

Interrupted orthodontic force results in less root resorption than continuous force in human premolars as measured by microcomputed tomography

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Abstract

Introduction. Root resorption is an undesirable but very frequently occurring sequel of orthodontic treatment. The aim of this study was to compare root resorption caused by either continuous (CF) or interrupted (IF) orthodontic force.

Material and methods. The study was performed on human subjects on 30 first upper and lower premolars scheduled for extraction for orthodontic reasons. During four weeks before extraction 12 teeth were subjected to either CF or IF. The force was generated by a segmental titanium-molybdenum alloy cantilever spring that was activated in buccal direction. Initially a force of 60 CentiNewton was used in both CF and IF groups, the force in the former, however, was reactivated every week for 4 weeks. There was no reactivation of force in the IF group after initial application. A morphometric analysis of root resorption was performed by microcomputed tomography and the extent of tooth movement was measured on stone casts. Furthermore, a Tartarate-Resistant Acidic Phosphatase activity (TRAP), the marker enzyme of osteoclasts and cementoclasts, was determined by histochemical method. The Mann-Whitney U test was used to compare the difference in measured parameters between treatment and control tooth groups.

Results. The number of resorption craters was significantly higher and their average volume almost twice as large in the CF compared to the IF group ($p < 0.05$). However, the distance of tooth displacement was similar for both groups. Cementoclasts were detected with the TRAP technique on the surface of two teeth only; both were subjected to continuous force.

Conclusions. The use of IF leads to less destruction of root structure as opposed to continuous force while the same tooth movement was achieved. (*Folia Histochemica et Cytobiologica* 2014, Vol. 52, No. 4, 289–296)

Key words: human premolars; root resorption; continuous and interrupted orthodontic force; microcomputed tomography; TRAP; cementoclasts; reparative cementum

Introduction

Root resorption is an unwanted and frequently occurring sequel as a result of orthodontic treatment. The

application of orthodontic force causes compression of periodontal ligament (PDL) fibers resulting in the creation of the hyaline zone (necrotic tissue), which is subsequently eliminated by macrophages and multinucleated giant cells. In some patients the resorptive process can lead to shortening of the root. Among the factors that may cause orthodontically-induced inflammatory root resorption, treatment-related factors such as direction, duration and magnitude of the orthodontic force are considered important [1, 2].

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While the result of fixed orthodontic appliances is based on the action of continuous force (CF), the application of interrupted forces (IF) was proposed as a more advantageous alternative [3, 4]. This can be explained that the magnitude of CF remains approximately at the same level, while the magnitude of the IF declines between activations [3]. It has been suggested that the application of IF may result in less detrimental effects on the root surface by providing resting periods that are necessary for the reorganization of the PDL and spontaneous restoration of cementum, *i.e.* healing of resorption lacunae [4–6]. The aim of this study was to test this hypothesis in a clinical setting. For that purpose, the extent of root resorption caused by application of either continuous or interrupted orthodontic force to human premolars scheduled for extraction for orthodontic reason was analyzed by means of computed microtomography. A further aim was to assess the presence of odontoclasts/cementoclasts in resorptive craters to evaluate if the active root resorption is still continuing after 4 weeks.

Material and methods

Patients and their treatment. Thirty maxillary and mandibular first premolars obtained from 12 teenagers (7 boys, 5 girls; mean age 12.7 ± 2.3 years), all of whom required extraction of 2–4 teeth as determined by their orthodontic treatment plan were used for the study. The study was approved by the Ethics Committee of the Medical University of Gdansk (NKEBN/194/2006) and written informed consent was obtained from the parents. Criteria for participation were the following:

1. Good general health.
2. No medicaments prescribed and taken during the experiment.
3. Lack of radiographic signs of idiopathic resorption.
4. No previous dental treatment of the qualified teeth.
5. No previous orthodontic treatment of the qualified teeth.
6. The patient's ability to come in for treatment once a week for 4 weeks.
7. Being diagnosed for orthodontic treatment requiring 2 to 4 premolar extraction(s).

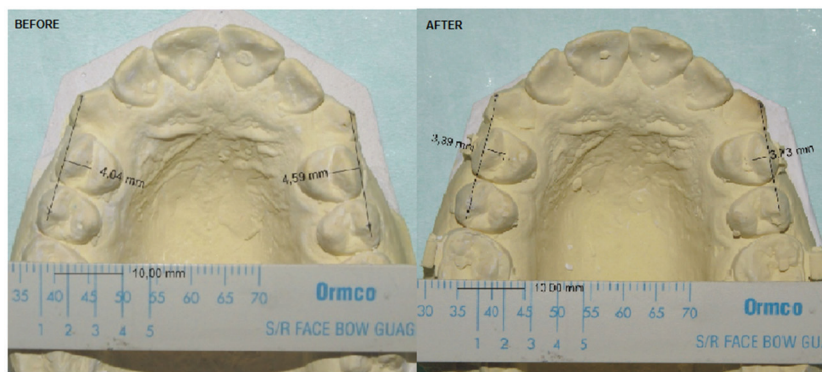
The teeth were randomly divided into 3 groups. Both the CF and IF group consisted of 12 upper and lower first premolars in 12 patients, control group consisted of teeth from 6 patients. Age and gender divisions were not statistically different between groups (CF: 12.3 ± 1.9 ; IF: 12.8 ± 2.3 ; control: 13.2 ± 3.0 years, mean age \pm SD, respectively). A power analysis had determined the minimum number of teeth that would be sufficient for statistical analysis. The study had a split mouth design with the CF application on one side and the IF on the other side of the arch in each

patient. The availability of bicuspid was dictated by the orthodontic treatment plan. Therefore in some patients only two first premolars could be used for the study, in others all four first premolars were available. The control group consisted of 6 first premolars randomly selected amongst the twelve patients, which were not subjected to orthodontics forces. An initial force of 60 CentiNewton was applied to all teeth in both groups at the start of the study. However, in the CF group the force was reactivated every week for 4 weeks, while no reactivation was applied to the teeth of the IF group. One operator treated all patients.

The orthodontic appliance consisted of 0.018-in slot Roth bands (Dentaurum, Ispringen, Germany) on the first molars and 0.018-in slot Roth brackets (Dentaurum) on the first premolars. The right and left first premolars were randomly assigned to the CF and IF groups. An orthodontic force of 60 cN was applied using a 0.017×0.025 -in titanium-molybdenum alloy cantilever spring (TMA, Ormco Inc, Orange CA, USA). The force magnitude was measured to the desired force levels with a strain gauge (Correx, Haag-Streit, Koeniz, Switzerland). Anchorage was secured by a transpalatal bar in the upper arch and a lingual bar in the lower arch. The patients were scheduled for follow-up visits every 7 days for force reactivation in the CF group.

To assess the amount of the tooth movement, dental plaster casts of the maxilla and mandible, obtained before and 4 weeks after buccal tooth movement were used. Using a sharp pencil, reference points on the buccal cusp tips of the adjacent teeth were marked. Another reference point was marked in the middle of the sulcus on the occlusal surface of the first premolar. All plaster casts were photographed twice using a tripod-mounted Sony Alpha 100 camera (Sony Corp., Tokyo, Japan). The images were analyzed with CorelDraw 9 (Corel Corp., Ottawa, Canada) software. All dimensions were calibrated using an Ormco ruler (Ormco Inc.). The distance between the line connecting buccal cusp tips of the adjacent teeth and the reference point (the middle of the sulcus on the occlusal surface of the first premolar) was measured accurate to 0.01 mm (Figure 1). After the experimental period the teeth were extracted exercising extra care to prevent damage to the root cementum.

Analysis of root resorption. A three-dimensional analysis of the morphology of the roots was made and volumetric measurements of root resorption craters were assessed using X-ray microtomography and a desktop X-ray microfocus CT scanner (SkyScan 1072, SkyScan, Aartselaar, Belgium) [7, 8]. The scanning procedure was completed at 10 watts, 100 kilovolts, 98 microamperes, using a 1.0-mm aluminum filter with $\times 15$ magnification resulting in a pixel size of a $19.1 \mu\text{m} \times 19.1 \mu\text{m}$. More than four hundred 2-D projections were secured during the acquisition for each tooth. All data were then transformed into new 2-D image pro-



Group	Number of teeth	Tooth movement on stone casts (mm)			
		mean	SD	min	max
I continuous force	12	1.542	0.505	0.775	2.300
II interrupted force	12	1.541	0.465	0.590	2.090

Figure 1. Measurements of the buccal movement of premolars in relation to the reference points before and after 4 weeks of force application. Bottom table presents buccal displacement of the premolars

jections of axial cross-sections of 1024 × 1024-pixel format and slice thickness of 13 μm. Images in 3-D format were achieved by the juxtaposition of 2-D images of adjacent slices. The morphology of root surfaces was assessed using TView software (SkyScan bvba, Aartselaar, Belgium), which allowed for the observation of all two-dimensional (2D) axial slices and identification of root resorption craters. The length of craters was measured with an accuracy of 1 μm. The Computer Tomography Analyzer (CTAn) software (SkyScan) was used for the determination of the volume of the craters. Microtomography and TView software allowed for accurate location of resorption craters along the root surface, generally very similar to the method described in detail by Ballard et al. [1] and in our pilot study [8]. Each root resorption crater was identified, the position recorded and its coordinates noted. All resorption lacunas were assessed in relation to their position on the root (apical, middle and cervical thirds) and their relationship was established to the root’s surface (buccal, lingual, distal or mesial). The length of the root was determined as the distance between the initial vertical axial slice (cemento-enamel junction) and final vertical axial slice (apex) with a precision of 1 μm. The magnitude of the resorption craters on the root surface was determined by analyzing adjacent scans with an accuracy of 1 μm as shown in Figure 2.

Assessment of TRAP activity. After the experimental period the teeth were carefully extracted to prevent iatrogenic damage to the root cementum. Subsequently, each tooth was immediately immersed (to facilitate rapid and immediate penetration of the fixation solution) in 10% neutral buffered formalin for at least one month. The specimens were

then demineralized in the acid/EDTA solution for 2 weeks (Dilute HCl and Disodium ethylenediaminetetraacetic acid, Cal-Ex, Fischer Scientific, Hampton, NH, USA), and afterwards routinely processed and embedded in paraffin. Serial sections (7 μm thick) of the roots were cut in a bucco-lingual direction perpendicular to the long axis of the tooth. Tartarate-Resistant Acidic Phosphatase activity (TRAP), the marker enzyme of osteoclasts and cementoclasts, was detected by a histochemical method [9] on histological sections of 4 teeth subjected to CF, 4 teeth subjected to IF and 2 control teeth. At the location of resorption lacunas (identified on the basis of microCT analysis) in every 10th section, assessment of TRAP activity was made using Acid Phosphatase, Leukocyte (TRAP) Kit (Cat. No 386A-1KT, Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer’s protocol.

Statistical analysis. Statistica 8 software (StatSoft, Tulsa, OK, USA) was used for statistical analysis and GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA, USA) was used for the generation of plots. Shapiro-Wilk W test was used to check the normality of distribution of values in groups; for groups which did not pass this test, Mann-Whitney U test was used to compare the differences in measured parameters (number and volume of root resorption craters of teeth subjected to various orthodontic forces relative to the crater position) between treatment and control tooth groups. Fisher’s 2 × 2 exact test was used for comparison of number of craters between groups. Pearson’s chi-squared test (χ²) was applied to compare the number of root resorption craters between groups. The level of statistical significance was set at p < 0.05.

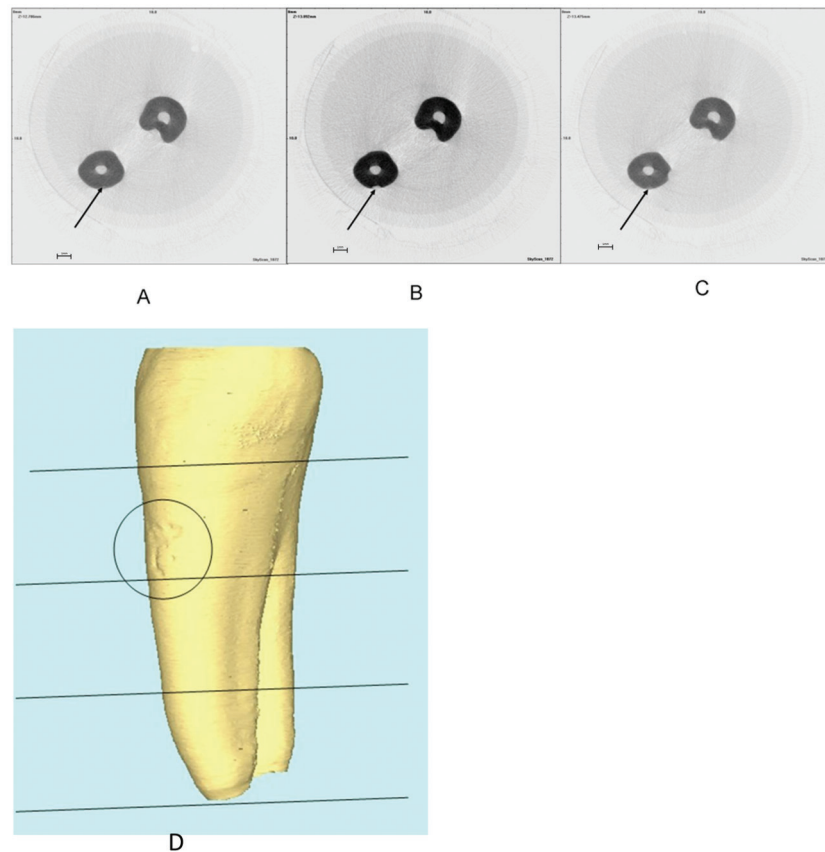


Figure 2. Cross-sectional and longitudinal views of microscans of the buccal root of a tooth that was exposed to continuous force. The cross sections visualize the beginning (A), middle part (B) and apical border (C) of a root resorption crater (arrows); D. At the micro CT scan the horizontal lines denote the division of the three root areas into cervical, middle and apical ones. The resorption crater (circle) is present on the cervical buccal surface

Results

The measurements made on stone casts showed that the mean effective buccal tooth displacement was very similar for the continuous (1.542 mm) and the interrupted (1.492 mm) force groups (Figure 1).

Table 1 represents the analysis of 13383 axial slices of 41 root surfaces in 30 first premolars which revealed 99 resorption craters (each with a volume $> 0.01 \text{ mm}^3$) present on 2860 axial slices (*i.e.* 21.4% of all 'microcomputed' slices). The resorption craters were detected in 12/12 teeth in the CF group and 11/12 in the IF group ($p = 0.54$, Fisher's test). For comparison, only two root resorption craters were found in 2/6 teeth of the control group ($p = 0.2$ for control *vs.* CT and $p = 0.24$ for control *vs.* IF, Fisher's test). The number and total volume of craters in the CF *vs.* the IF group were higher by 43% and 89%, respectively ($p < 0.05$; Table 1 and Figure 3).

The mean total volume of root resorption craters per tooth was significantly higher for the CF group

Table 1. Number of resorption craters and their total volume in all studied teeth

Group	Number of teeth	Number of resorption craters	Total volume of resorption craters [mm^3]
I, continuous force	12	57	5.225
II, interrupted force	12	40*	2.768
III, control	6	2	0.047

*significantly different from group I, test χ^2 ; $\chi^2 [1] = 4.341$, $p < 0.037$

than the IF group (Table 2). This can also be seen in Figure 3. When the specific surfaces of the roots were analyzed based on data collected from all groups, the mean crater volume on the buccal surface was significantly higher compared to all other surfaces combined (Figure 4), particularly in the cervical third of the roots (Figure 1D).

The histochemical detection of TRAP activity, the marker enzyme of osteoclasts, revealed the presence

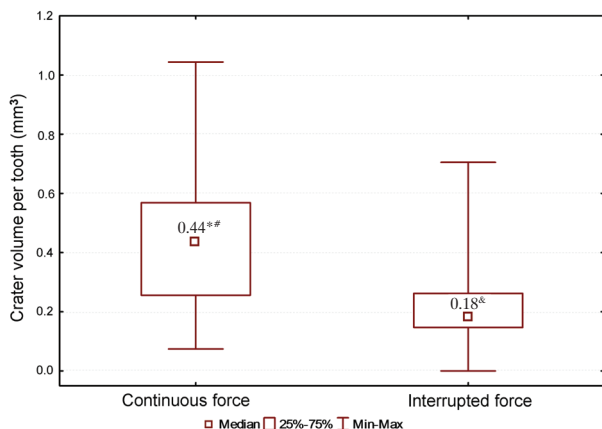


Figure 3. Boxplot comparison of a median crater volume per tooth in the continuous force and interrupted force groups. *Significantly different in relation to group II (IF), test U, $U = 38.5, p < 0.05$; #significantly different in relation to group III (control teeth), test U, $U = 0.0, p < 0.001$; &significantly different in relation to group III, test U, $U = 4.0, p = 0.003$. For technical reasons very low value of median volume of craters in the group III ($Q50 = 0.024$) has not been shown

of cementoclasts on the surface of only two of ten examined teeth, which had been subjected to continuous force (Figure 5). Moreover, in about half of the resorption craters of all teeth reparative cementum was observed.

Discussion

The results indicated that the application of continuous force induced volumetrically larger root resorption craters than interrupted force, while tooth movement was similar for both groups. Therefore, the hypothesis stated in the Introduction was accepted. Almost 50 years ago Reitan [10] reported that IF resulted in less root resorption, yet little proof has been presented substantiating his observation [8]. Introduction of a pause in force application during tooth movement is believed to allow the resorbed cementum to heal [1, 2, 4, 6, 11, 12]. This resting period may be obtained ei-

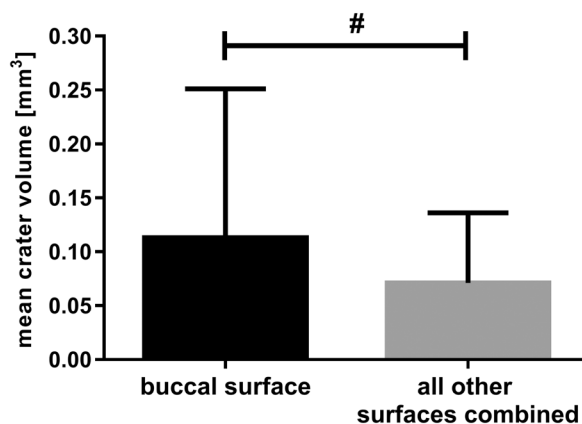


Figure 4. Mean crater volume on the buccal surface compared to all other surfaces combined. Figure is based on data from all three groups (CF + IF + Control). #Significantly different in relation to the other surfaces, test U, $U = 694, p = 0.047$

ther by removing the appliance for a few hours every day (intermittent force) or by decreasing the force to a certain level (interrupted force). Although the daily period of force application and the duration of studies were different, all the authors have suggested that intermittent or interrupted forces resulted in less resorption [1, 2, 4, 6, 11, 13]. However, Owman-Moll et al. [14] found no difference in root resorption in 32 maxillary premolars of 16 patients that were buccally moved with either CF or IF force (60 cN). The discrepancy between our results and theirs may be due to the accuracy of measurements. Owman-Moll et al. used light microscopy [14], whereas our observations were based on the very precise method of micro-computed tomography. It is of interest to note that tooth displacement for both groups was the same and similar to that reported by Owman-Moll et al. [14], which suggests a comparable efficacy of CF and IF. Moreover, Roberts stated that the mean tooth displacement in young patients in buccal tipping is approximately $100 \mu\text{m}/\text{day}$, amounting to $2 \text{ mm}/\text{month}$ [15]. In translation movement this amounts to

Table 2. Crater volume per tooth in each group

Group	Number of teeth	Total crater volume per tooth			
		Mean \pm SD	Median value	Min	Max
I, continuous force	12	0.435 ± 0.260	0.443*#	0.074	1.044
II, interrupted force	12	0.231 ± 0.174	0.182&	0.0	0.705
III, control	6	0.008 ± 0.014	0.024	0.0	0.034

*significantly different in relation to group II, test U; $U = 38.5; p < 0.05$; #significantly different in relation to group III, test U; $U = 0.0; p < 0.001$; &significantly different in relation to group III, test U; $U = 4.0; p < 0.003$

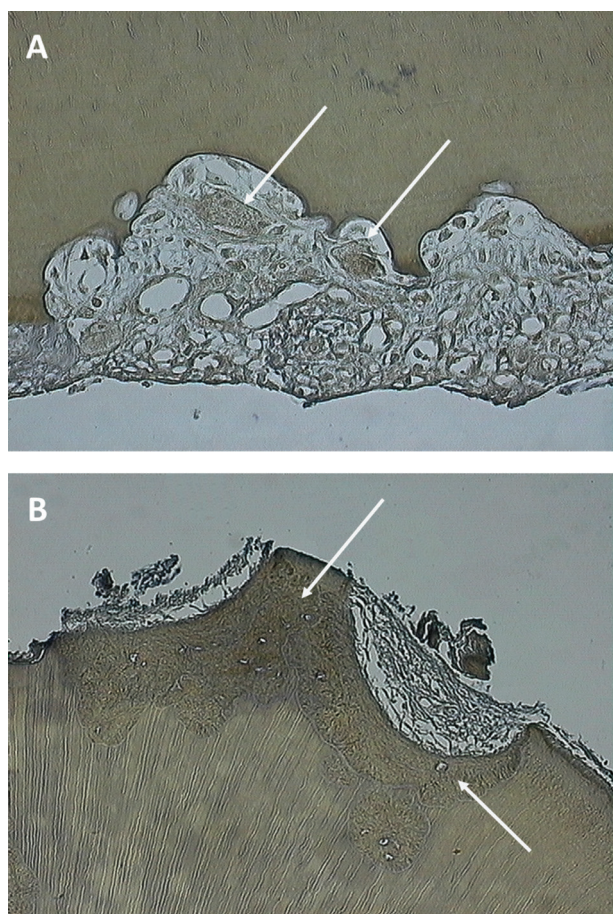


Figure 5. Morphology of resorptive craters. **A.** Histochemical detection of TRAP activity in 2 cementoclasts (arrows) in resorptive lacunas found in a tooth of CF group, magnification $\times 40$. **B.** Repair of the resorptive craters with cementum (arrows) found in a tooth of the IF group, magnification $\times 20$

0.7 mm/month. In our study the combined translation and buccal tipping obtained a mean displacement of 1.5 mm per month, which is comparable to Roberts' studies [15].

Almost no osteoclastic activity was observed in either experimental group, yet after four weeks reparative cementum was present in resorption craters in both the IF and CF groups. The much shorter duration of the cementoclastic/odontoclastic activity when compared with the tissue-forming function of osteoblasts/cementoblasts/odontoblasts can be offered as an explanation for this observation [5]. Gonzales et al. [13] in an animal model observed formation of reparative cementum after 4 weeks of continuous force and a gradual increase in the volume of reparative cementum until the 16th week after cessation of the force. The appearance of cellular cementum in the resorption craters is considered to

be a hallmark of a healing process since reparative cementum is more resistant to root resorption [13]. Root resorption as a result of orthodontic treatment consists of a series of repetitive resorption and healing processes throughout the treatment period. The presence of resorption craters with reparative cementum may be indicative of some protective mechanism against severe resorption during the next reactivations [16]. However, the potential for reparative cementum in resorptive lacunas related to orthodontic force application needs further investigation. Moreover, in about half of the resorption craters reparative cementum was observed. The histochemical detection of TRAP activity, the marker enzyme of osteoclasts, identified the presence of only a few cementoclasts on two teeth. Both had been subjected to continuous force. Nevertheless, this method appeared suitable for the detection of osteoclast.

Our sample was rather small, yet we did observe some repair signs after 4 weeks of force application, which seems to validate the conclusion that the formation of secondary cementum must have started before that period. We also propose that the repair process starts earlier when interrupted force is used. To test this hypothesis conclusively, however, a new investigation is needed. Moreover, longitudinal studies are needed to show whether resorption craters can be partially or fully repaired depending on the type of applied orthodontic force.

By definition, physiologic resorption concerns the roots of primary teeth only, whereas root resorption of permanent teeth is considered to be pathologic [17]. However, some authors have suggested that physiologic root resorption and repair can occur as a part of a tooth drift and changes in bone metabolism [16, 18]. The data from this study support this hypothesis as resorption craters in the control teeth were observed, albeit the number and volume of these craters were significantly smaller than those observed by other authors [1, 19, 20]. Roberts proposed that root resorption followed by cementum repair can be considered a form of root remodeling akin to bone turnover [16].

Root resorption has usually been measured using two-dimensional radiography techniques [21, 22], light microscopy [10, 14] and scanning electron microscopy [11, 23]. While radiographs are still a good diagnostic tool for clinical assessment of root resorption, for research purposes more precise quantitative measurements can be obtained with the use of 3D techniques such as stereo-imaging with SEM images or confocal laser scanning microscopy and X-ray microtomography [6, 7, 24]. Our study

provides quite accurate data on the number, volume and location of resorption craters on roots by means of microcomputed tomography.

Our data seem to validate the opinion that root resorption occurs as a result of compression of the periodontal ligament [1, 24, 25], since we found the largest average volume of resorption craters on the buccal surface in the cervical area of roots, as a result of buccal tooth movement. However, the resorption craters were also observed on the mesial and distal root surfaces, which suggest that compression of the periodontal ligament fibers on those surfaces also occurs during buccal tooth movement. Furthermore, von Bohl et al. [26] noticed that focal hyalinizations were found not only on the pressure side of the displaced tooth but also laterally to it. Our results seem to collaborate those of Ballard et al. [1], who also used microtomography to assess the extent of root resorption on upper bicuspid after application of continuous or intermittent force for 8 weeks. They reported less root resorption in the intermittent force group, the bucco-cervical region being the most severely affected. The forces they used (225 cN) were considerably greater than 60 cN in our study. Furthermore, no data was gathered to determine the efficacy of either system on the extent of tooth movement. In addition, in the study reported here a control group was added, which received orthodontic brackets but no force application.

In a recently published microtomographic study Aras et al. [2] concluded that intermittent force caused less root resorption than continuous force. The design of the orthodontic appliance in their study was the same as in our study, except the springs on the continuous force side were calibrated every 14th or 21th day. The initial force was 150 g on both sides, while the appliance on the intermittent side was removed for 3 days before day 14 or 21. In our study, weekly reactivations ensured true continuous force, although not at a constant level, the average force reduction after one week being 17%. Infrequent reactivation (every 2 or 3 weeks) may result in a greater declining force and a lack of continuity. A light continuous force still seems to be the state of the art in orthodontic mechano-therapy and accordingly our research was focused on light forces (60 g). Aras et al. [2] reported that intermittent force causes less tooth movement than continuous, which was not corroborated by our data. As the measurement method of tooth movement in Aras' study was similar, the difference in results may have been caused by removing the appliance for 3 days. Lastly, Gibon et al. [4] also reported no difference in tooth movement between interrupted and continuous forces, which is consistent with our

findings. In future studies it is recommended to increase the sample size and observe the repair at earlier and later time periods.

Conclusions

Human bicuspid subjected to orthodontic forces and retrieved after 4 weeks and subsequently analyzed by means of microcomputed tomography demonstrated that an interrupted force resulted in significantly lower root resorption compared to continuous force application. Both techniques were similarly effective and accomplished the same amount of tooth movement.

Acknowledgment

The studies presented in this report were partially funded by research grant to M.S. from the Polish Ministry of Science and Higher Education (grant no. 2211/P01/2007/32).

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Submitted: 5 December, 2014

Accepted after reviews: 18 December, 2014

Available as AoP: 19 December, 2014