Repair of root resorption 4 and 8 weeks after application of continuous light and heavy forces on premolars for 4 weeks: A histology study

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Introduction: Repair of root resorption cavities has been studied under light microscopy, scanning electron microscopy, and transmission electron microscopy. The aim of this investigation was to demonstrate the use of microcomputed tomography (micro-CT) to assist in the identification of the region of interest for light microscopy preparation. This study also qualitatively illustrated the root resorption craters with 4 or 8 weeks of retention after 4 weeks of continuous light or heavy orthodontic force application. Methods: Four patients who required bilateral extractions of maxillary first premolars as part of their orthodontic treatment were divided into 2 groups (groups I and II) of 2. The maxillary left and right first premolars were loaded with light (25 g) or heavy (225 g) orthodontic force for 4 weeks. After 4 or 8 weeks of retention, the maxillary first premolars were extracted. The extracted teeth were investigated with micro-CT. By using 3-dimensional images created by the micro-CT, the largest resorption craters on the buccal and lingual sides were identified. Parasagittal sections of these resorption craters were studied histologically under hematoxylin and eosin staining. Results: The use of micro-CT improved the efficiency and accuracy of histologic techniques. Comparatively, less root resorption was repaired by new cementum after heavy orthodontic force application and short retention time. The reparative processes seemed to depend on time, with longer retention time yielding the most amount of repair. Reparative cementum was a mixture of acellular and cellular cementum. Reparative processes seemed to commence at the central part of the resorption cavity and expand to the periphery. Conclusions: Root resorption cavities have the potential to repair regardless of the orthodontic force magnitude. Correlative microscopy with micro-CT and conventional light microscopy adds a new dimension to current root resorption investigation techniques. (Am J Orthod Dentofacial Orthop 2010;138:727-34)

Budvik and Rygh1,2 carried out studies on the repair of orthodontically induced inflammatory root resorption after application of 50 g of force for 21 days using light microscopy and transmission electron microscopy. They showed that the transition of active root resorption into a process of repair was associated with the invasion of fibroblast-like cells from the circumference of the resorption crater into the active root resorption site even with a light force. The formation of new tooth-supporting structures was seen in the periphery of the resorption lacunae, whereas active resorption by multinucleated odontoclast-like cells took place in the central parts. When orthodontic force was discontinued, the reparative process was similar to early cementogenesis during tooth development. It has been suggested that the epithelial cell rests of Malassez might have a significant role in mediating repair cementogenesis.3,4

The resorptive defects are repaired by the deposition of new cementum and the reestablishment of new periodontal ligament.2,3,5-8 In a histologic study by Owman Moll and Kurol,9 50 cN of buccally directed force was applied on maxillary first premolars for 6 weeks and passively retained up to 7 weeks. It was found that the repaired cementum was more of the cellular type than the acellular type. The acellular cementum occurred more often in the early phase of healing.3 The repair process did not vary with the location or the type of

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tissue, either on the buccal or the lingual side of the tooth, or in the cervical or apical parts of the root.9

There are various patterns of repair in the resorption cavity. Depositions of cementum from both the periphery of the resorption cavity inward and the center outward have been identified.1,2,7,10 Brudvik and Rygh1,2 illustrated in their histologic studies that the repair process started in the periphery of the root resorption lacunae, while active resorption was still occurring beneath the main more centrally located and overcompressed syalinized zone. Owman Moll and Kurol9 showed that healing cementum only occurred centrally in the bottom of the cavity and centrally and peripherally on 1 lateral wall of the cavity. This could be due to different sectioning orientations, or the specimens were collected at different times.

The aim of this investigation was to demonstrate the use of microcomputed tomography (micro-CT) to assist in the identification of the region of interest for light microscopy preparation. We also qualitatively studied the histology of root resorption craters after 4 or 8 weeks of retention after 4 weeks of continuous light or heavy orthodontic force application.

MATERIAL AND METHODS

Eight first premolars were collected from 4 orthodontic patients (2 boys, 2 girls; age range, 10.3-15.3 years; mean, 13.7 years) who required bilateral maxillary first premolar extractions as part of their fixed appliance orthodontic treatment. They were recruited according to strict selection criteria as described previously.11,12 Ethics approvals were obtained from the Medical Faculty Ethics Committee of the University of Ondokuz Mayis in Samsun, Turkey, and the Human Research Ethics Committee of the University of Sydney in Surry Hills, New South Wales, Australia (EK:347 and 12-2005/1/8783, respectively). All subjects and their guardians consented to participate in this study after verbal and written explanations had been provided.

The subjects were divided into 2 groups (groups I and II) of 2 subjects in each group. They received a light conventional orthodontic force of 25 g on the maxillary left first premolar and a heavy conventional orthodontic force of 225 g on the maxillary right first premolar for 4 weeks. The premolars were then placed in retention, without orthodontic force application, for 4 or 8 weeks in groups I and II, respectively (Fig 1).

The 0.022-in slot SPEED brackets (Strite Industries, Cambridge, Ontario, Canada) were bonded on the buccal surfaces of the maxillary first permanent molars and the experimental maxillary first premolars (Fig 2, A). Self-ligating brackets were selected to allow for standardized ligation of the experimental teeth. A buccally directed light orthodontic force of 25 g was induced by a 0.016-in titanium-molybdenum alloy cantilever spring (Rematitan, Dentaurum, Ispringen, Germany) (Fig 2, A and B). A buccally directed heavy orthodontic force of 225 g was induced by a 0.017-in × 0.025-in titanium-molybdenum alloy cantilever spring (Beta III Titanium, 3M Unitek, Monrovia, Calif) (Fig 2, C). The force magnitude was measured with a strain gauge (Dentaurum). Light-cured band cement (Transbond Plus, 3M Unitek) was bonded onto the occlusal surfaces of the mandibular first permanent molars to minimize occlusal trauma to the maxillary first premolars during the experimental period (Fig 2, D).

After 4 weeks of force application, the cantilever springs were removed, and the maxillary first premolars were retained by a custom-made passive retention wire bonded on the palatal surfaces of the maxillary first and second premolars and first molars with 0.0175-in multi-stranded stainless steel wire (TwistStraight, 3M Unitek) (Fig 3). The maxillary first premolars were then extracted after either 4 or 8 weeks of retention. All extractions were performed by an oral surgeon who was instructed to avoid any forceps contact on the cervical cementum. Immediately after extraction, the teeth were stored in individual containers containing 10% buffered formalin for tissue fixation.

The extracted teeth were placed in a moist chamber of 10% buffered formalin and scanned by a desktop x-ray micro-CT system (SkyScan 1172, Aartsellaar, Belgium). Micro-CT is a variant of the medical CT scan system that allows nondestructive imaging of the internal microstructure of materials with high spatial resolution. The x-ray source was an air-cooled, sealed microfocus x-ray tube with a focal spot size less than 8 μm. The x-ray tube was operated at 100 kV and 100 μA with aluminum and copper filters. The x-ray detector consisted of a 1024 × 1024 pixel 16-bit charged coupled device camera with fiber-optic coupling to an x-ray scintillator. All teeth were scanned from the cementoenamel junction to the apex with resolution ranging from 14.38 to 16.11 μm pixel size. Scanning of the teeth was done with 360° rotation around the vertical axis and a single rotation step of 0.2°. At each rotation step, an x-ray absorption radiograph was acquired. A total of 1800 x-ray absorption radiographs were acquired for each tooth and saved in 16-bit tagged image file format (TIFF).

After acquisition, an axial slice-by-slice reconstruction was performed with specific software (version 1.4.2, Nrecon, Aartsellaar, Belgium). Two-dimensional images were generated as 1024 × 1024 pixel bite-map images.
having an 8-bit greyscale dynamic range. Then, Studio Max software (version 1.2, Volume Graphics, Heidelberg, Germany) was used to collate the axial 2-dimensional slices to form a 3-dimensional (3D) reconstruction of the images.

After micro-CT analysis, the teeth were prepared for histologic study. The samples were decalcified in 10% formic acid in phosphate-buffered formalin for approximately 4 weeks. The largest resorption craters on the buccal and lingual surfaces of each tooth were identified on the 3D images produced by the micro-CT scans (Fig 4, A). By using these 3D images as a reference map, the specimens were grossly sectioned into 2 pieces for 1-rooted premolars or 4 pieces for 2-rooted premolars (Fig 4, B and C). The samples were then embedded in paraffin (Fig 4, D). Parasagittal sections of the mesiodistal aspects of the teeth were cut at 5 µm with a microtome (Leica Microsystems, Düsseldorf, Germany). Serial sections were stained with hematoxylin and eosin. From each specimen, the glass-containing sections with the longest root resorption crater length coronally and apically was chosen for the investigation. Color photographs were exposed in a light microscope with 1.25, 4, 10, and 20 times objective magnifications (OM). Histology was assessed for root resorption and associated repair.

RESULTS

In group I, with 4 weeks of retention after 4 weeks of light orthodontic force, the buccal root showed 2 main areas of root resorption with associated repair: the apical third of the buccal root and the buccal surface of the buccal root at the cervical region (Fig 5). On the lingual surface of the buccal root near the tooth apex, the resorption crater appeared to have extended into the dentin and was repaired by new cementum (Fig 5). Cementocytes were also seen trapped in the new cementum; this might indicate repaired cementum of the cellular type. A layer of cementoblast-like cells overlaid the new cementum. However, the reparative cementum did not restore the anatomic contour of the root. Active root resorption craters were also evident near the root apex. The resorption crater was multiloculated and infiltrated with blood cells. The resorption crater on the buccal surface had also extended into the dentin (Fig 5).
The newly formed cementum in the bottom of the resorption cavity had no trapped cementocytes that indicated acellular cementum. However, the superficial layer of reparative cementum had incorporated cementocytes that indicated cellular cementum.

After 4 weeks of retention after 4 weeks of heavy orthodontic force, resorption craters were also found on the buccal surface of the cervical region of the buccal root and the apical region of the lingual root (Fig 6). Resorption craters were repaired with new cementum, and many inflammatory infiltrates were present in the adjacent area (Fig 6). The reparative cementum seemed to be thicker in the center of the resorption crater than at the periphery. With the same OM, the amount of reparative cementum seemed to be less than that in the light orthodontic force group with the same retention period (Figs 5 and 6). In addition, the majority of reparative cementum was the acellular type. The resorption crater was not fully repaired to the root shape. Interestingly, in 1 resorption crater, cementum deposition was beyond the crater’s level (Fig 7).

In group II, with 8 weeks of retention after 4 weeks of light orthodontic force, a shallow resorption crater with reparative cementum was found on the buccal surface of the cervical level of the root (Fig 8). The new cementum layer had almost repaired the resorption cavity, unlike in the 4-week retention group. Some cementocytes were enclosed in the newly formed cementum. There seemed to be no evidence of inflammatory infiltrates, blood cells, cementoblast-like cells, and clastic cells at this OM. This might indicate that the resorption and reparative processes had stopped.

At 8 weeks of retention after 4 weeks of heavy orthodontic force, a significant amount of reparative cementum was evident (Fig 9). Unlike in the previous group, the reparative cementum was not clearly distinct from the existing cementum by the differences in stain intensities and histomorphologies. The reparative cementum in this group displayed strongly stained incremental lines (Fig 10). A few cementocytes were included in the newly formed cementum (Fig 9).

**DISCUSSION**

Histologic preparation is technique-sensitive, and isolation of a small region of interest such as root resorption craters can be time-consuming. Micro-CT allowed nondestructive imaging of the tooth samples.
The resultant 3D visualization permitted easy identification of regions of interest—e.g., root resorption craters—which made the histologic sectioning more accurate and efficient. In this study, the micro-CT images showed that the regions of interest, the largest root resorption craters, were located in the buccal-cervical and lingual-apical root surfaces. These results also showed that cells and tissues were well preserved in the histologic slides after the samples were scanned by the micro-CT scanner. This was because the specimen remained either immersed in buffered formalin or in a moist buffered formalin chamber. Therefore, micro-CT scanning can optimize histologic sectioning techniques that could be used in future research studies on hard tissues.

Previous studies have shown that the volume of root resorption craters was greater after heavy force application. It is clinically fortunate that this investigation showed evidence of reparative cementum even after the application of heavy force, especially when the sample teeth were retained for a longer period of time. This illustrated the protective mechanism against adverse effects from orthodontic forces. Although this study was not quantitative, the extent of reparative cementum that filled the resorption cavities was greater in the longer retention group; this indicated that the reparative process depends on time. At the same OM, the least amount of reparative cementum was found in the teeth with the heavy force retained for 4 weeks followed by the teeth with the light force.
retained for 4 weeks, the teeth with the light force retained for 8 weeks, and the teeth with the heavy force retained for 8 weeks. This correlated with our volumetric study that found the largest mean root resorption crater volume in the teeth with the heavy force retained for 4 weeks. By the end of 8 weeks, there was little evidence of inflammatory infiltrates histologically, especially in the light force group; this might indicate deceleration of the reparative process. However, the periodontal ligaments were not totally intact in the sample, so the inflammatory infiltrates could not be fully investigated. The properties of cementum were found to be closely related to bone. The cells that were involved in the cementum resorption and repair process were similar to those found in bone biomechanics. These included fibroblasts, osteoblast-like cells, osteoclast-like cells, and inflammatory cells. Similar reversal lines that indicated periods of bone remodeling were seen with the healing of the resorbed cementum. Because the specimens were extracted teeth, the reestablishment of the periodontal ligament could not be investigated.

Bosshardt and Schroeder found that the reparative cementum was principally cellular in teeth. Other authors suggested that the reparative cementum was a combination of cellular and acellular cementum. The resorption craters were initially repaired by acellular cementum; this was followed by continuous repair with cellular cementum. In our investigation, the majority of the reparative cementum was the cellular type. Some resorption craters were repaired with a combination of cellular and acellular cementum, whereas few resorption craters showed entirely acellular cementum. Acellular cementum appeared to

Fig 5. Histologic section of the buccal root of a tooth subjected to 4 weeks of light force followed by 4 weeks of retention (1.25 × OM). Blue and yellow arrows indicate areas of resorption craters. Bar = 1 cm. Blue-bordered insert indicates active cementum reparative process with acellular cementum lining the base of the resorption cavity (AC) and cementocytes trapped in the superficial layer of newly formed cementum (arrows) (20 × OM). Bar = 1 mm. Yellow-bordered insert shows root resorption crater after 4 weeks of light orthodontic force applied after 4 weeks of retention with cellular cementum (C) overlaid by a layer of cementoblast-like cells (arrow) (20 × OM). Bar = 1 mm.

Fig 6. Histologic section of the buccal root of a tooth subjected to 4 weeks of heavy force followed by 4 weeks of retention (1.25 × OM). Yellow arrow indicates the resorption crater. Bar = 1 cm. Yellow-bordered insert shows root resorption crater after 4 weeks of heavy orthodontic force applied after 4 weeks of retention with minimal repair. Resorption cavity was filled with inflammatory infiltrate. Reparative cementum was thicker in the central part (black arrows) than the peripheral part (green arrows) of the resorption cavity (20 × OM). Bar = 1 mm.

Fig 7. Reparative cementum deposited outside the resorption cavity (arrows) (4 × OM). Bar = 5 mm.
first deposit in the bottom of some resorption cavities followed by reparative cementum with cementocyte entrapment. However, the exact sequence of events could not be elucidated due to the lengthy retention time intervals, 4 and 8 weeks, and the small sample size. There seemed to be a tendency that the type of reparative cementum depended on the orthodontic force. The heavy-force group appeared to show more acellular reparative cementum, whereas the light-force group showed more of the cellular type. However, the sample size was not large enough to support this hypothesis.

Henry and Weinmann identified 2 structural types of root resorption repair: anatomic and functional. Anatomic repair was characterized by the restoration of the root surface to its original contour, and functional repair occurred when the exposed dentin had been covered by a thin layer of repair cementum. Functional repairs were seen more often in the specimens that were retained for 4 weeks, and anatomic repairs were found in the samples that were retained for 8 weeks. This possibly indicated that the structural type of root resorption repair was time-dependent and not orthodontic force-dependent. Occasionally, cementum repaired outside the contour of the root resorption crater appeared as hypercementosis.

Brudvik and Rygh believed that the reparative process began in the periphery of the root resorption craters, whereas active resorption continued beneath the centrally located overcompressed hyalinized zone. Our results were not consistent with their findings and showed that the amount of reparative cementum was thicker in the central area than at the periphery of the root resorption crater. This agreed with the study of Langford and Sims. They found that the process of root resorption repair commenced centrally and extended peripherally, and that resorption continued to spread at the periphery of the resorption site. This can be explained by the fact that the squeezing of tissues between the tooth and the bone surface was less pronounced in the central area of the resorption crater than the periphery because of the resilience of the tissue. Therefore, cell proliferation and regeneration of tissue were more pronounced in the deep central part of the resorption cavity. Owman Moll and Kurol also

Fig 8. Root resorption crater after 4 weeks of light orthodontic force applied after 8 weeks of retention with almost full repair of the resorption crater with cellular cementum (C) (20 × OM). Bar = 1 mm.

Fig 9. Root resorption craters after 4 weeks of heavy orthodontic force applied after 8 weeks of retention with large amounts of reparative cementum. Small amounts of cementocytes were included in the newly formed cementum (20 × OM). Bar = 1 mm.

Fig 10. Reparative cementum showed incremental lines (arrows) that might indicate active and resting periods of cementum repair; this was similar to the reversal lines in bone remodeling (20 × OM). Bar = 1 mm.
found reparative cementum in the central and central peripheral areas of the resorption craters.

CONCLUSIONS

1. The use of micro-CT definitely improves the efficiency and accuracy of histologic techniques.
2. During the cementum reparative process, the cells involved seem to be similar to the cells involved in bone biomechanics; for the same OM, less of the root resorption cavity seemed to be repaired by new cementum after heavy orthodontic force application and short retention time when compared with light orthodontic force with the same retention time; the reparative processes seem to depend on time, with a longer retention time yielding the most amount of repair; reparative cementum is a mixture of acellular and cellular cementum; and the reparative process seems to commence at the central part of the resorption cavity and expand to the periphery.

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