Analysis of Orthodontically Induced Root Resorption Using Micro-Computed Tomography (Micro-CT)

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ABSTRACT
Objective: To establish a protocol for assessing orthodontically induced tooth root resorption (OITRR) in human premolars using micro-computed tomography.

Materials and Methods: Ten extracted maxillary first premolars were obtained from eight healthy adolescent patients; five of these premolars were extracted before any orthodontic movement was applied to them, and the other five premolars were involved in nonextraction orthodontic treatment for 1 year before treatment plan modification lead to extraction treatment. Using reconstructed scanned images, we measured several key resorption lacunae parameters, including the number, volume, and depth on each surface, as well as its extension coronoapically.

Results: Orthodontic treatment resulted in a significantly greater tooth root resorption lacunae volume, number, maximum depth, and coronoapical extension as well as areas of dentin de-mineralization subjacent to the resorption lacunae than in normal premolars.

Conclusions: We have established a protocol to evaluate OITRR quantitatively and have provided a method to predict further resorption based on dentine demineralization. (Angle Orthod. 2009; 79:91–96.)

KEY WORDS: Tooth root resorption; Orthodontic treatment; Micro-CT; Resorption lacuna; Demineralization

INTRODUCTION
Orthodontically induced tooth root resorption (OITRR) is a relatively common side effect that has received considerable attention, although the causes and mechanism remain poorly characterized. The incidence has been found to be 28.8% of affected incisors. Several studies have detailed the prevalence and severity of OITRR in order to better define predisposing factors, highly susceptible teeth, and individuals at greater risk. Lupi et al found that the frequency of incisors showing any grade of OITRR increased from 15% before treatment to 73% after treatment. Also, the number of teeth with moderate and severe OITRR increased from 1% before treatment to 25% after treatment. Another study found that 4% of orthodontic patients experience “generalized” resorption of more than 3 mm, and about 5% of adults and 2% of adolescents are likely to have at least one tooth that resorbs more than 5 mm during treatment.

Minor OITRR in the first 6 months is a strong indicator of progressive root resorption by the end of orthodontic treatment. Progressive OITRR can lead to a compromised crown to root ratio and compromised function. Research has shown that OITRR can occur within 35 days. With forces as light as 50 g, progressive apical resorption was accompanied with areas of cellular cementum repair. Teeth are also prone to additional root loss during relapse as a result of jiggling by light muscular forces. Incidence of severe OITRR lacunae is more frequent in the apical one third.

Investigations have examined changes in physical properties of human premolar cementum, wherein the
application of orthodontic forces altered the mineral content of cementum. It has been shown that OITRR is related to the degree of force applied to the affected teeth. In addition, clinically significant changes demonstrated a trend toward an increase in the mineral composition (calcium, phosphorus, and fluoride) of cementum at various areas of periodontal ligament (PDL) compression. Overall decrease in the calcium concentration of cementum occurred with the application of heavy orthodontic forces that correspond to areas of PDL tension. OITRR has been associated with local damage of the PDL due to overcompression. Retardation and stagnation of the blood flow in such pressure zones lead to sterile necrosis of the soft tissue. This may be due to the presence of greater root resorption in the area of compressive forces on the PDL than greater combined compressive and tensile forces.

Micro-computed tomography (Micro-CT) is an x-ray imaging technology used to visualize mineralized tissues in three dimensions and at incredibly high resolution (and magnification). Currently, the in vivo analysis of dental tissues by micro-CT is possible only in laboratory animals because of the small diameter imager gantry restrictions (68 mm diameter maximum); thus, the evaluation of human dental material by micro-CT is currently only possible ex vivo. It has been used to study the root resorption in animals and to study periodontal ligament hydrostatic pressure with areas of root resorption through finite element analysis. However, there is no established protocol that comprehensively evaluates tooth root resorption in human teeth.

The aim of this study was to establish a protocol to investigate the severity and volume of root resorption in extracted human first premolars after undergoing 1 year of regular orthodontic treatment, which involved multidirectional tooth movement, representing the actual clinical tooth movement and forces applied in the clinical environment. We set out to establish a protocol to quantitatively assess and predict further OITRR. We used high-resolution Micro-CT to measure OITRR and other geometric aspects of the extracted teeth.

**MATERIALS AND METHODS**

**Sample Collection**

Teeth were extracted from adolescent patients undergoing orthodontic treatment at the University of Alberta. This research was approved by the Health Research Ethics Board at the University of Alberta. Ten maxillary first premolars were obtained; five control teeth were extracted before orthodontic treatment commenced, and five test teeth were from patients who underwent 1 year of treatment before these premolars were extracted. Extraction was as a result of modifying the treatment plan in order to resolve space deficiency, severe midline discrepancy, or excessive incisor proclination.

The patients were medically and dentally healthy except for their malocclusion. The control group had an average age of 14 years 4 months ± 1 year 9 months (3 males, 2 females), and the test group had an average age of 15 years 5 months ± 1 year 7 months (3 females). All patients had Class I malocclusion with mild to moderate crowding (4–6 mm). Patients underwent orthodontic treatment involving the expansion of dental arches, using progressive sequences of nitinol archwires (0.014, 0.016, 0.018 round and 0.016 × 0.022 rectangular) in an MBT (McLaughlin Bennett Trevisi) preadjusted bracket system with a slot size 0.022 (3M Unitek, Monrovia, Calif). The treatment plan was modified from nonextraction to extraction to improve lip competence in all cases. Teeth were extracted using periosteomes and stored in 70% ethanol for disinfection. As most extraction decisions were made early in the course of treatment, our study sample size was small as these were the only cases that presented in our clinic and met our study criteria for selection as detailed earlier.

**Micro-CT Scanning of Teeth**

Extracted tooth samples were air dried for 30 minutes and scanned axially in a Skyscan 1076 Micro-CT imager using the vendor-supplied imaging control software (Version 2.6.0; Skyscan N.V., Kontich, Belgium). Image projections were acquired at a resolution of 9 μm using an x-ray source potential of 100 kVp, ampere of 100 μA, and power of 10 W. An aluminum filter of 1.0-mm thickness was used to increase the mean photon energy of the source x-ray beam; three scan projections was the average per step, through the 180° of rotation at 0.5° step increments. Raw image data were reconstructed using NRecon software Version 1.4.4 (SKYSCAN, Kartulzersweg 3B 2990 Kentich, Belgium) using a modified Feldkamp back-projection technique. The tooth was selected as the region of Interest, using a 0.0 to 0.06 image to cross-section software threshold.

**Analysis of Root Resorption**

Reconstructed images of scanned teeth were analyzed using CT analyzer (Version 1.6.1.0, Skyscan N.V., Kontich, Belgium). Resorption lacunae were analyzed by sampling every 50th slice over the length of the tooth, starting with 226 slices, which is equivalent to the average of the biological width dimension 2.04 mm², below the cementoenamel junction (CEJ) and proceeding to the root apex. Measured indices included the location and number of resorption lacunae, height of resorption lacunae (μm), deepest point of resorption (μm), zone of...
ROOT RESORPTION AFTER ORTHODONTIC TREATMENT

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<th>Table 1. Resorption Lacunae Measurement Data Obtained from 9-μm Tooth Slices (by micro-computed tomography)</th>
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Tooth demineralization (μm), and volume of each resorption lacuna and percentage of tooth root resorbed (to that of original tooth volume). Height of resorption lacunae was calculated by determining the number of 9-μm slices over which resorption was evidenced. The deepest point of resorption was determined by measuring the distance perpendicular from an estimated root periphery, based on root shape, to the deepest point of the resorption lacuna. Zone of tooth demineralization was determined by measuring the distance perpendicular from the lacuna periphery to the furthest point of visualized density difference in the tooth root. Volume of a lacuna was calculated by selecting the surface area of the lacuna, including the estimated root periphery, over all the slices the lacuna was present, and allowing the analysis software to calculate the volume. To calculate the percentage of tooth root resorbed, an original tooth volume was estimated based on the volume of the tooth root, as determined by analysis software, and adding to it the volume of all resorption lacunae found. Reconstructed three-dimensional renderings of analyzed teeth were viewed using computed tomography (CT) volume Realistic 3D-Visualization (Version 1.10.0.5, Skyscan NV).

Statistical Analysis

Data are displayed in Table 1. Nonparametric statistical analyses were conducted using SPSS software (SPSS, Version 13, SPSS Inc, Chicago, III). The Mann-Whitney test was used to compare the difference in measured parameters between treatment and control tooth groups. The Friedman test was performed to examine the difference between the four tooth surfaces for treatment and control, respectively. Differences were detected using a Wilcoxon signed-rank test and conducted as a pairwise comparison. The significance level was set at .05.

RESULTS

Distribution of Root Resorption Lacunae

A significantly greater number of resorption lacunae were found on the root surfaces of treatment teeth compared with control teeth (Figure 1). Of particular importance was the observation that control teeth had resorption lacunae located only on the mesial, distal, and palatal surfaces, whereas treatment teeth had lacunae located on all surfaces. Using the Mann-Whitney test, significant differences were detected between the control and treatment groups on all surfaces ($P = .008, .015, .005, and .007$ on mesial, distal, buccal, and palatal surfaces, respectively) (Figure 2A). Within the treatment teeth, there was a significant difference in the number of resorption lacunae among different surfaces ($P = .020$). There were a significantly greater number of resorption lacunae on the mesial surface of the teeth compared with the buccal or palatal surfaces. Thus, significant differences were detected in the quantity of resorption lacunae based on the distribution on different root surfaces between orthodontically treated and untreated control teeth ($P = .009$, the typical values are 25 and 1, respectively).

Resorption Lacuna Height and Depth

Control and treatment samples showed variability in the average coronoapical height and average depth of resorption lacunae, with greater variability seen in the control group (Table 1). The height and depth of lacuna in the treatment teeth were significantly larger than those found in the control teeth ($P = .047$ and $.009$ for height and depth, respectively) (Figure 2B).

Resorption Lacuna Volume

Both control and treatment teeth showed average resorption lacunae volume < 1 mm³, with greater variability in lacunae volume in the treatment sample (Figure 2C). The treatment teeth had significantly greater (more than four times as large) resorption lacunae volume compared with the control group.

Percentage of Tooth Root Volume That Underwent Resorption

To determine the extent of tooth resorption that had occurred, the clinical tooth root volume was estimated by adding the total volume of resorption lacuna in a
Figure 1. A three-dimensional rendering of control and treatment teeth using micro-computed tomography (at an orthotropic resolution of 9 μm) facilitating the visualization and aspect location of resorption lacunae (arrows).

tooth to the calculated clinical tooth root volume from the CEJ. Both control and treatment teeth had low percentages of resorption based on the entire tooth volume; however, the treatment samples had on average a 52-fold significantly greater resorption of the total tooth structure than the control teeth after 1 year of orthodontic manipulation ($P = .009$) (Figure 2D).

Tooth Root Dentin Demineralization Subjacent to Resorption Lacunae

Focal zones of demineralized dentin were observed subjacent to many (but not all) tooth root resorption lacunae. Demineralized dentin presented consistently as semicircular zones deeper and beneath the resorption lacunae and were measured to be at a lower Hounsfield unit threshold (ie, a lower CT density/attenuation value), which was readily visualized as a lighter shade of grey-scale compared with the darker fully mineralized dentin matrix (Figure 3). A striking observation was that zones of demineralized dentin were only present in orthodontically treated tooth samples. Of the control samples, most of which exhibited a basal level of tooth resorative activity, no subjacent zones of demineralized dentin were evidenced. On the other hand, all treated teeth exhibited demineralized zones of dentin, which varied greatly in radial area from 0.12 to 275 μm² and in relation to the size of the resorption lacunae. It was noted that when present subjacent to larger resorption lacunae, a larger area of demineralization was seen.

DISCUSSION

Previous clinical research has mostly investigated root resorption caused as a result of controlled orthodontic tooth movement.21-23 By using samples obtained from patients undergoing regular orthodontic treatment of 1 year’s duration before tooth extraction, this study provides a powerful insight into how orthodontic treatment contributes to tooth resorption; such insights have not been available from short duration controlled unidirectional force studies.

The results of this study are consistent with the published literature showing that teeth without any forces have fewer resorption lacunae than teeth that have undergone orthodontic treatment. Also consistent is the finding that the mesial and distal surfaces of treat-
Figure 2. All values represent mean ± standard deviation. (A) Total number of resorption lacunae counted by tooth surface in control and treatment teeth; M, mesial; B, buccal; P, palatal; D, distal tooth aspect. (B) Height and depth of resorption craters in control and treatment teeth. (C) Resorption lacunae volume in control and treatment teeth. (D) Percentage of total estimated tooth volume that has been resorbed apical to cementoenamel junction in control and treatment teeth.

Figure 3. Illustration of dentin demineralization subjacent to root resorption lacunae by micro-computed tomography (at an orthotropic resolution of 9 μm); M, mesial; B, buccal; P, palatal; D, distal tooth aspect.

Ed teeth have considerably more resorption lacunae than the other surfaces.²⁴ That is probably due to the possibility that mesial or distal surfaces of the tooth root occupy a greater amount of the surface area than the other surfaces; hence, a greater probability of resorption occurs at these surfaces.

Previous studies that quantified root resorption looked at the volumes of resorption lacunae²⁴,²⁵; however, in this study, in addition to volume of the resorption lacunae, we investigated the size of the resorption lacuna based on height and depth. Our results are consistent with published results²⁴,²⁵ that mean resorption lacuna depth, length, and volume in the treatment teeth were greater than in the control teeth.

To our knowledge, this study is also the first to quantify the extent of tooth resorption in a subset of orthodontically treated teeth for 1 year. Clinically, root resorption is quantified by examining pantographs or periapical radiographs. However, small changes in root resorption
cannot be accurately quantified by those methods. Using the micro-CT, we were able to show that even in clinically normal teeth without macroscopic resorption, microscopic resorption can still be measured and quantified. In teeth that have been orthodontically treated for 1 year, the average amount of resorption was calculated to be 0.9% of the tooth root volume.

An exciting and novel observation of our study was the identification of focal zones of demineralized dentin subjacent to many tooth root resorption lacunae. These zones likely represent ongoing loci of tooth root resorption, with odontoclastic/cementoclastic resorptive activity resulting in focal acid production, and in further dentin demineralization. Furthermore, these zones likely represent the full extent of future tooth root loss. As demineralized zones are not always associated with every resorption lacuna, it is possible that the absence of subjacent demineralization will indicate lacuna that have reached quiescence. Hence, the advent of high-resolution imaging technology that is capable of quantifying such demineralized foci in situ and in vivo may, in the future, be capable of predicting the full extent of subsequent root loss as a consequence of orthodontic manipulation.

CONCLUSIONS
- We have established a protocol to evaluate quantitatively OITRR and have provided a way to predict further resorption based on dentine demineralization.
- The limited field of view, high resolution, low-dose cone beam CT or CT machines could be used to follow this protocol for clinical evaluation of in vivo OITRR.

ACKNOWLEDGMENT
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REFERENCES