Validation of two-dimensional measurements of root resorption craters on human premolars after 28 days of force application

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SUMMARY The aims of this study were to develop a three-dimensional (3D) mathematical model of a typical root resorption crater and to correlate two-dimensional (2D) surface area measurements to 3D volumetric measurements of root resorption craters created under light and heavy orthodontic forces. Data were obtained from a previous study of 36 first premolars from 16 subjects requiring extraction of these teeth as part of their orthodontic treatment. Buccal tipping forces of 25 or 225 g were applied for an experimental period of 28 days. After extraction, the samples were prepared for scanning electron microscopy (SEM) imaging, image processing and analysis. Surface area (2D) and volumetric (3D) measurements of all craters were obtained. A mathematical analysis of the 2D/3D relationship enabled the determination of an appropriate digital model for the shape, type and dimensions of resorption craters, which was also able to distinguish between a 'hemispheric' model versus a 'layered' model of craters.

The results demonstrated that 2D and 3D measurements were strongly correlated ($r = 0.991^{**}$). Within the light and heavy force groups, the measurements were also strongly correlated ($r = 0.978^{**}$ and $r = 0.994^{**}$, respectively). For a 28 day experimental period, 2D measurements of root resorption craters were found to be as reliable as 3D measurements.

Introduction

Root resorption has been used as a universal term that describes a pathological process for which no single aetiological component is engaged in the expression (Brezniak and Wasserstein, 2002). It has been defined as the active removal of mineralized cementum and dentine (Brudvik and Rygh, 1994). The study of this phenomenon over the years has concluded that root resorption could be an idiopathic and unpredictable adverse effect of orthodontic treatment. The detection of root resorption has been mainly through radiographs (Ketcham, 1929), light microscopy (Reitan, 1974) and scanning electron microscopy (SEM) (Jones and Boyde, 1972; Kvam, 1972a, b). Although the importance of identifying high-risk patients and their various ways of management has been highlighted, quantitative evaluation of root resorption is still relatively poor. There remain several flaws in two-dimensional (2D) surface measurements for this three-dimensional (3D) phenomenon.

It has been previously reported that most external root resorption could be self-limiting. For this reason, approximately 70 per cent of all defects seen in mature teeth are anatomically repaired (Henry and Weinmann, 1951; Andreasen, 1988). However, the mechanism behind this self-limiting phenomenon has not been fully explored.

The aims of this study were to develop a 3D mathematical model of a typical root resorption crater created under both light and heavy orthodontic forces and to correlate accurate 2D surface area measurements to 3D volumetric measurements of root resorption craters.

Materials and method

A sample of 36 teeth from 16 patients (10 males, six females, mean age 13.9 years, range 11.7–16.1 years) requiring at least bilateral maxillary or mandibular first premolar extraction for orthodontic treatment was collected. Ethical approval and informed consent from all patients were obtained. They also complied with a strict selection criterion excluding any local or systemic conditions that may predispose to root resorption (Srivicharnkul *et al.*, 2005). The clinical set-up for the application of forces has been previously described (Srivicharnkul *et al.*, 2005).

The patients were allocated equally into a light or heavy force group with a buccal tipping force of 25 or 225 g applied to the first premolars using a cantilever spring from the ipsilateral first molar. Within the same patients, the contralateral sides served as the control (0 g). After an experimental period of 28 days, the premolars were extracted, disinfected and cleaned (Malek et al., 2001). The samples were then bench dried and carbon coated for SEM imaging with a XL30 SEM (Phillips, Eindhoven, The Netherlands). A motorized jig (Figure 1) was designed so that all surfaces of the root could be studied without constant pumping and venting of the vacuum chamber. Using stereo imaging, all craters detected on the buccal and lingual surfaces of the samples were digitally obtained and stored as TIFF images. The imaging surface of the root was positioned to line up parallel to the horizontal during imaging to minimize parallax errors. A further shading correction (Chan et al., 2004) also eliminated the innate



Figure 1 The 360 degree motorized rotating jig that was installed in the scanning electron microscope (SEM) chamber enabled the viewing and imaging of all surfaces of the root in the SEM chamber without time-consuming and constant pumping and venting.

curvature present, which could induce errors in quantitative measurements. Surface area and volumetric recordings of these craters were obtained using a modification of a commercial computer software (Analysis Pro 3.1 Soft Image System, SIS, Münster, Germany). For surface area (2D) measurements, absolute straight-on images were captured, whereas for volumetric (3D) measurements, stereo pairs of images at 6 degree angulations were taken. This methodology has been described previously (Chan *et al.*, 2004). The analysis software was previously calibrated with a Vickers hardness tester using four different metallic rods and has proven its accuracy and reproducibility (Chan *et al.*, 2005). The profiles for surface area and volumetric measurements were compared and correlated. A digital model of a typical crater was mathematically derived.

Statistical analysis

Univariate analysis of variance (one-way ANOVA) was performed using the Statistical Package for Social Sciences (SPSS for Windows, version 11, SPSS Inc, Chicago, Illinois, USA). During statistical evaluation, the raw data were transformed for the residual plots to conform to normality. The square root of the surface area (srtarea) and cube root of the volumetric readings (crtvol) were used to create the full model for statistical analysis. This allowed correlation of the quantitative data between 2D and 3D measurements.

Results

Comparing the area and volumetric measurements

The profiles of the surface area (2D) measurements demonstrated very similar results to the volumetric (3D) measurements (Table 1). The 2D measurements were strongly correlated to the 3D measurements (r = 0.991**) (Table 2). Within the light and heavy force groups, the

measurements were also strongly correlated ($r = 0.978^{**}$ and $r = 0.994^{**}$, respectively) (Table 3).

Digital model of a resorption crater

The values obtained for the area and volumetric measurements were analysed mathematically to test for either a layered or hemispheric model. If a layered model of craters is appropriate, then a graph of volume against area should be linear. On the other hand, if a hemispheric model is appropriate, then a graph of volume against area should show a curvature, while a graph of cube root volume against square root area should be linear. The appropriate graphs are shown in Figure 2. Figure 2a does in fact show a curved relationship between volume and area (even more pronounced if the 'outlier', the largest crater, is ignored), whereas Figure 2b shows a relationship that is essentially linear. These observations are supported by the correlations obtained between the sets of measurements: the correlation between volume and area was 0.97 (0.96 with the outlier removed), whereas the correlation between cube root volume and square root area was 0.99. These results indicate that the craters tend to conform to a hemispheric model rather than a layered model, although the very largest craters may not conform. This demonstrates that typical craters tend to be hemispheric models (area less than $2 \times 10^6 \,\mu\text{m}^2$ and volume less than $300 \times 10^6 \,\mu\text{m}^3$) (Figure 3a), whereas very large craters tend to be layered (Figure 3b).

Discussion

Previous studies investigating the quantitative value of root resorption utilized 2D measurements. A series of studies by Owman-Moll (1995) and Owman-Moll et al. (1995, 1996a, b) performed on 144 adolescents and 200 premolar teeth reported that tooth movements and severity of root resorption were not significantly affected by doubling the force magnitude from 50 to 100 g. It was also noted that the amount of root resorption was greater after 7 weeks with a force magnitude of 50 g compared with 100 g. Although the authors could not explain this phenomenon, their results were in agreement with Stenvik and Mjör (1970), who reported that an increased force caused a decrease in the frequency of root resorption when premolars were intruded. They observed that root resorption increased after the application of light forces of 35 g when compared with heavy forces of 250 g. They all contributed this phenomenon to other idiopathic individual variations, e.g. the metabolic responses of the subjects. They then concluded that root resorption does not seem to be very force sensitive. Despite these observations, they still cautioned that their results should not mislead clinicians into using heavy forces.

However, in a recent study measuring the volume of resorption craters (Chan and Darendeliler, 2005) it was demonstrated that the mean volume of resorption in the light force group was 3.49-fold more than in the control

		п	Mean		Standard deviation
(a) Descriptive statistics					
Volume total					
0 control 1 light 2 heavy		108 54 54	2.796 9.781 32.428		21.081 33.098 84.085
Total		216	11.950		48.813
Surface area					
0 control 1 light 2 heavy Total		108 54 54 216		0.025 0.090 0.270 0.102	
Cube root volume					
0 control 1 light 2 heavy Total	108 54 54 216		0.174 0.591 1.220 0.540		0.768 1.394 2.124 1.438
Square root area					
0 control 1 light 2 heavy Total		108 54 54 216	0.033 0.113 0.258 0.109		0.155 0.280 0.455 0.301
	Sum of squares	df	Mean square	F	Significance
(b) ANOVA					
Volume total					
Between groups Within groups Total	31949.468 480334.235 512283.703	2 213 215	15974.734 2255.090	7.084	0.001
Surface area					
Between groups Within groups Total	2.171 26.216 28.388	2 213 215	1.086 0.123	8.821	0.000
Cube root volume					
Between groups Within groups Total	39.546 405.053 444.600	2 213 215	19.773 1.902	10.398	0.000
Between groups Within groups Total	1.812 17.730 19.542	2 213 215	0.906 0.083	10.884	0.000

 Table 1
 Descriptive statistics (a) and one-way ANOVA (b) for raw and transformed volume and area measurements by force group.

group, and in the heavy force group was 11.59-fold more than in the control group. The heavy force group had a 3.31fold more total resorption volume than the light force group. The buccal cervical and lingual apical regions demonstrated significantly more resorption craters than the other regions. Although there was more resorption recorded in the light group, the difference in the amount of resorption between the light and control groups was not significantly different.

The method of quantitative analysis of craters described in earlier studies may not be sufficiently adequate to truly reflect the amount of resorption that occurred. Serial sectioning and selective sampling of the resorbed area may have caused resorption regions to be missed. Parallax error was not taken into consideration and could have markedly distorted the data. The selection criteria for the premolars were not strict and external factors that may predispose to root resorption did not seem to be excluded. Ligature wires used to engage the wires into the bracket could have distorted the force system by introducing undesired moments (Chan and Darendeliler, 2004).

On the other hand, Reitan (1964, 1974, 1985) has always advocated the use of light orthodontic forces during treatment in order to increase cellular activity in the surrounding tissues and to reduce the risk of root resorption. This was later confirmed by King and Fischlschweiger (1982). They found in an investigation in rats that light forces produced

	Volume total	Surface area	Cube root volume	Square root area
Volume total				
Pearson correlation	1.000	0.971**	0.868**	0.879**
Significance (two-tailed)		0.000	0.000	0.000
n	216	216	216	216
Surface area				
Pearson correlation	0.971**	1.000	0.916**	0.945**
Significance (two-tailed)	0.000		0.000	0.000
n	216	216	216	216
Cube root volume				
Pearson correlation	0.868**	0.916**	1.000	0.991**
Significance (two-tailed)	0.000	0.000		0.000
n	216	216	216	216
Square root area				
Pearson correlation	0.879**	0.945**	0.991**	1.000
Significance (two-tailed)	0.000	0.000	0.000	
n	216	216	216	216

Table 2	Correlations	between raw	and transformed	volume and	area measurements.
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**Correlation is significant at the 0.01 level (two-tailed).

 Table 3
 Correlations between raw and transformed volume and area measurements in (a) the light force and (b) the heavy force groups.

	Volume total	Surface area	Cube root volume	Square root area
(a) Light force group				
Volume total				
Pearson correlation	1.000	0.947**	0.889**	0.902**
Significance (two-tailed)		0.000	0.000	0.000
n	54	54	54	54
Surface area				
Pearson correlation	0.947**	1.000	0.874**	0.941**
Significance (two-tailed)	0.000		0.000	0.000
n	54	54	54	54
Cube root volume				
Pearson correlation	0.889**	0.874**	1.000	0.978**
Significance (two-tailed)	0.000	0.000		0.000
n	54	54	54	54
Square root area				
Pearson correlation	0.902**	0.941**	0.978**	1.000
Significance (two-tailed)	0.000	0.000	0.000	
n	54	54	54	54
	Volume total	Surface area	Cube root volume	Square root area
(b) Heavy force group				
Volume total				
Pearson correlation	1 000	0 974**	0.878**	0.880**
Significance (two-tailed)	1.000	0.000	0.000	0.000
n	54	54	54	54
Surface area				
Pearson correlation	0.974**	1.000	0.933**	0.949**
Significance (two-tailed)	0.000		0.000	0.000
n	54	54	54	54
Cube root volume				
Pearson correlation	0.878**	0.933**	1.000	0.994**
Significance (two-tailed)	0.000	0.000		0.000
n	54	54	54	54
Square root area				
Pearson correlation	0.880**	0.949**	0.994**	1.000
Significance (two-tailed)	0.000	0.000	0.000	

**Correlation is significant at the 0.01 level (two-tailed).



Figure 2 Graphs of (a) volume plotted against area for control, light and heavy groups testing for a layered model and (b) cube root volume plotted against square root area for control, light and heavy groups testing for a hemispheric model.

insignificant root resorption, whereas intermediate or heavy forces resulted in substantial crater formation. This result was in agreement with earlier findings, both in animals (Dellinger, 1967; Kvam, 1972a) and in humans (Harry and Sims, 1982), as well as the findings of the present study.

However, it should be borne in mind that in the present study, the force was only applied for a period of 28 days. A longer period of force application may result in the formation of deeper craters. In that case, 2D measurements may not be sufficiently accurate to evaluate the extent of the resorption. This present investigation correlated accurate 2D versus 3D measurements of root resorption. With the elimination of parallax errors, missed or distorted craters and other



Figure 3 (a) A digital hemispheric model. (b) A digital layered model.

confounding factors, it has been demonstrated that if 2D measurements are conducted adequately, they could be as reliable as 3D measurements in a 28 day force application study. The mathematical model derived for resorption craters also allows understanding of the morphology of resorption craters of differing sizes.

The present research has attempted to shed some light on the effect of 2D versus 3D measurements and force magnitude on root resorption. After a 28 day experimental period, the typical root resorption crater conformed more to a hemispheric model, whereas if the craters were larger they conformed more to a layered model. Does the thickness of cementum and dentine at various levels of the root play a role in self-limiting the depth of these craters? Is there an inner dentine layer that is 'hard' enough to resist resorption? Is the mechanism behind root resorption different between a light and heavy force group? What happens after 28 days? These questions remain to be addressed in future studies and investigations.

Conclusions

In this study, when light (25 g) and heavy (225 g) forces were applied for 28 days, 2D measurements of root resorption craters were as reliable as 3D measurements. A typical crater (area less than $2 \times 10^6 \,\mu\text{m}^2$ and volume less

than $300 \times 10^6 \ \mu\text{m}^3$) in this study conformed more to a hemispheric model; larger craters tended to be layered.

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