the reparative root resorption lacunae.

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