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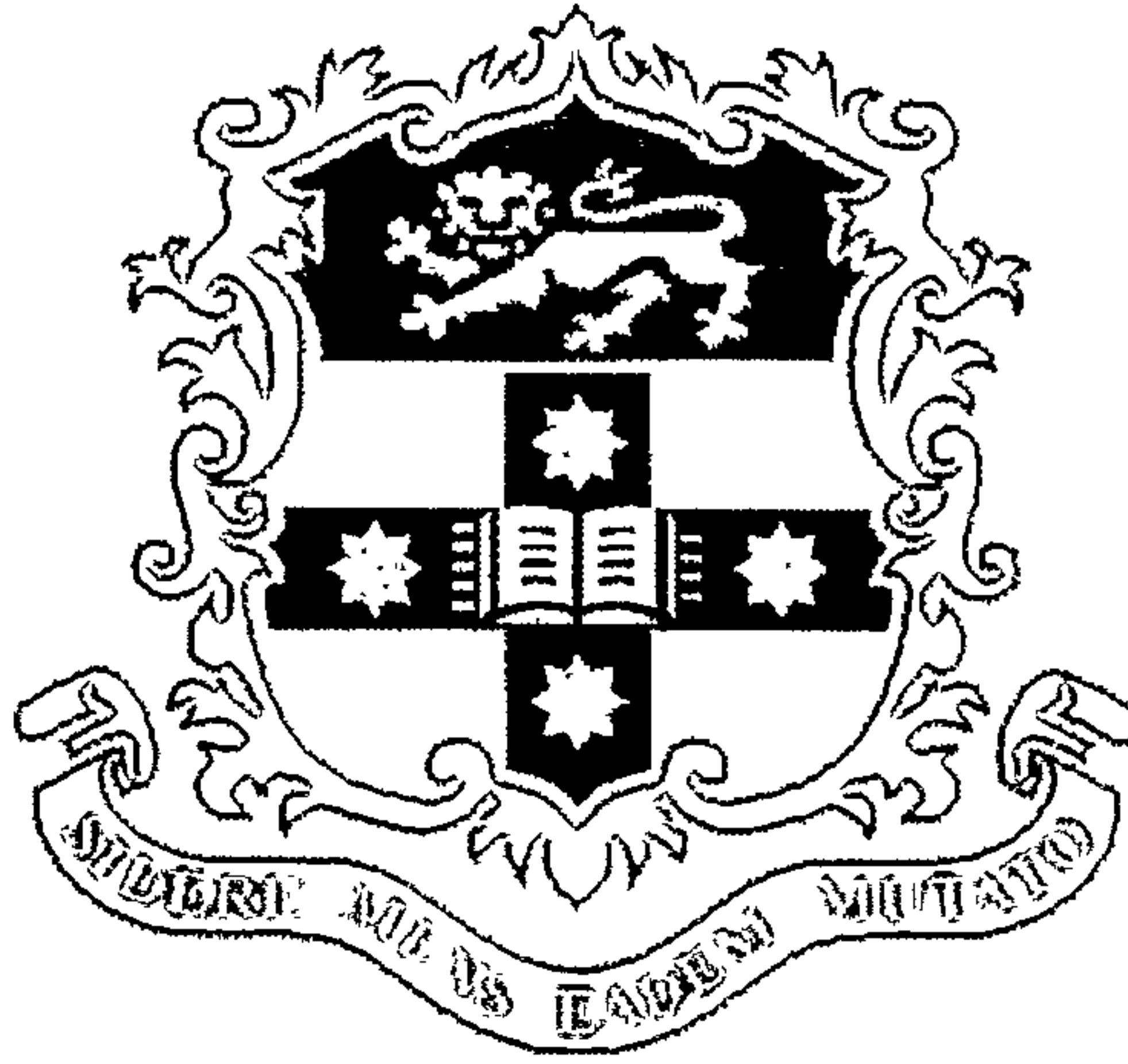
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8. Manuscript I



REPAIR OF ROOT RESORPTION FOUR AND EIGHT WEEKS FOLLOWING THE APPLICATION OF CONTINUOUS LIGHT AND HEAVY FORCES FOR FOUR WEEKS: A MICRO-CT STUDY

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8.1 Abstract

INTRODUCTION: Orthodontically induced inflammatory root resorption is a pathologic process related to local injury of the periodontal ligament which occurs in association with the removal of hyalinised tissue. Studies have shown that a reparative process in the periodontium commenced when the applied orthodontic force was discontinued or reduced below a certain level. The aim of this study was to evaluate the amount of cementum repair by quantitative assessment of the volumetric changes of the root resorption craters after four and eight weeks retention periods following four weeks of continuous light and heavy orthodontic force applications. The impact of age, amount of tooth movement and fluoride exposure on the extent of root resorption was also investigated.

METHODS: Forty patients who required bilateral extractions of maxillary first premolars as part of their orthodontic treatment were recruited in this study. They were divided into four groups (Groups IL, IIL, IH, and IIH), each group consisted of 10 subjects. The maxillary first left and right premolars of all subjects were loaded with either light (25 grams) (Groups IL and IIL) or heavy (225 grams) (Groups IH and IIH) orthodontic force. After four weeks of orthodontic force loading, the upper left first premolars were extracted and served as the positive control group whilst the upper right first premolars were placed in retention for a further four or eight weeks before extraction and served as the experimental group. The extracted teeth from all of the groups were investigated by micro-computed tomography (micro-CT). Cementum repair was assessed by measuring the volumetric changes of resorption craters using specially designed computer software. Amount of tooth movement was also measured on study casts that were taken prior to the experiment and following tooth extractions.

RESULTS: (1) Root resorption continued for another four weeks after orthodontic force had ceased. The resorptive activity was more pronounced following the application of heavy forces. (2) Passive retention following four weeks of light orthodontic force application had the least root resorption crater volume (cube root scale). (3) The total amount of the cementum repaired was not dependent on the magnitude of orthodontic force nor on the retention time with the parameters set in this study ($p>0.05$). This may indicate a concurrence of resorption and repair during passive retention. (4) The majority of the reparative process seemed to have occurred after four weeks of passive retention following the application of four weeks of heavy orthodontic forces. (5) The volume of root resorption craters was positively dependent on the amount of tooth movement ($p=0.02$) and negatively correlated with the chronological age ($p<0.01$).

CONCLUSION: Reparative processes occur after both light and heavy orthodontic force applications but the temporal patterns of repair differ for different force applications.

KEYWORD: Orthodontic force, retention, root resorption, repair, micro-CT

8.2 Introduction

Root resorption is described as an inevitable side-effect of orthodontic tooth movement. However, there exists a reparative process in the periodontium when the applied orthodontic force is discontinued or reduced below a certain level.^{1,2} This healing process seems to increase over time and can occur as early as the first week of retention following orthodontic treatment.³⁻⁵

Orthodontically induced inflammatory root resorption can be influenced by a wide range of biological and or mechanical factors.^{6,7} Biological factors are not within the control of the clinician and these include age, gender, rate of metabolism and abnormal tooth and root morphology.^{7,8} Mechanical factors, such as duration of the treatment, amount of tooth movement, magnitude of the applied force, are attributed to the nature of the orthodontic appliance and could be controlled by both the clinician and patient.^{6,9} The magnitude of orthodontic force has been shown to have an influence on the severity of root resorption.^{10,11} Reitan^{12,13} advocated the use of light orthodontic force in order to reduce the risk of root resorption. King and Fischlschweiger¹⁴ found minimal root resorption with light force whereas intermediate or heavy force resulted in substantial resorption crater formation in rats.

Extensive qualitative studies have been carried out to investigate root resorption and repair using light microscopy, scanning electron microscopy and transmission electron microscopy.^{3,15-18} Quantitative analysis of root resorption could be accomplished by radiographs but these two dimensional (2D) studies were limited to measuring only the amount of root apex loss and were highly inaccurate because of the magnification error and low reproducibility.¹⁹ Histological studies were

technique sensitive and quantitative measurement of root resorption was not reliable as loss of material occurs during histological sectioning. With the advances of computed tomography, a three dimensional (3D) reconstruction of small specimens such as the root resorption crater with high spatial resolution could be created using micro-computed tomography (micro-CT). The associated computer software enabled quantification of volumetric changes of root resorption craters with a high degree of accuracy.

The aim of the present investigation was to quantitatively assess the volumetric changes of the root resorption craters after four or eight weeks of retention following four weeks of continuous light or heavy orthodontic force application. This may provide a guideline for the optimum level of orthodontic force that could be applied clinically to result in the greatest amount of cementum repair. The biological and mechanical factors such as age, amount of tooth movement and fluoride exposure that could influence the extent of root resorption and repair were also investigated.

8.3 Subjects & Methods

The sample consisted of 80 maxillary first premolar teeth from 40 orthodontic patients (14 boys and 26 girls, age range 11.6 – 18.2 years, mean 15.2 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.^{11,20} Ethics approvals were obtained from the Medical Faculty Ethics Committee of the University of Ondokuz Mayıs and Human Research Ethics Committee of the University of Sydney (Nos. 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 given. The amount of fluoride exposure during the experimental period for each subject was documented.

The subjects were divided into four groups (Groups IL, IIL, IH and IIH) comprising 10 subjects within each group (Figure 1). The left and right maxillary first premolars of all subjects were exposed to either light (25 grams) (Groups IL and IIL) or heavy (225 grams) (Groups IH and IIH) orthodontic force for four weeks. The maxillary left first premolars served as the positive control group and were extracted after four weeks of force application, whilst the maxillary right first premolars were placed in retention, without orthodontic force application, for either four (Groups IL and IH) or eight weeks (Groups IIL and IIH) and served as the experimental group.

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 maxillary first premolars. Self-ligating brackets were selected to allow for standardised ligation of the experimental teeth. In the light force groups (Groups IL

and IIL), 25 grams of buccally directed force was induced by a 0.016-in beta-titanium-molybdenum alloy (TMA[®]) cantilever spring (Rematitan[®], Dentaaurum, Ispringen, Germany) (Figures 2a, b). In the heavy force groups (Groups IH and IIH), 225 grams buccally directed force was induced by a 0.017-in × 0.025-in TMA[®] cantilever spring (Beta III Titanium, 3M Unitek[™], Monrovia, Calif). The force magnitude was measured with a strain gauge (Dentaaurum, Ispringen, Germany). Light cured band cement (Transbond[™] Plus, 3M Unitek[™], Monrovia, Calif) was bonded onto the occlusal surfaces of the lower first permanent molars to minimise occlusal trauma to the maxillary first premolars during the experimental period (Figure 2c).

Following four weeks of force application, the cantilever springs were removed and the teeth of the positive control group were extracted. The maxillary right first premolars (experimental group) were retained by custom made passive retention wires bonded on the palatal surface of the maxillary first and second premolars and first molars using 0.0175-in multi-stranded stainless steel wires (TwistStraight, 3M Unitek[™], Monrovia, Calif) (Figure 3). They were then extracted following either four weeks (Groups IL and IH) or eight weeks (Groups IIL and IIH) of retention. All extractions were performed by one oral surgeon who was instructed to avoid any forceps contact on the cervical cementum. Immediately after extraction, the teeth were stored in individualised containers containing sterilised de-ionised water (Milli Q[®], Millipore, Bedford, Mass) which has previously been found to be an appropriate storage medium.²¹

All visible signs of periodontal ligament and soft tissue fragments on the extracted

teeth were removed by wet gauze before they were 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 non-destructive 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 $<8\mu\text{m}$. The X-ray tube was operated at 100kV and 100 μA with the aluminium and copper filters. The X-ray detector consisted of a 1024×1024 pixel 16-bit charged coupled device camera with fibre-optic coupling to an X-ray scintillator. All teeth were scanned from the cemento-enamel junction to tooth apex with the resolution ranging from 11.45 to 16.03 μm pixel size. Scanning of the teeth was done with a 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 as a 16-bit tagged image file format (TIFF) files.

After acquisition, an axial slice-by-slice reconstruction was performed with specific software, NRecon (version 1.4.2, Aartsellaar, Belgium). Two dimensional images were generated as 1024×1024 pixel bitmap (BMP) images having an 8-bit greyscale dynamic range. Then, the VG Studio Max software (version 1.2, Volume Graphics, GmbH, Heidelberg, Germany) was used to collate the axial 2D slices to form a 3D reconstruction of the images (Figure 4a and b). Each resorption crater was isolated (Figure 4c) and the axial slices of each resorption crater were exported to the Convex Hull 2D software (CHull2D, University of Sydney, Australia) which measured the volume of each resorption crater. Because micro-CT cannot differentiate the repaired cementum from the existing cementum, the volumetric

amount of repaired cementum was deduced from the intra-individual differences in the volumes of the resorption craters between the positive control and experimental teeth.

Impressions of the maxillary dentition were taken with polyvinylsilosane impression material (Imprint II Regular Body, 3M™ ESPE™, MN) at three time points: prior to placement of orthodontic appliance, following extraction of the maxillary left premolars and following extraction of the maxillary right premolars. Impressions were poured with die stone (Vel-mix, Kerr, Orange, Calif). The distance of buccal movement of the upper first premolars was measured on the stone cast with a digital vernier calliper (Masel, Bristol, Penna) (Figure 5).

Statistical Analysis

Analysis of variance (ANOVA) was used to determine the significance of differences among the four groups (Groups IL, IIL, IH and IIH). Univariate analysis of variance was performed to investigate the effect of covariates (age, fluoride exposure, volume of root resorption craters in the positive control group) on the volume of root resorption craters in the experimental group. Pearson's correlation coefficient was used to calculate the possible correlations between age, fluoride exposure, amount of tooth movement and root resorption crater volume. During statistical analysis, the raw data was transformed for the residual plots to conform to normality. The cube root of the volumetric readings was used to create a model for statistical analysis. Essentially, this replaces each volume by the radius of an equivalent hemispherical crater. This approach was also used in previous studies.^{10,22}

The difference in volumes between the control and experimental groups was not cube root transformed because the differences were closer to being normally distributed and some data contains negative values. One subject in Group IH displayed unusually large root resorption volumes and was excluded in the analysis. The “*p*” values quoted in the analyses were two-tailed and the significance level of any individual test was chosen as five per cent. The Statistical Package for Social Sciences (SPSS for Windows, version 12, SPSS, Chicago, Ill) was used to carry out all statistical calculations.

Measurement Error

The volumes of the resorption craters were remeasured for all four groups (Groups IL, IIL, IH and IIH) three months after the initial measurement. The measurement error was calculated as the standard error from repeated measurements on the same subject. ANOVA was used to calculate the mean square error. The standard error of measurement was then derived from the square root of the mean square error. The standard errors of measurement for the positive control and experimental groups were 0.069 mm³ and 0.038 mm³ respectively. The standard errors of measurement following cube root transformation for the positive control and experimental groups were 0.448 mm and 0.490 mm respectively. Measurement error can also be expressed as a coefficient of variation which is a percentage expressed by the standard deviation divided by the mean. The coefficients of variation for the positive control and experimental groups were 58.6 and 24.2 per cent respectively for the ordinary scale and 14.3 and 9.07 per cent respectively on the cube root scale.

8.4 Results

Positive Control Group (Cube Root Scale)

The mean cube root volumes of the resorption craters were significantly different between all four groups ($p=0.03$) with Group IL displaying the lowest mean cube root volume of resorption craters ($0.351\pm0.117\text{mm}^3$) and Group IH displaying the highest mean cube root volume of resorption craters ($0.488\pm0.110\text{mm}^3$) (Table I, Figure 6).

Experimental Groups (Cube Root Scale)

The mean resorption crater volumes of the teeth on cube root scale in all four experimental groups were very similar ($p=0.32$) (Table I, Figure 6). Groups IL and IIL showed the lowest mean cube root volume of resorption craters and Group IH showed the highest mean cube root volume of resorption craters. One subject in Group IH had an extraordinary amount of cube root volume of resorption craters which was considered as an outlier.

Repaired Cementum (Ordinary Scale)

The amount of repaired cementum was calculated as the intra-individual differences between the positive control and experimental groups. The mean values of repaired cementum were not statistically significantly different across the four groups ($p=0.48$) (Table I, Figure 7). Despite the insignificant differences among the four groups, Group IIL displayed a positive mean repaired cementum volume of $0.033\pm0.058\text{mm}^3$ whereas Groups IL, IH and IIH displayed negative mean repaired cementum volumes of $-0.050\pm0.064\text{mm}^3$, $-0.106\pm0.289\text{mm}^3$ and $-0.036\pm0.075\text{mm}^3$ respectively.

Effect of covariates on the volume of root resorption craters in the experimental group

Age had a significant effect ($p=0.01$) on the cube root volume of resorption craters in the experimental group (Table II). However, fluoride exposure, cube root volume of resorption craters in the control group and the amount of tooth movement in the control and experimental groups had no significant effect on the cube root volume of resorption craters in the experimental group.

Correlations between Variables

Pearson's Correlation analysis showed significant correlations between several parameters (Table III). The amount of tooth movement was significantly correlated with the cube root volume of resorption craters in the positive control group ($p=0.02$). There were significant negative correlations between age, cube root volumes of resorption craters in the positive control and experimental groups, and the amount of tooth movement in the positive control group ($p<0.02$). Strong correlation was also found in the cube root volumes of resorption craters between the positive control group and the experimental group ($p=0.01$). The amount of tooth movement in the positive control group was also significantly correlated to the cube root volume of resorption craters in the experimental group ($p=0.01$). No correlation was found between fluoride exposure and any tested variables.

8.5 Discussion

The magnitude of orthodontic force was shown to have an influence on the severity of root resorption^{11,23-25} and light orthodontic forces were advocated to reduce the adverse tissue reaction.¹²⁻¹⁴ In addition, previous studies demonstrated that the reparative process increases over time.^{3,4,26,27} Therefore, it would be expected that light orthodontic force and a lengthy retention period would result in a more favourable cementum reparative process. The present study supported this by having both the positive amount of reparative cementum displayed in Group IIL and the lowest cube root volume of resorption craters present in the light force groups (Groups IL and IIL) (Table 1). However, the differences of the root resorption volumes for both the experimental teeth and repaired cementum among all four groups were not statistically significant (Table 1, Figure 7). Therefore, the results of this study could not clearly demonstrate whether or not the total amount of the reparative cementum was dependent on the magnitude of the orthodontic force or the retention time.

The cementum reparative process occurred when the orthodontic force was ceased or decreased below a certain level.¹ However, even when the active orthodontic force has ceased, root resorption continues in the area of hyalinised tissue.¹⁸ This was explained by the fact that osteoclastic activity does not decrease until all necrotic tissue in the periodontal ligament has been resorbed.¹⁸ Therefore, the persistence and removal of the necrotic tissue determined the root resorption and repair process.¹⁸ In light of this observation, whether the teeth were subjected to light force or no orthodontic forces (retention period), resorption and repair occurred simultaneously. This could explain the similarity of the amount of repaired

cementum and the mean cube root volumes of resorption craters on the experimental teeth between the light force groups (Groups IL and IIL) (Table 1). The area of hyalinised tissue was larger and more persistent under heavy orthodontic force.²⁸ Therefore, the resorption process may have dominated over the reparative process and this was shown by the higher mean cube root volume of resorption craters on the teeth in Group IH when compared to the light force groups (Groups IL and IIL) (Table 1). Group IH also showed larger, but not statistically significant, mean cube root volume of resorption craters on the experimental teeth than Group IIH (Table 1). In addition, the amount of repaired cementum was less, but not statistically significant, in Group IH when compared to Group IIH (Table 1). This may indicate that after four weeks of retention, the reparative process surpassed the resorption process in the heavy force groups.

Previous studies^{5,29} showed that the reparative process increased over the first four weeks of retention, then slowed down in the fifth and sixth weeks and finally reached a plateau phase. In the current study, the retention periods were determined to be either four or eight weeks in order to highlight the differences in the amount of cementum repaired. However, the present study showed a continuous increase in cube root volume of resorption craters in the first four weeks of retention (Figure 8). One explanation was that resorption and repair processes occurred simultaneously and resorption dominated over repair especially in the heavy force group. The present study also did not detect any differences in the cube root volume of resorption craters between the four and eight week retention periods after the application of light orthodontic force. This indicated that the repair process might have started to reach a steady rate from four weeks which was earlier than suggested

in a previous study.⁵ In addition, Brudvik and Rygh¹⁷, in a rodent model, showed that root resorption repair occurred during the second and fourth week and reparative processes finished at the fourth week. The lesser mean cube root volume of resorption craters found on the teeth of long retention, heavy force group (Group IIH) when compared to the teeth of short retention, heavy force group (Group IH) seemed to suggest that the majority of the reparative process occurred after four weeks of retention. Therefore, there was a difference in the onset of the reparative process for the heavy orthodontic force application when compared to the light orthodontic force application. Further studies are needed to investigate different retention time intervals (e.g. 6, 10, 12 weeks) in order to extrapolate the reparative process through time.

Many biological and mechanical factors were found to influence the severity of orthodontically induced inflammatory root resorption. However, there were limited studies that have documented factors that influence the amount of reparative cementum. In this study, the significant correlation between the cube root volumes of resorption craters in the positive control group and the experimental group highlighted the individual susceptibility to root resorption. Therefore, an individual reaction pattern to root resorption may play an important role in influencing the degree of cementum repair. The wide individual variation displayed in the results contributed to the similarity in the mean amount of repaired cementum between all groups. Some studies^{30,31} have shown that root resorption increased with age while others have disproved this finding.^{32,33} In the present investigation there was a strong negative correlation between age and the cube root volumes of resorption craters in both the positive control and experimental groups. This indicated that the

root resorption volume decreased with age. Fluoride has been documented to increase osteoid formation and suppress osteoclastic activity which may result in smaller root resorption craters.³⁴ However, this study did not show a significant correlation between the subject's fluoride exposure and the cube root volumes of resorption craters. Therefore, fluoride may only influence the depth of the resorption crater as suggested by Taylor *et al.*³⁵ Unlike biological factors, mechanical factors can be controlled by the clinician to minimise the adverse effect of root resorption and allow initiation of repair. Significant positive correlations were found between the amount of tooth movement in the positive control group and the cube root volumes of resorption craters in the positive control and experimental groups. This suggested that the longer the distance the tooth was moved the greater the amount of root resorption volume. This confirmed the previous findings that have shown a positive relation between the distance of tooth movement and the severity of orthodontically induced inflammatory root resorption.^{6,36,37} The amount of tooth movement in the experimental group did not display a significant correlation with the cube root volume of resorption craters in the experimental group. One possible explanation could be the possibility of relapse during the retention period that contributed to additional continuous resorption during the retention phase.

Although the Miro-CT provided a high spatial resolution image of the teeth studied, it was not possible to differentiate the repaired cementum from the existing cementum. This could only be accomplished by investigating the structural changes histologically. In this study, it was assumed that the intra-individual differences in the volumes of the resorption craters between the teeth of the positive control and experimental groups were estimates of the amount of reparative

cementum. Brudvik and Rygh¹⁷ indicated that new mineralised cementum was observed on the resorbed root surface by 21 days of force application. Therefore, the present study also assumed that following four weeks of force application and four weeks of retention, there would be an adequate time period for the cementum to remineralise and be adequately detected by the micro-CT.

8.6 Conclusions

1. Root resorption continued for another four weeks after orthodontic force was ceased. The resorptive activity was more pronounced following the application of heavy forces.
2. Passive retention following four weeks of light orthodontic force application had the least root resorption crater volume (cube root scale).
3. The reparative processes were different between light and heavy orthodontic force application. It reached a steady rate after four weeks of passive retention following the application of four weeks of light force whereas the majority of the reparative process occurred after four weeks of passive retention following the application of four weeks of heavy orthodontic force.
4. The volume of root resorption craters was positively dependent on the amount of tooth movement and negatively correlated with chronological age.

8.7 Acknowledgement

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8.8 References

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8.9 List of Tables

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Table I Statistical analysis of the volumes of root resorption craters and repaired cementum for Groups IL, IIL, IH and IIH

Groups	Positive Control Teeth Cube Root Scale (mm)				Experimental Teeth Cube Root Scale (mm)				Repaired Cementum Ordinary Scale (mm ³) (Positive Control – Experimental)			
	Mean	Standard Deviation	Standard Error		Mean	Standard Deviation	Standard Error		Mean	Standard Deviation	Standard Error	
IL	0.351	0.117	0.037		0.442	0.134	0.042		-0.050	0.064	0.020	
IIL	0.450	0.072	0.023		0.445	0.072	0.023		0.003	0.058	0.018	
IH	0.488	0.110	0.037		0.522	0.093	0.031		-0.106	0.289	0.091	
IIH	0.398	0.101	0.032		0.460	0.103	0.032		-0.036	0.075	0.024	
p-value	0.03				0.32				0.48			

Table II Univariate analysis of variance on the effect of covariates on the cube root volume of resorption craters in the experimental group

Tests of Between-Subjects Effects

Dependent Variable: Cube Root Volume (CRV) in Experimental Group

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.198*	8	.025	3.467	.006
Intercept	.148	1	.148	20.785	.000
age	.058	1	.058	8.104	.008
Fluoride exposure	.016	1	.016	2.228	.146
CRV in Positive Control Group	.001	1	.001	.079	.780
Tooth Movement in Positive Control Group	.017	1	.017	2.320	.138
Tooth Movement in Experimental Group	.004	1	.004	.503	.484
Group	.010	3	.003	.482	.697
Error	.214	30	.007		
Total	8.866	39			
Corrected Total	.411	38			

*R Squared = .480 (Adjusted R Squared = .342)

Table III Pearson’s Correlation between age, fluoride exposure, cube root volume of resorption craters and the amount of tooth movement

		Correlations					
		age	Fexp	cuberoot Exp tot vol	cuberoot Ctrl tot vol	CTotMvt	ExpTotMvt
age	Pearson Correlation	1	-.022	-.581**	-.448**	-.362*	-.217
	Sig. (2-tailed)	.	.894	.000	.004	.024	.185
	N	39	39	39	39	39	39
Fexp	Pearson Correlation	-.022	1	.267	.178	.126	.020
	Sig. (2-tailed)	.894	.	.101	.278	.446	.905
	N	39	39	39	39	39	39
cuberoot Exp tot vol	Pearson Correlation	-.581**	.267	1	.393*	.422**	.153
	Sig. (2-tailed)	.000	.101	.	.013	.007	.351
	N	39	39	39	39	39	39
cuberoot Ctrl tot vol	Pearson Correlation	-.448**	.178	.393*	1	.370*	.202
	Sig. (2-tailed)	.004	.278	.013	.	.020	.217
	N	39	39	39	39	39	39
CTotMvt	Pearson Correlation	-.362*	.126	.422**	.370*	1	-.295
	Sig. (2-tailed)	.024	.446	.007	.020	.	.068
	N	39	39	39	39	39	39
ExpTotMvt	Pearson Correlation	-.217	.020	.153	.202	-.295	1
	Sig. (2-tailed)	.185	.905	.351	.217	.068	.
	N	39	39	39	39	39	39

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

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Figure 1 Diagrammatic explanation of sample groupings

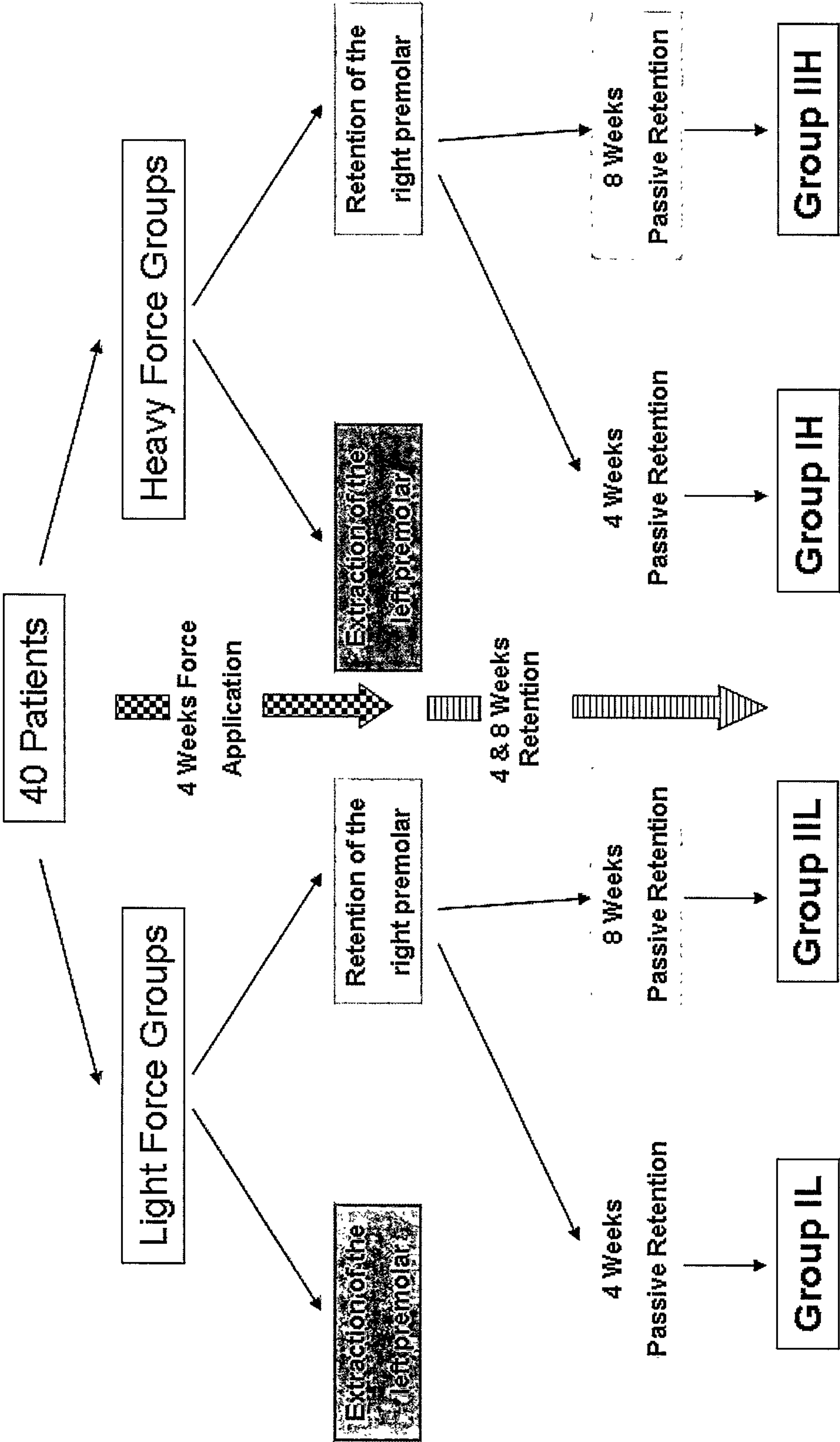


Figure 2 Intraoral photographs of the experimental set-up (a) buccal view, (b) maxillary occlusal view, (c) mandibular occlusal view

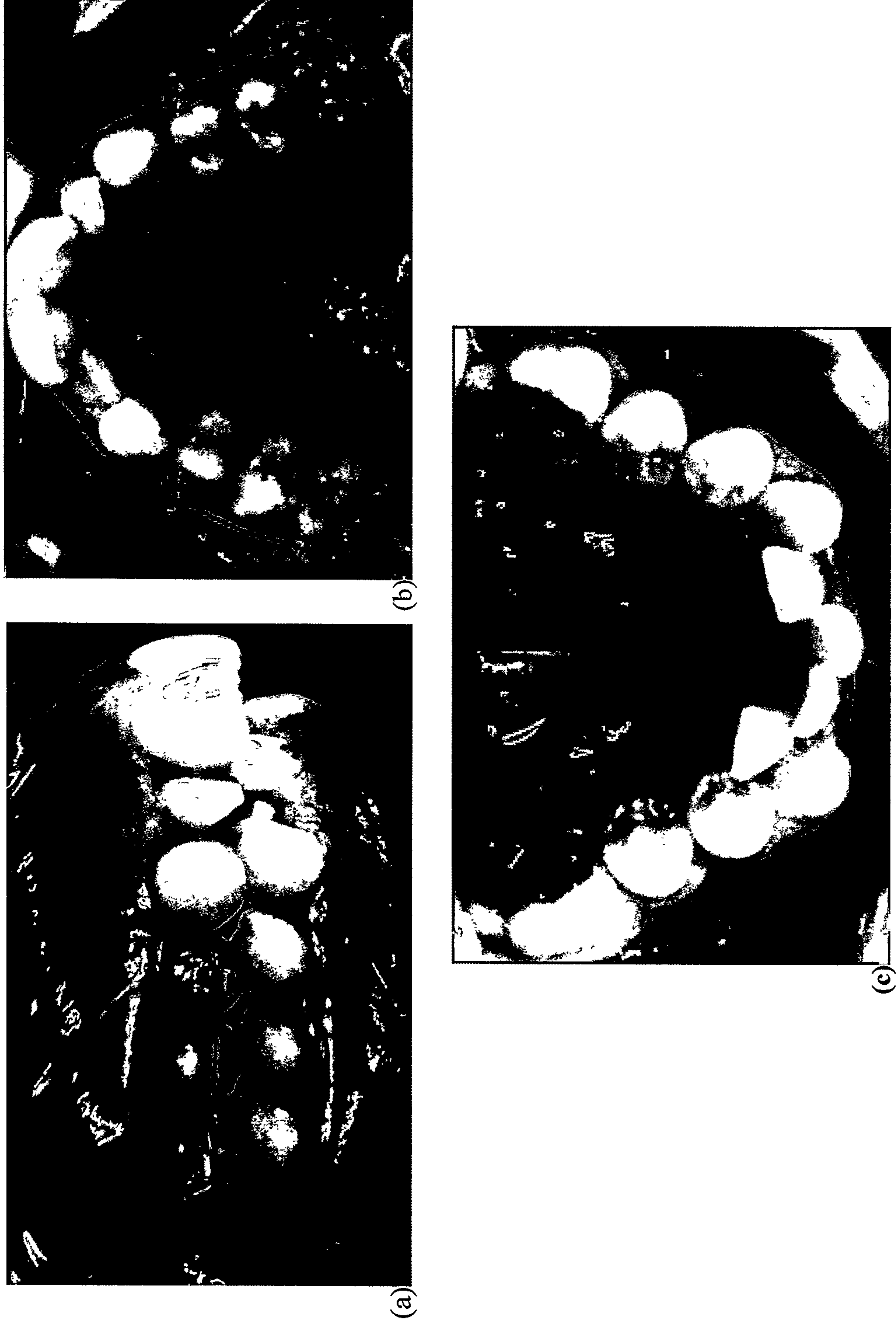


Figure 3 Occlusal view of the passive retention wire on the palatal side of the maxillary right posterior quadrant (arrow)



Figure 4 Micro-CT images of the (a) maxillary left first premolar (positive control) (b) maxillary right first premolar (experimental) (c) root resorption crater in detail

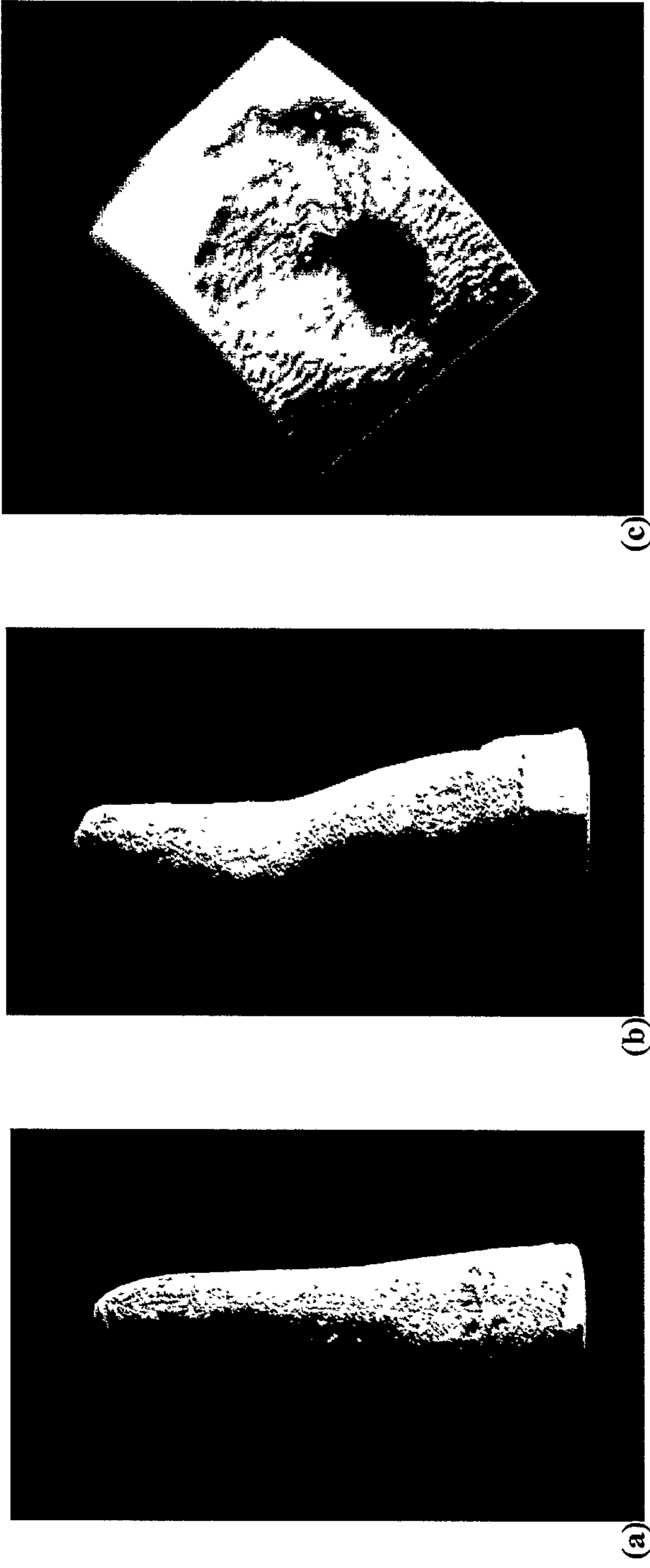


Figure 5 Methodology for tooth movement measurement

1. Impressions were poured with die stone (Velmix, Kerr, Orange, CA, USA)
2. Study models were scanned with Epson Expression 1600 flatbed colour image scanner (Epson, Australia) at 600 d.p.i., 24 bit grayscale
3. Study model images were printed on paper
4. A line (Line A) was drawn between either the mesiopalatal cusps of upper second molars, upper cuspid tips or the distoincisoral corners of upper lateral incisors (depending on the eruption of these teeth)
5. The distance of Line A was measured and a perpendicular line (Line B) to halfway of Line A was drawn
6. Perpendicular lines (Lines C and D) to Line B were drawn to the palatal cusps of upper left and right first premolars.
7. The distance of Lines C and D were measured with digital vernier calliper (Masel, Bristol, Pennsylvania, USA)
8. The amount of tooth movement was calculated by the differences of the distance of Lines C and D between two different time points.

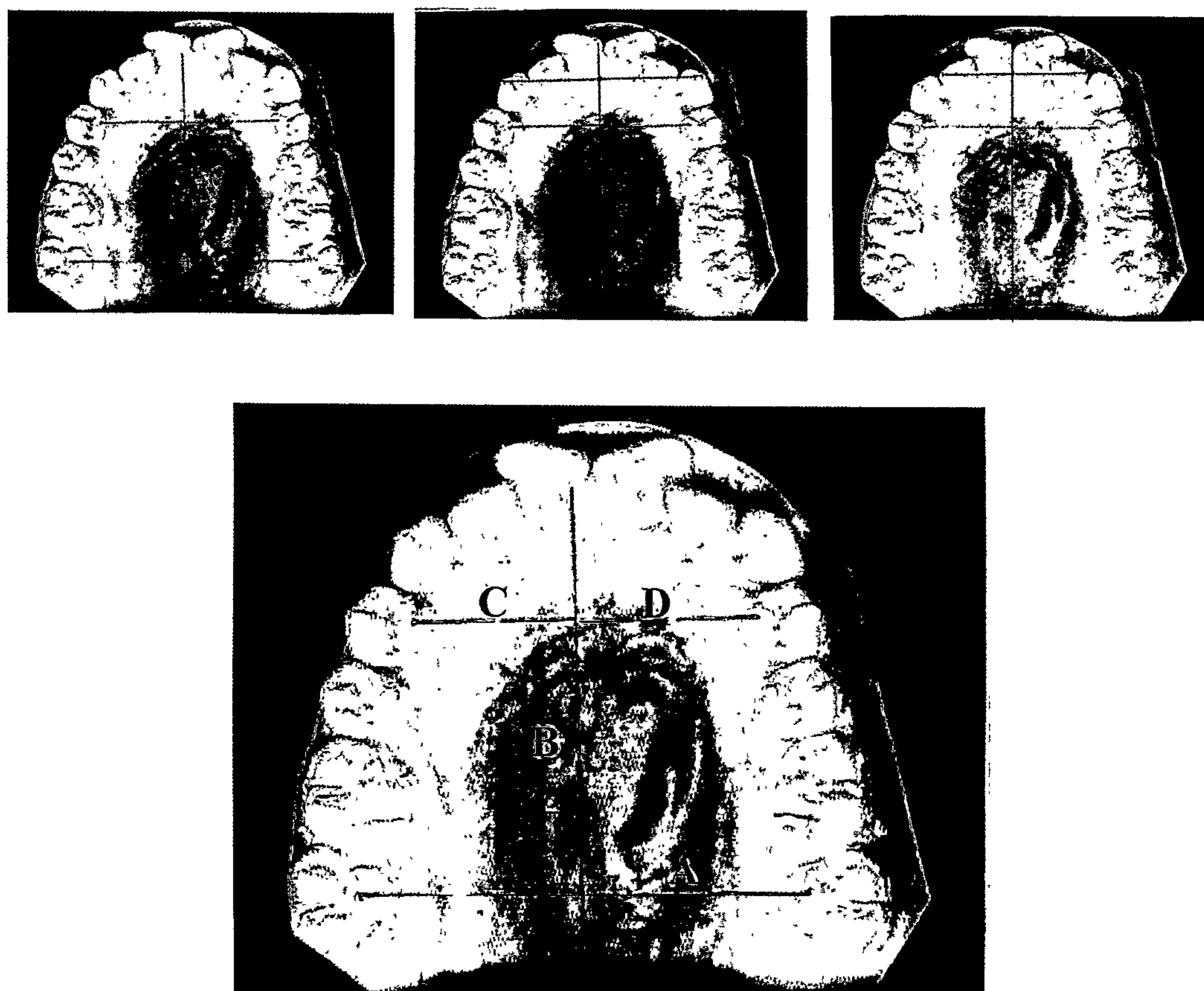


Figure 6 Box-plot of the distribution of the cube root volumes of resorption craters for the positive control and experimental groups

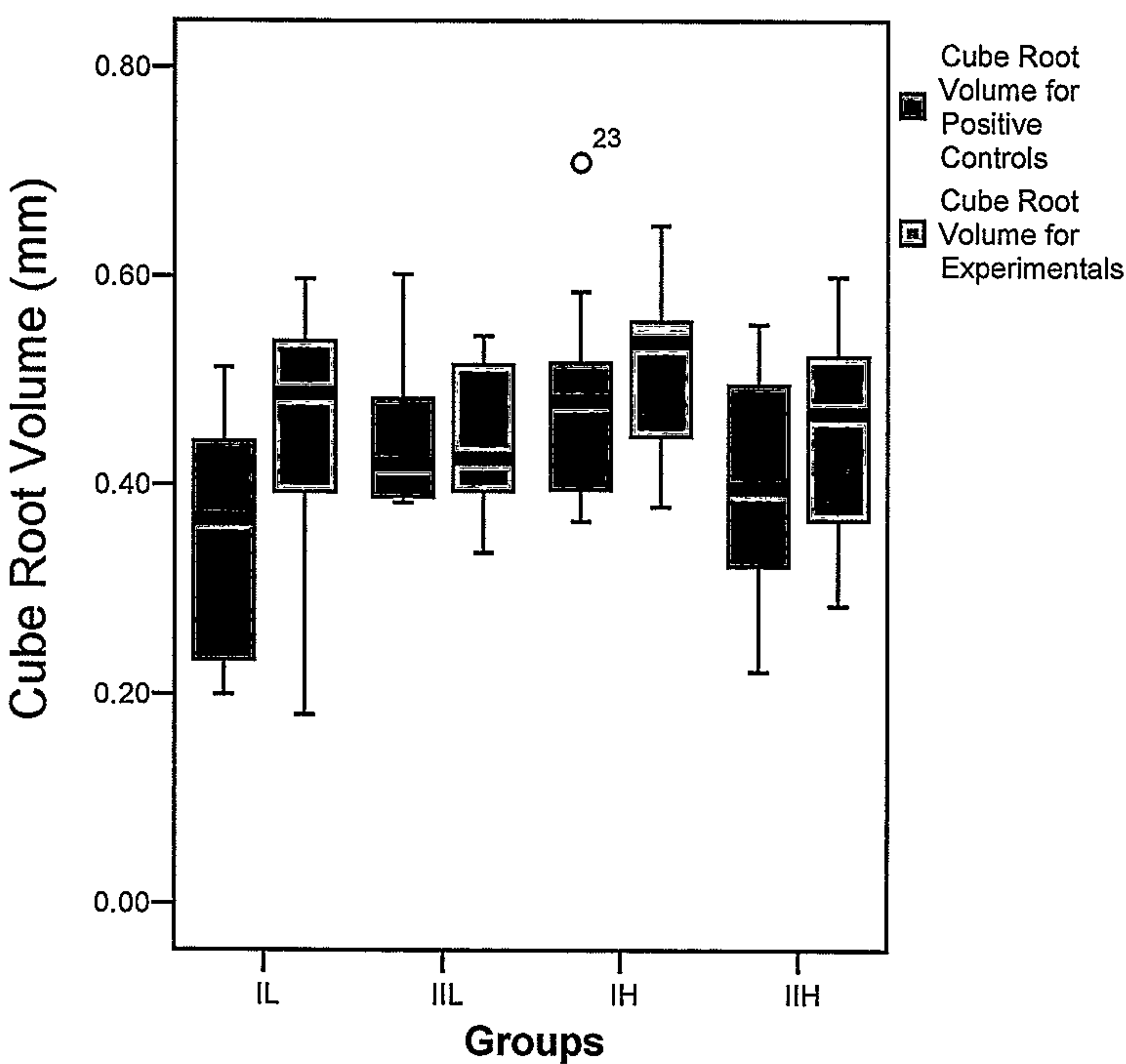


Figure 7 Box-plot of the distribution of the differences in the resorption crater volume between the positive control and experimental groups

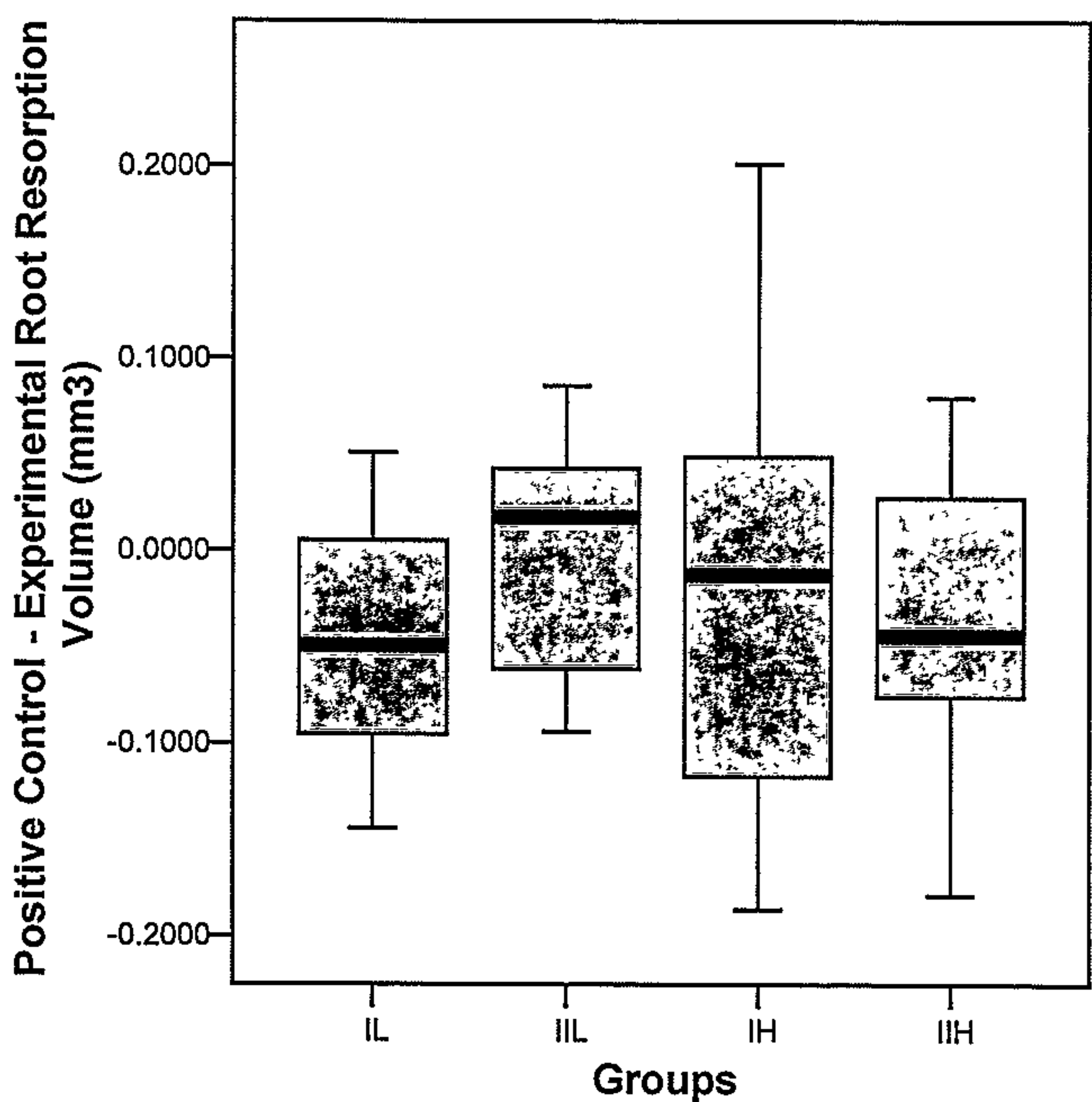
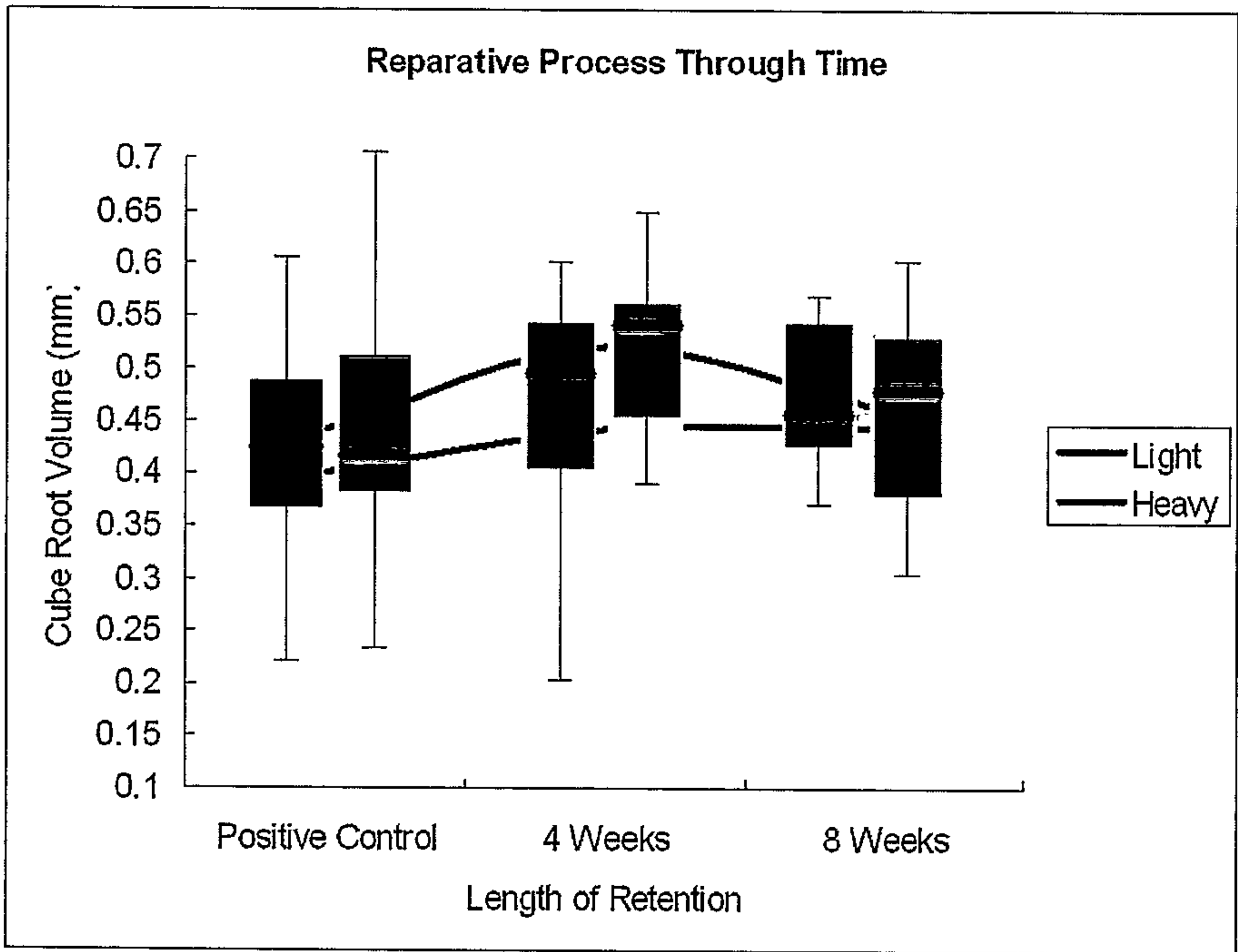
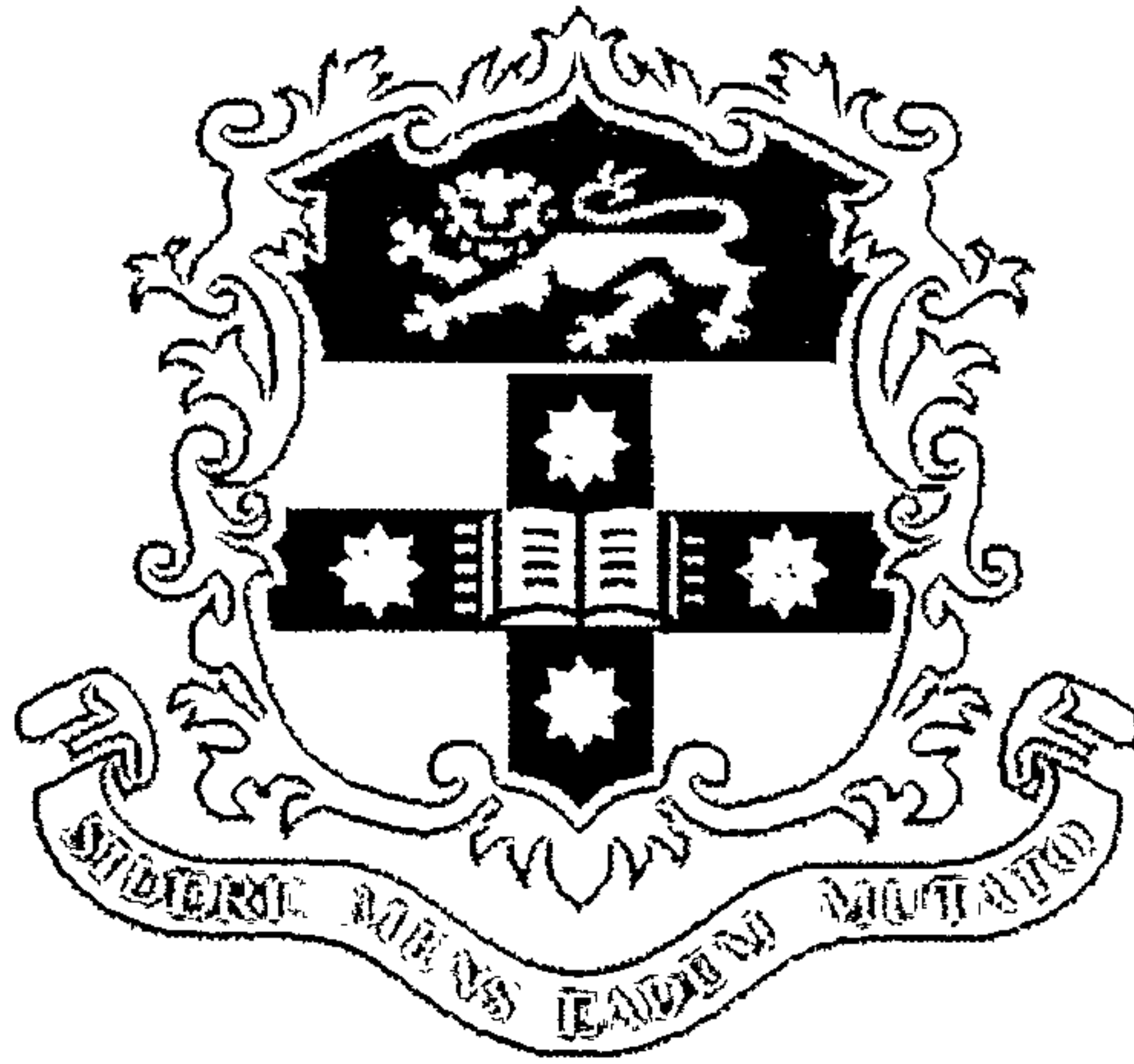


Figure 8 Temporal pattern of repair for light and heavy force groups. Note that the control cube root volumes of resorption craters were the average cube root volumes of resorption craters of Groups IL and IIL for the light force group and Groups IH and IIH for the heavy force group



9. Manuscript II



REPAIR OF ROOT RESORPTION FOUR AND EIGHT WEEKS FOLLOWING THE APPLICATION OF CONTINUOUS LIGHT AND HEAVY FORCES FOR FOUR WEEKS: A HISTOLOGY STUDY

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9.1 Abstract

INTRODUCTION: Repair of root resorption cavity has been studied under light microscopy, scanning electron microscopy and transmission electron microscopy. The aim of the present investigation was to demonstrate the use of micro-computed 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 after four or eight weeks of retention following four 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 recruited in this study. They were divided into two groups (Groups III and IV), each group consisted of two subjects. The maxillary first left and right premolars of all subjects were loaded with light (25 grams) or heavy (225 grams) orthodontic force respectively for four weeks. After four or eight weeks of retention, the upper first premolars were extracted for Groups III and IV respectively. The extracted teeth from all of the groups were firstly investigated by micro-CT. Using the three dimensional images created by the micro-CT, the largest resorption crater on the buccal and lingual sides were identified. Parasagittal sections of these resorption craters were studied histologically under hematoxylin and eosin staining.

RESULTS: (1) The use of micro-CT has improved the efficiency and accuracy of histological techniques. (2) Comparatively a reduced amount of the root resorption cavity was repaired by new cementum after heavy orthodontic force application and short retention time. (3) The reparative processes seemed to be time dependent with longer retention time yielding the most amount of repair. (4) Reparative cementum was a mixture of acellular and cellular cementum. (5) Reparative processes seemed

to commence at the central part of the resorption cavity and expand to the periphery.

CONCLUSION: Root resorption cavities have the potential to repair regardless of the orthodontic force magnitude. Correlative microscopy using micro-CT and conventional light microscopy adds a new dimension to the current root resorption investigation techniques.

KEYWORD: Orthodontic force, retention, root resorption, repair, micro-CT, histology

9.2 Introduction

Brudvik and Rygh^{1,2} carried out rodent studies on the repair of orthodontically induced inflammatory root resorption following application of 50 grams 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 in the presence of light force. The formation of new tooth supporting structures was seen in the periphery of the resorption lacunae while active resorption by multinucleated odontoclast like cells took place in the central parts. When orthodontic force was discontinued, the reparative process was similar to the early cementogenesis occurring 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 re-establishment of new periodontal ligament.^{2,3,5-8} In a human histological study by Owman Moll and Kurol⁹, 50cN of buccally directed force was applied on maxillary first premolars for six weeks and passively retained up to seven weeks. It was found that the type of repaired cementum was more of the cellular cementum than the acellular type. The acellular cementum occurred more often in the early phase of healing.⁹ The repair process did not vary with the location or type of tissue neither on the buccal or lingual side of the tooth nor in the cervical or apical parts of the root.⁹

There are various patterns of repair within the resorption cavity. Depositions of

cementum both from the periphery of the resorption cavity inward and or from the centre outward have been identified.^{1,2,7,10} In particular, Brudvik and Rygh^{1,2} illustrated in their histological study 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 over-compressed hyalinised zone. Owman Moll and Kurol⁹ revealed that healing cementum only occurred centrally in the bottom of the cavity and central-peripherally on one of the lateral walls of the cavity. This could be due to different sectioning orientations or the specimens were collected at different time-points.

The aim of the present investigation was to demonstrate the use of micro-CT to assist in the identification of the region of interest for light microscopy preparation. This study also qualitatively studied the histology of root resorption craters after four or eight weeks of retention following four weeks of continuous light or heavy orthodontic force application.

9.3 Subjects & Methods

Eight first premolar teeth were collected from four orthodontic patients (two boys and two 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 Mayıs and the Human Research Ethics Committee of the University of Sydney (Nos. 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 two groups (Groups III and IV) comprising of two subjects within each group (Figure 1). Subjects received a light conventional orthodontic force of 25 grams on the upper left first premolar and a heavy conventional orthodontic force of 225 grams on the upper right first premolar for four weeks. The premolars were then placed in retention, without orthodontic force application, for four or eight weeks in Groups III and IV respectively.

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 (Figure 2a). Self-ligating brackets were selected to allow for standardised ligation of the experimental teeth. A buccally directed light orthodontic force of 25 grams was induced by a 0.016-in. Titanium Molybdenum Alloy (TMA[®]) cantilever spring (Rematitan[®], Dentaaurum, Ispringen, Germany) (Figures 2a and b). A buccally directed heavy orthodontic force of 225

grams was induced by a 0.017-in. $\times$ 0.025-in. TMA[®] cantilever spring (Beta III Titanium, 3M Unitek[™], Monrovia, Calif) (Figure 2c). The force magnitude was measured with a strain gauge (Dentaurum, Ispringen, Germany). Light cured band cement (Transbond[™] Plus, 3M Unitek[™], Monrovia, Calif) was bonded onto the occlusal surfaces of the lower first permanent molars to minimise occlusal trauma to the maxillary first premolars during the experimental period (Figure 2d).

Following four 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 using 0.0175 in. multi-stranded stainless steel wire (TwistStraight, 3M Unitek[™], Monrovia, Calif) (Figure 3). The maxillary first premolars were then extracted following either four or eight weeks of retention. All extractions were performed by one oral surgeon who was instructed to avoid any forceps contact on the cervical cementum. Immediately after extractions, the teeth were stored in individualised containers containing 10 per cent buffered formalin for tissue fixation.

Micro-CT

The extracted teeth were placed in a moist chamber of 10 per cent buffered formalin (Figure 4) and scanned by a desktop X-ray micro-CT system (SkyScan 1172, Aartsellaar, Belgium). Unlike scanning electron microscopy in which the specimen requires invasive preparation, micro-CT is a variant of the medical CT scan system that allows non-destructive 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 $<8\mu\text{m}$. The X-ray tube was operated

at 100kV and 100 μ A with the aluminium and copper filters. The X-ray detector consisted of a 1024 $\times$ 1024 pixel 16-bit charged coupled device camera with fibre-optic coupling to an X-ray scintillator. All teeth were scanned from the cemento-enamel junction to tooth apex with the resolution ranging from 14.38 to 16.11 μ m pixel size. Scanning of the teeth was done with a 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 as a 16-bit tagged image file format (TIFF).

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

Histology

Following micro-CT analysis, the teeth were prepared for histological study. Samples were decalcified in 10 per cent formic acid in phosphate-buffered formalin for approximately four weeks. The largest resorption craters on the buccal and lingual surface of the tooth were identified on the three dimensional (3D) images produced by the micro-CT scans (Figure 5a). Using these 3D images as a reference map, the specimens were grossly sectioned into two pieces for one rooted premolars or four pieces for two rooted premolars (Figures 5b and c). The samples were then embedded in paraffin (Figure 5d). Parasagittal sections of the mesiodistal aspect of the teeth were cut at 5 μ m with a Leica Microtome (Leica

MicroSystems, Düsseldorf, Germany). Serial sections were stained with Harris hematoxylin and eosin (H&E). From each specimen, the glass containing sections with the longest root resorption crater length coronal-apically was chosen as a basis for the investigation. Colour photographs were exposed in a light microscope with 1.25×, 4×, 10×, and 20× objective magnifications (OM). Histology was assessed for the extent of the root resorption and the associated repair.

9.4 Results

Group III

Four weeks of retention following four weeks of light orthodontic force

The buccal root showed two 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 (Figure 6). On the lingual surface of the buccal root near the tooth apex, the resorption crater appeared to have extended into the dentine and has been repaired by new cementum (Figure 7). Cementocytes were also evident to be trapped into the new cementum which may indicate repaired cementum of the cellular type. A layer of osteoblast-like cells overlaid the new cementum. However, the reparative cementum did not restore the anatomical contour of the root. Active root resorption craters were also evident near the root apex (Figure 8). The resorption crater was multiloculated and infiltrated with blood cells and inflammatory tissues. The resorption crater on the buccal surface had also extended into dentine (Figure 9). The newly formed cementum in the bottom of the resorption cavity had no trapped cementocytes which indicated acellular cementum. However, the superficial layer of reparative cementum had incorporated cementocytes which indicated cellular cementum. A large amount of inflammatory infiltrates mixed with blood cells and osteoblast-like cells filled the resorption cavity. This may indicate an active reparative process.

Four weeks of retention following four 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 (Figure 10 and 11). Resorption craters were repaired with new cementum and a large amount of inflammatory

infiltrates (Figure 12). The reparative cementum seemed to be thicker in the centre 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 (Figures 7 and 12). In addition, the majority of reparative cementum was of the acellular type. The resorption crater was not fully repaired to the root shape. Interestingly, excess cementum deposition was evident associated with one of the resorption craters (Figure 13).

Group IV

Eight weeks of retention following four 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 (Figure 14). The new cementum layer had almost repaired the resorption cavity unlike the four weeks retention groups. Some cementocytes were enclosed in the newly formed cementum. There seems to be no evidence of inflammatory infiltrates, blood cells, osteoblast like cells and osteoclast like cells at this OM. This may indicate that the resorption and reparative processes had arrested.

Eight weeks of retention following four weeks of heavy orthodontic force

A significant amount of reparative cementum was evident (Figure 15a). Unlike the previous groups, the reparative cementum was not clearly distinct from the existing cementum by the difference in stain intensities and histomorphologies. The reparative cementum in this group displayed strongly stained reversal lines (Figures 16). Comparatively fewer amount of inflammatory infiltrates and blood cells were apparent. A small number of cementocytes were included in the newly formed cementum (Figure 15b).

9.5 Discussion

Histological preparation is technique sensitive and isolation of a small region of interest such as root resorption craters can be tedious. Micro-CT allowed non-destructive imaging of the tooth samples. The resultant 3D visualisation permitted easy identification of regions of interest, e.g. root resorption craters, which made the histological sectioning more accurate and efficient. In this study, the micro-CT images showed the regions of interest, i.e. the largest root resorption craters, were located in the buccal cervical and lingual apical root surfaces. The results of this study also showed that cells and tissues were well preserved in the histological 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 buffered formalin moist chamber. Therefore, micro-CT scanning can optimise histological sectioning techniques which could be utilised in future research studies on hard tissues.

Previous studies have shown that the volume of root resorption craters was greater after heavy force application.^{13,14} It is clinically fortunate that the present 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 human protective mechanism against adverse effects from orthodontic forces. Although this study was not quantitative in nature, the extent of reparative cementum that filled the resorption cavities was greater in the longer retention groups which indicated that the reparative process was time dependent. At the same OM, the least amount of reparative cementum was found in the heavy force group that was retained for four weeks followed by the light force

group retained for four weeks, the light force group retained for eight weeks and finally the heavy force group retained for eight weeks. This correlated with the current authors' volumetric study which found the largest mean root resorption crater volume in the heavy force group that was retained for four weeks. By the end of eight weeks, there were little evidences of inflammatory infiltrates histologically, especially in the light force groups, which may indicate deceleration of the reparative process. However, the periodontal ligaments were not totally intact in the sample, therefore, the inflammatory infiltrates cannot be fully investigated. The properties of cementum were found to be closely related to bone.¹⁵ 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 remodelling were seen with the healing of the resorbed cementum. Because the specimens were extracted teeth, therefore, the re-establishment of the periodontal ligament cannot be investigated.

Bosshardt and Schroeder¹⁶ found that the reparative cementum was principally cellular cementum in human teeth. Other authors¹⁷⁻²⁰ suggested that the reparative cementum were combinations of cellular and acellular cementum. The resorption craters were initially repaired by acellular cementum which was followed by continuous repair with cellular cementum.^{17,20} In the present investigation, the majority of the reparative cementum were of the cellular type. Some resorption craters were repaired with a combination of cellular and acellular cementum while very few resorption craters showed entirely acellular cementum. Acellular cementum appeared to firstly 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 lengthy retention time intervals, four and eight weeks, and small sample size. There seemed to be a tendency that the type of reparative cementum was orthodontic force dependent. The heavy force group appeared to show more acellular type of reparative cementum whereas the light force groups showed more of the cellular type. However, the sample size was not large enough to support this hypothesis.

Henry and Weinmann¹⁸ identified two structural types of root resorption repair, anatomic and functional. Anatomic repair was characterised by the restoration of the root surface to its original contour and a functional repair occurred when the exposed dentin has been covered by a thin layer of repair cementum. Functional repairs were seen more often in the specimens that were retained for four weeks and anatomic repairs were found in the samples that were retained for eight 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 which appeared as hypercementosis.

Brudvik and Rygh¹ believed that the reparative process began in the periphery of the root resorption craters while active resorption continued beneath the centrally located over-compressed hyalinised zone. The results of this study 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 was in concurrence with Langford.⁸ He 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 tissue between the tooth and bone surface was less pronounced in the central area of the resorption crater than the periphery due to the resilience of the tissue.⁹ Therefore, cell proliferation and regeneration of tissue were more pronounced in the deep central part of the resorption cavity. Owman Moll and Kurol⁹ also found reparative cementum in the central and central peripheral areas of the resorption crater.

9.6 Conclusions

1. The cells involved in the reparative process in human cementum were similar to the cells involved in bone biomechanics.
2. For the same OM, there was less of the root resorption cavity repaired by new cementum after heavy orthodontic force application and short retention time when compared to light orthodontic force with the same retention time.
3. The reparative processes seemed to be time dependent with longer retention time yielding the most amount of repair.
4. Reparative cementum was a mixture of acellular and cellular cementum.
5. The reparative process seemed to commence at the central part of the resorption cavity and expand to the periphery.
6. The use of micro-CT definitely improved the efficiency and accuracy of histological techniques.

9.7 Acknowledgement

We would like to thank the Australian Society of Orthodontics Foundation for Research and Education Inc and Australian Dental Research Foundation and Dentaurem Australia Pty Ltd for their sponsorship of this research. Gratitude is also expressed to Associate Professor Gang Shen at the Discipline of Orthodontics of the University of Sydney for reviewing this manuscript and Assistant Professor Mahmut Sumer at the Department of Oral Surgery, University of Ondokuz Mayıs for participation in this research study.

9.8 References

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Figure 16 Reparative cementum showed reversal lines (arrows) which indicated periods of cementum remodelling which was similar to the reversal lines that were seen in bone remodelling (20× OM).

Figure 1 Diagrammatic explanation of sample groupings

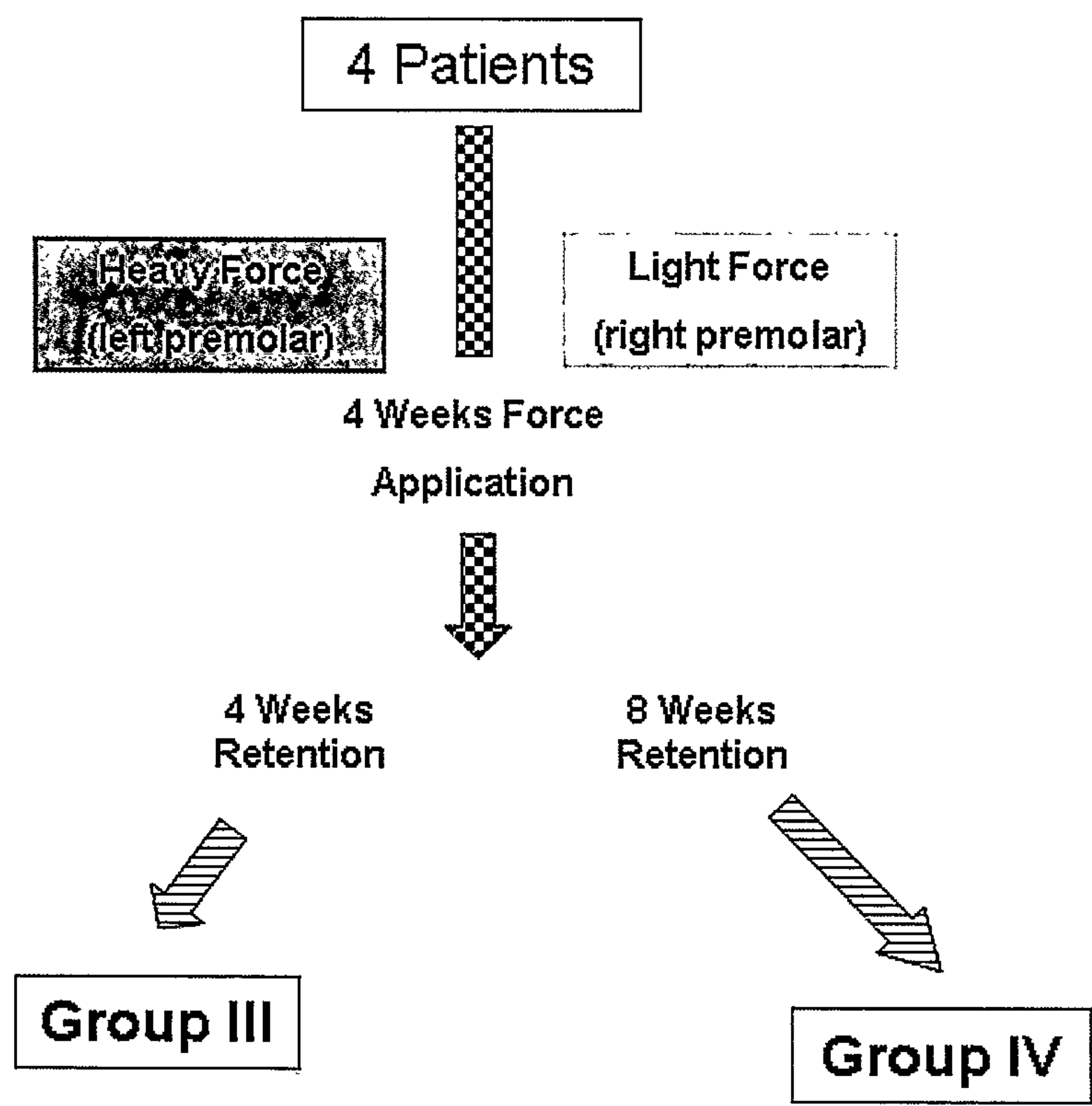


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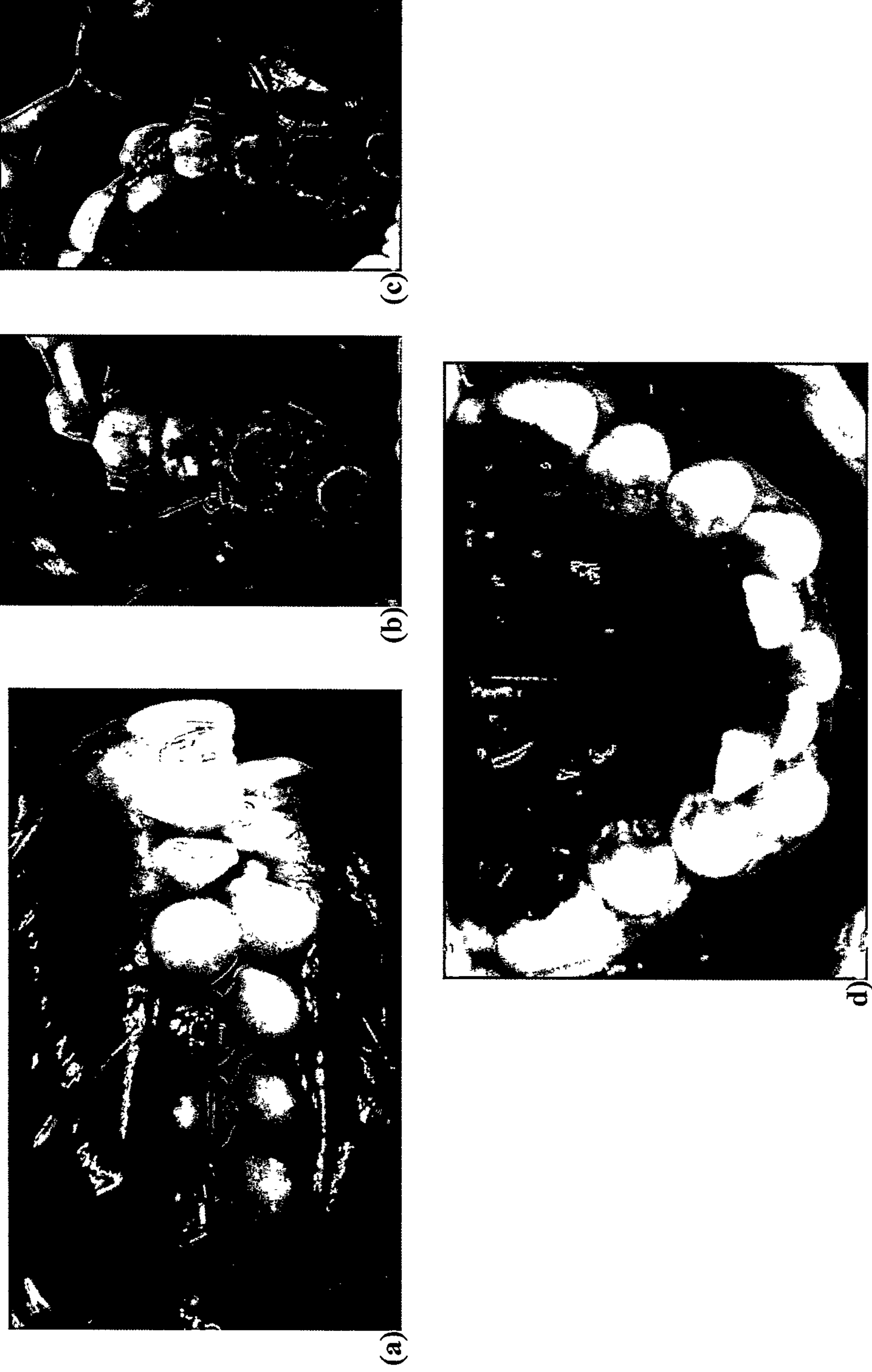


Figure 3 Occlusal view of the passive retention wire (arrows) on the palatal side of the maxillary posterior quadrants.



Figure 4 Specimen mounting in the Skyscan 1172 X-ray micro-CT system

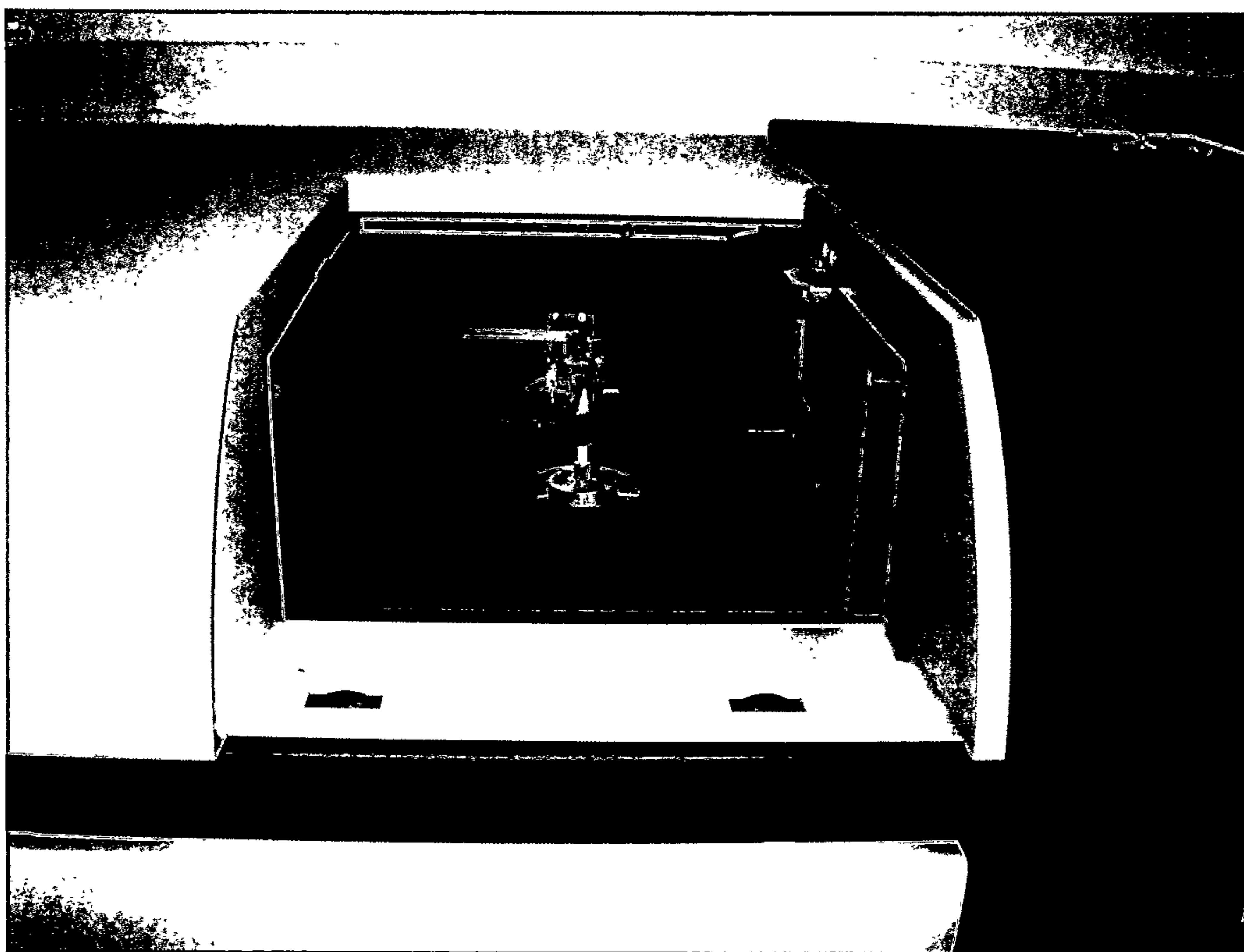


Figure 5 (a) Usage of micro-CT images to identify the regions of interest. Micro-CT images were used to show the direction of histological sectioning (b) single rooted premolars were sectioned parasagittally as shown by dotted line (c) two rooted teeth were first sectioned into buccal and lingual roots then sectioned parasagittally as shown by dotted line (d) the resultant specimen was embedded in paraffin wax.

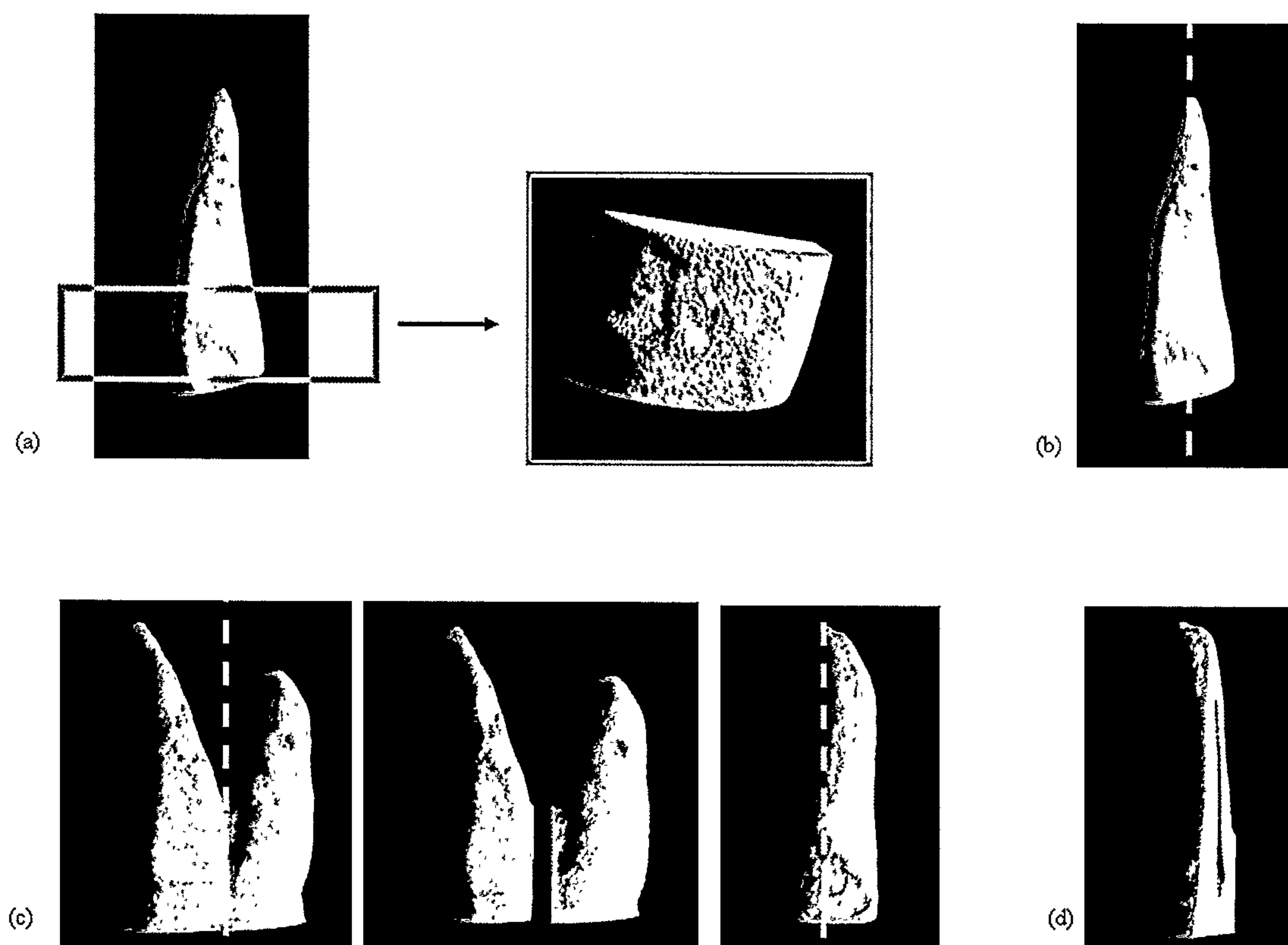


Figure 6 Histological section of the buccal root of a tooth subjected to 4 weeks of light force followed by 4 weeks of retention ($1.25\times$ OM). Arrows indicated areas of resorption craters.

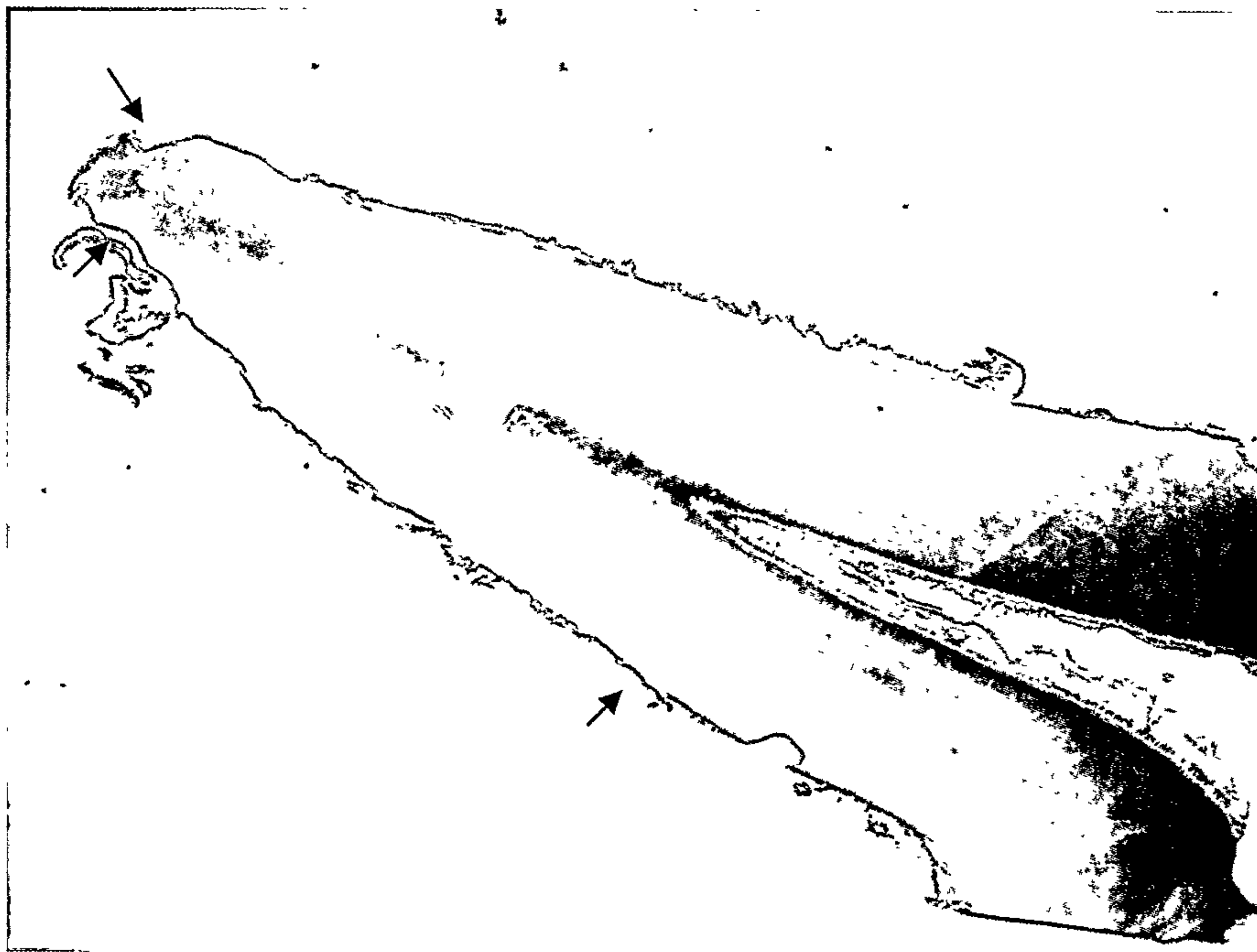


Figure 7 Root resorption crater after 4 weeks of light orthodontic force application following 4 weeks of retention showed cellular cementum (C) overlaid by a layer of mononuclear cells (arrow) ($20\times$ OM).

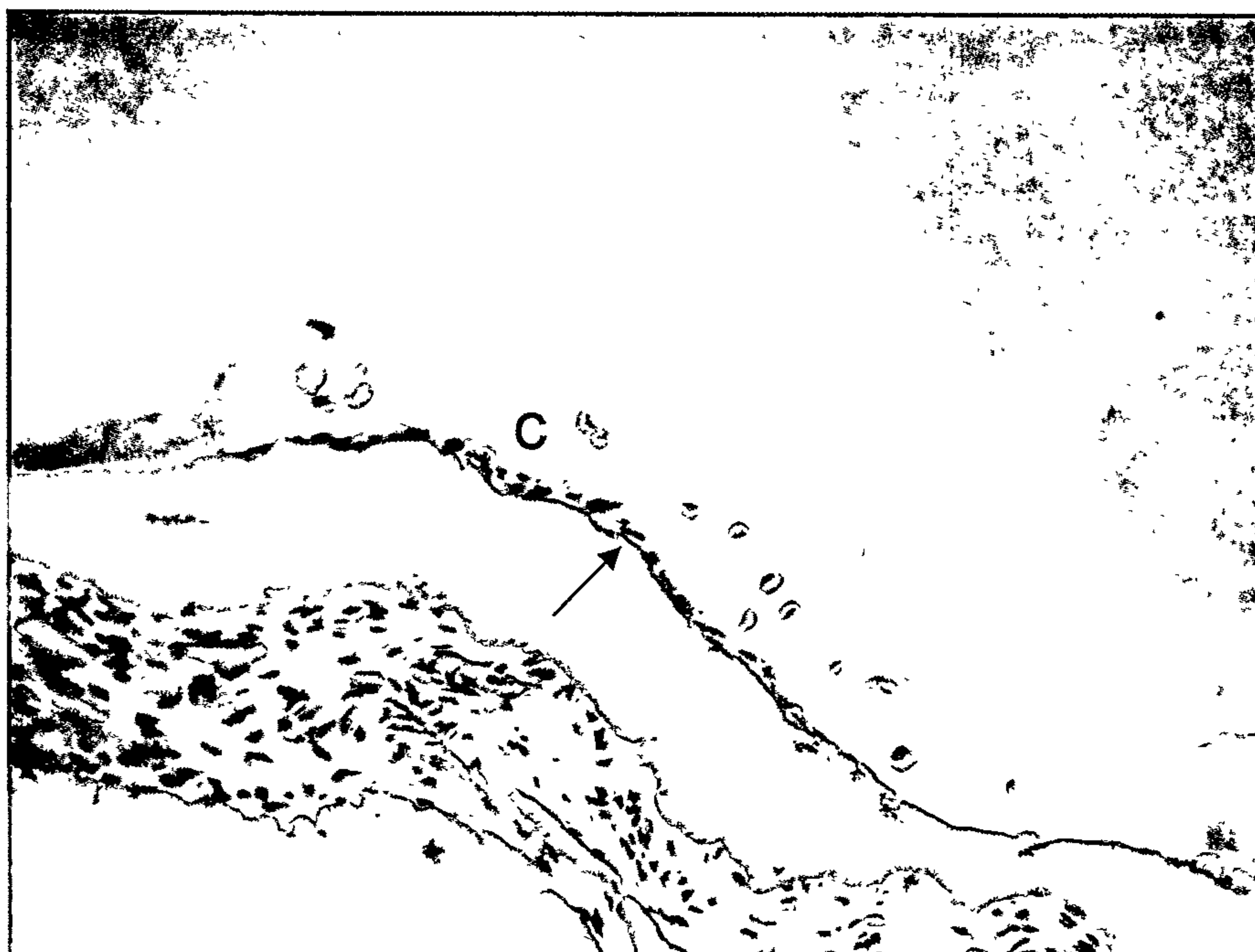


Figure 8 Active root resorption crater with inflammatory infiltrates, blood cells and minimal reparative cementum (20× OM).

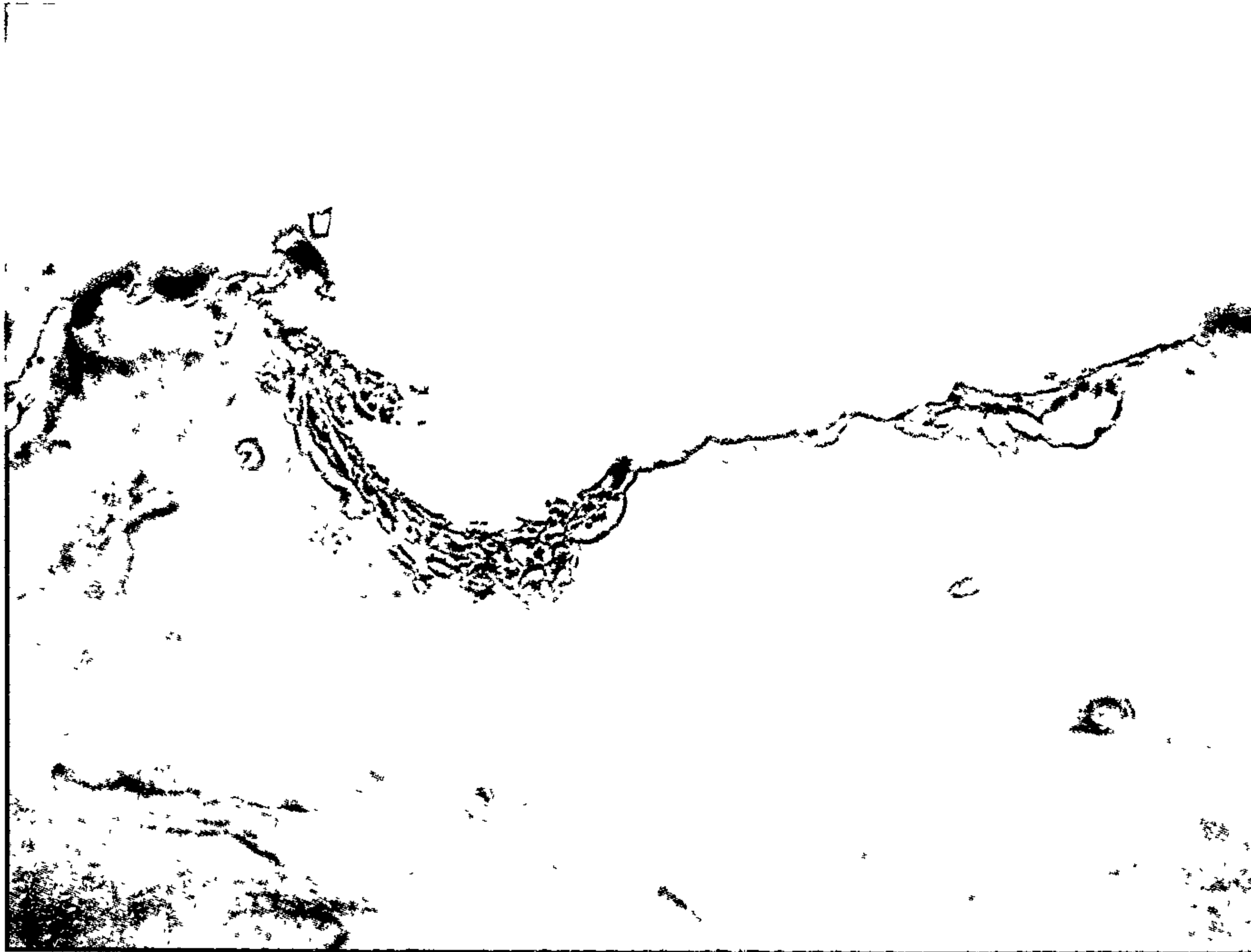


Figure 9 Active cementum reparative process with acellular cementum lining the base of the resorption cavity (AC) and cementocytes were trapped in the superficial layer of newly formed cementum (arrows) (20× OM).

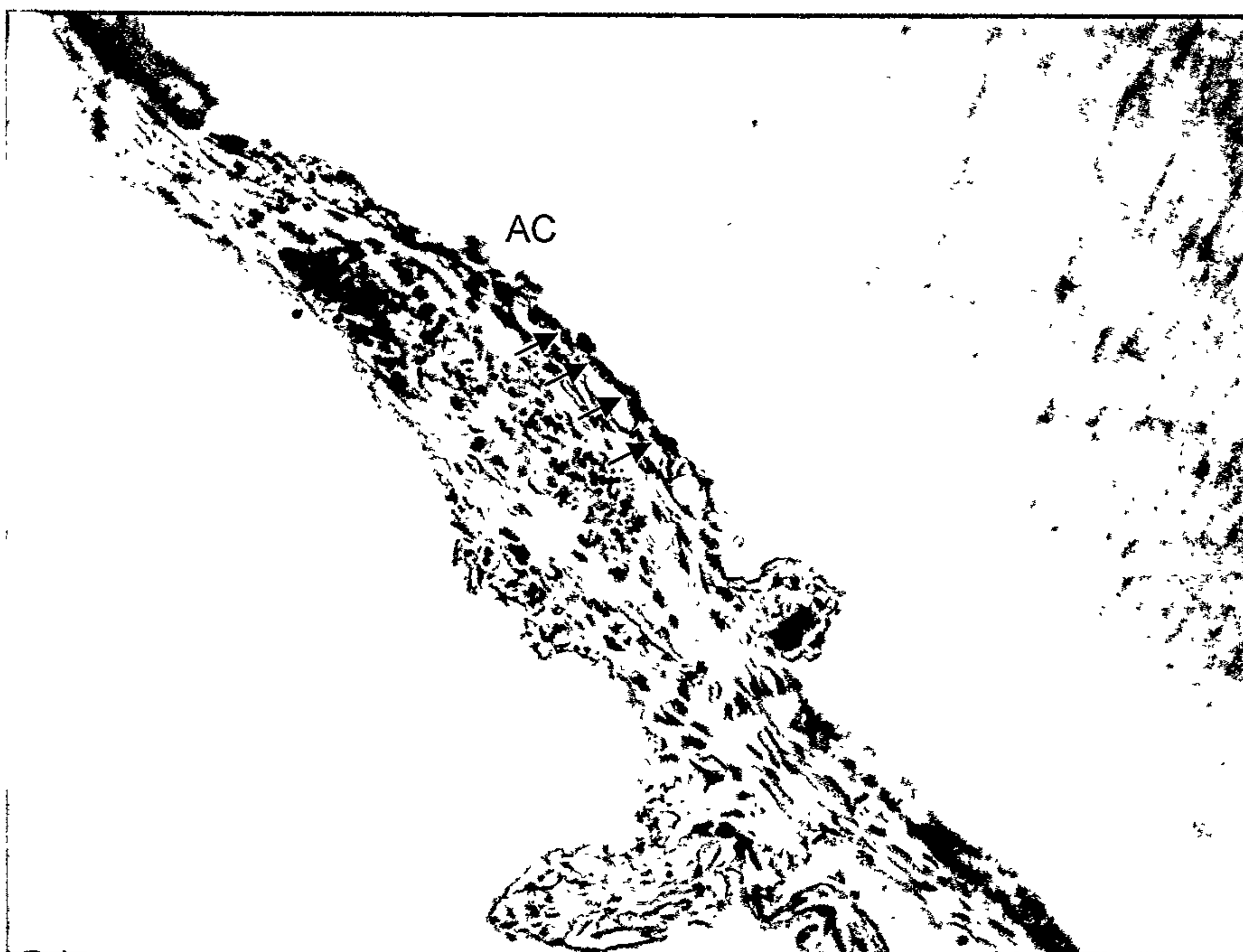


Figure 10 Histological section of the buccal root of a tooth subjected to 4 weeks of heavy force followed by 4 weeks of retention (1.25× OM). Arrow indicated the resorption crater.



Figure 11 Histological section of the lingual root of a tooth subjected to 4 weeks of heavy force followed by 4 weeks of retention (1.25× OM). Arrow indicated the resorption crater.

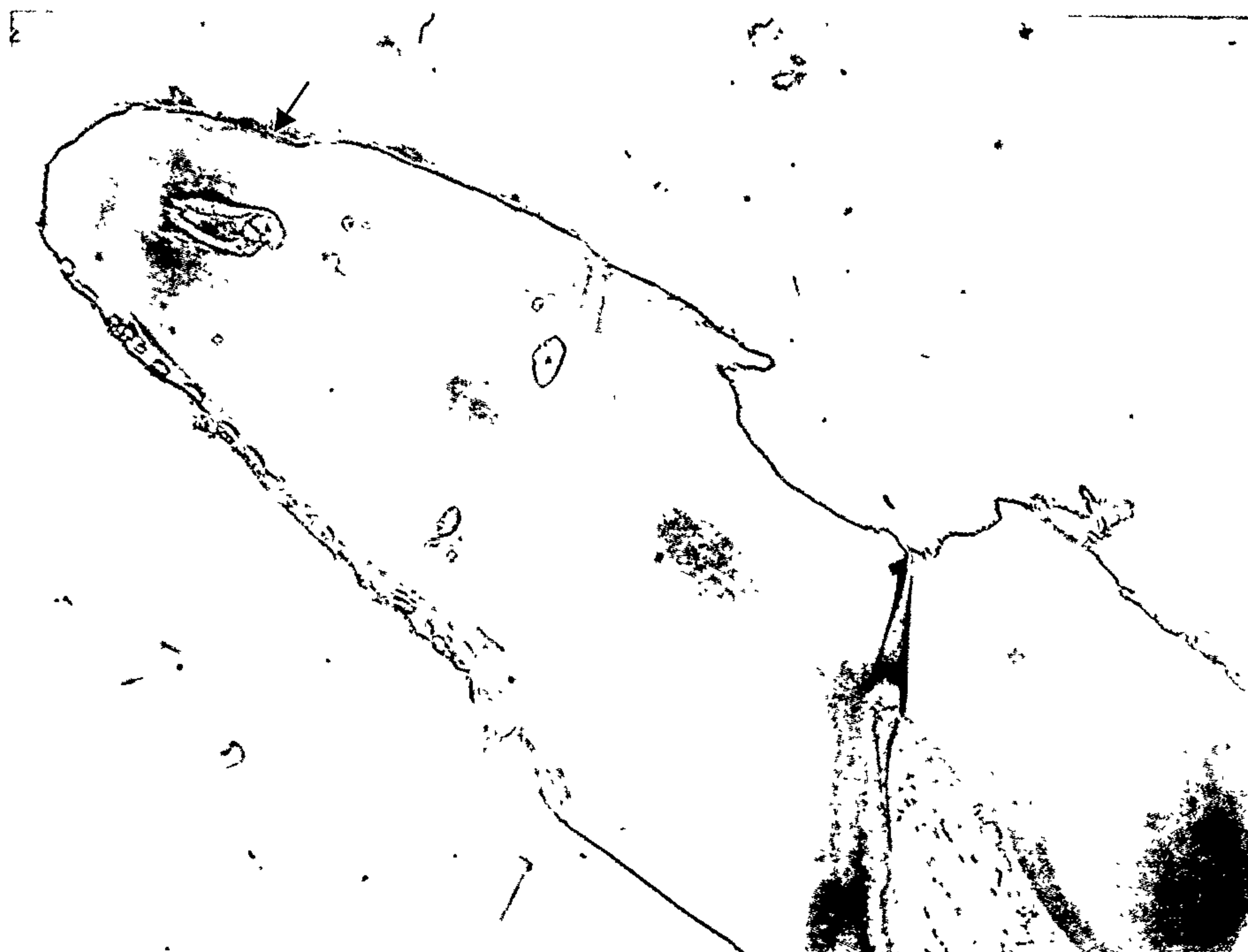


Figure 12 Root resorption crater after 4 weeks of heavy orthodontic force application following 4 weeks of retention showed minimal repair. Resorption cavity was filled with inflammatory infiltrate. Reparative cementum was thicker in the central part (black arrows) than the peripheral part (green arrows) of the resorption cavity (20× OM).



Figure 13 Reparative cementum deposited outside the resorption cavity (arrows) may indicate hypercementosis (4× OM).

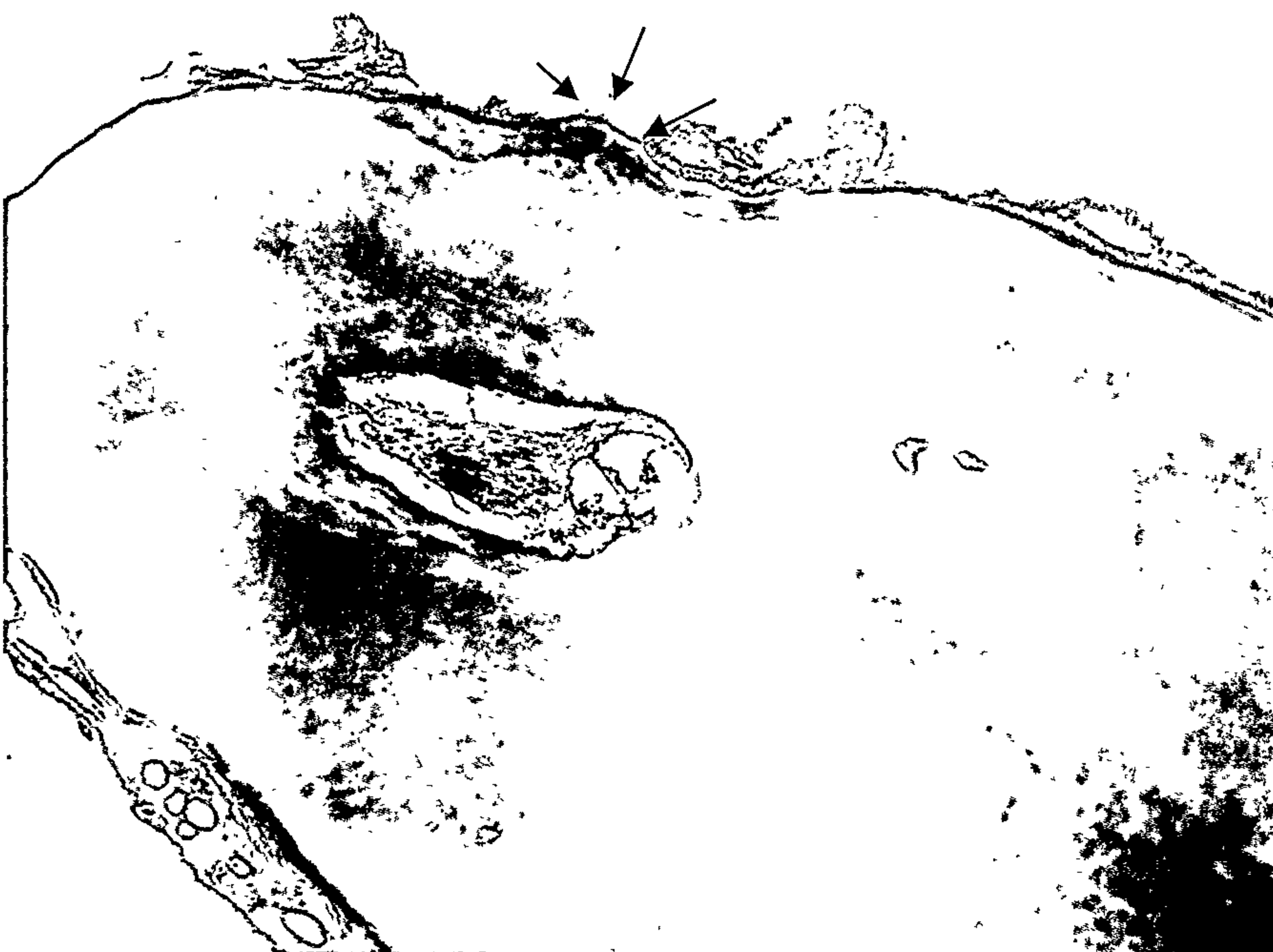


Figure 14 Root resorption crater after 4 weeks of light orthodontic force application following 8 weeks of retention showed almost full repair of the resorption crater with cellular cementum (C) (20× OM).

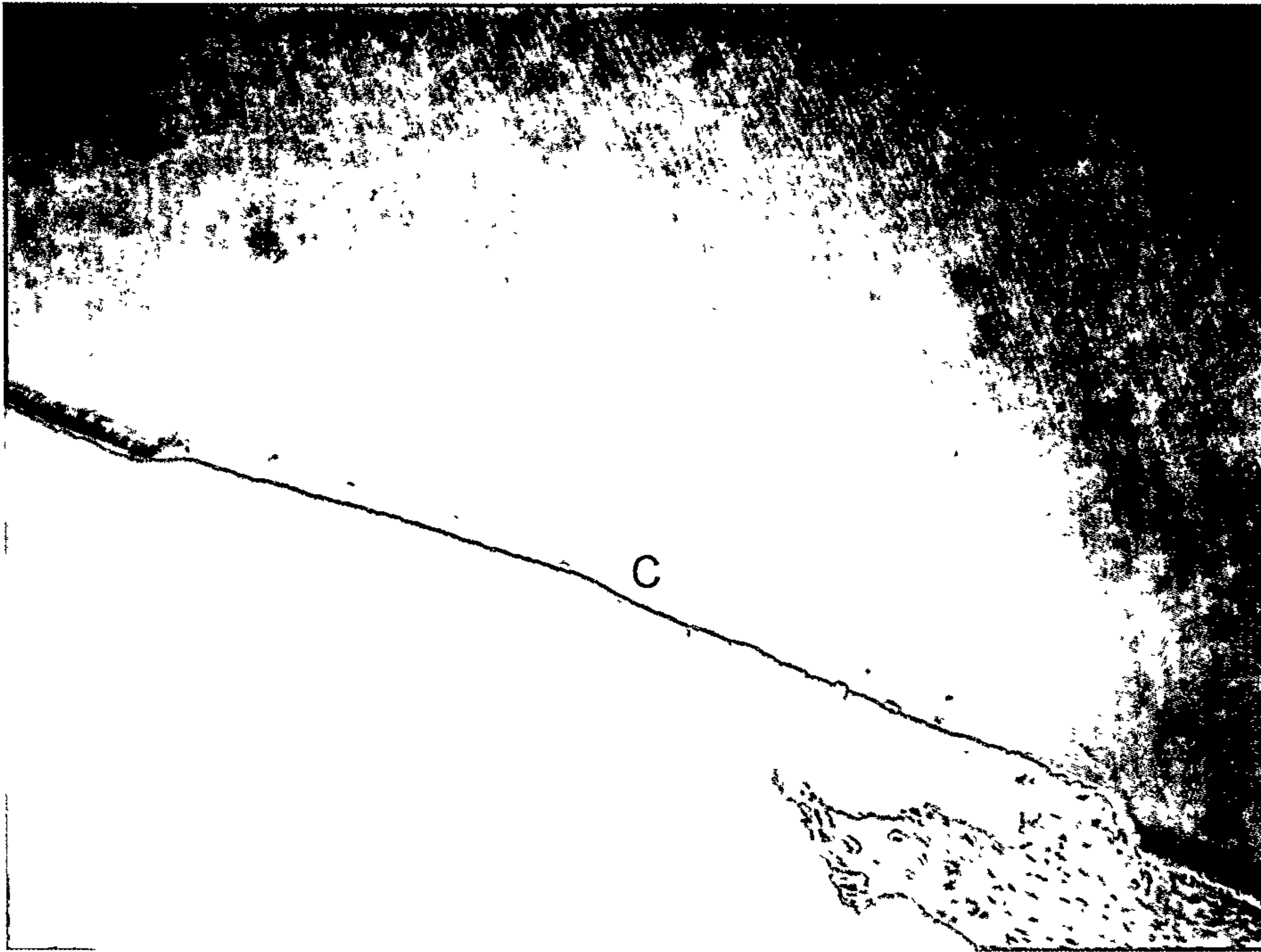


Figure 15 Root resorption craters after 4 weeks of heavy orthodontic force application following 8 weeks of retention showed large amounts of reparative cementum. Small amounts of cementocytes were included into the newly formed cementum (a) 10× OM (b) 20× OM.

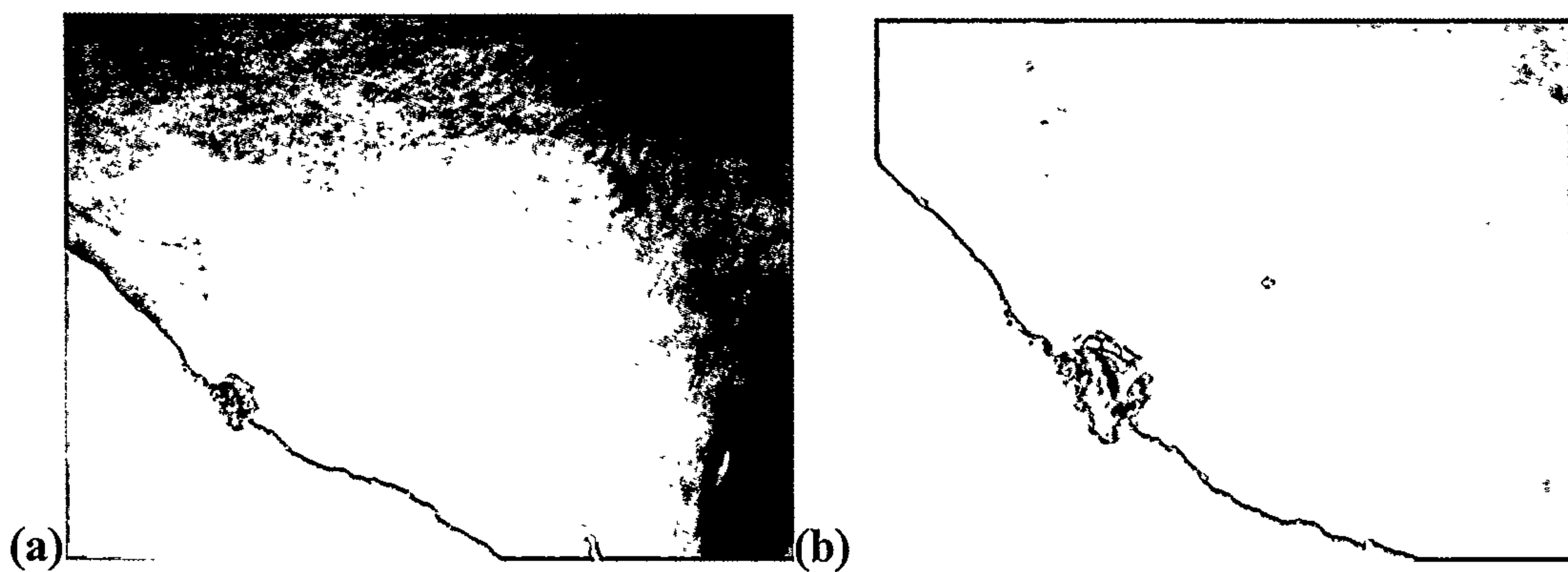
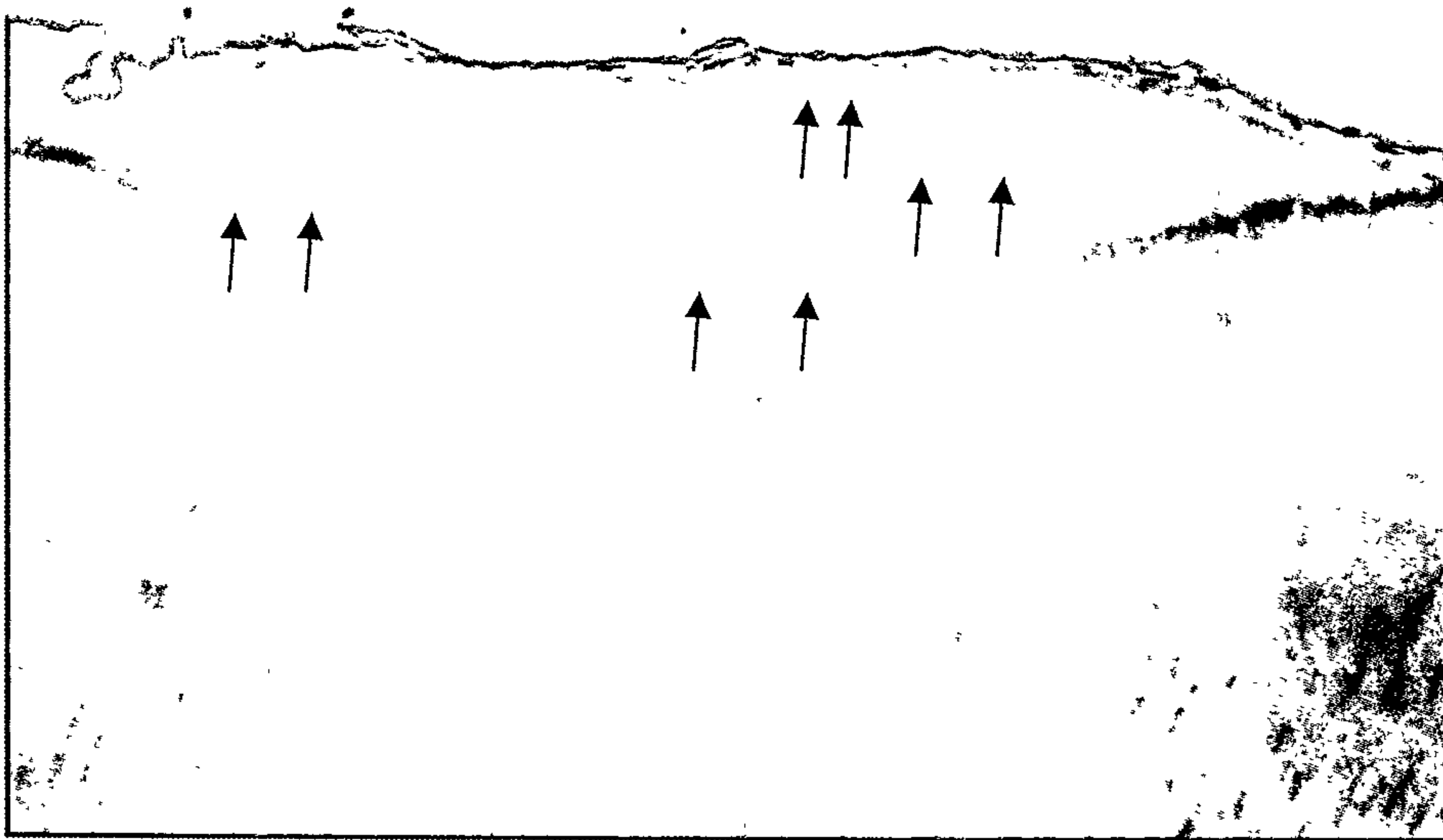


Figure 16 Reparative cementum showed reversal lines (arrows) which indicated periods of cementum remodelling which was similar to the reversal lines that were seen in bone remodelling (20× OM).



10. Future Directions

The results of the present experiment showed the least amount of root resorption occurred after eight weeks of passive retention following four weeks of light orthodontic force application. However, the differences between all study groups were not statistically significant. This could be attributed to the wide individual variations as displayed in the results of this study. The difference between Groups IIL and IH were quite close to being statistically significant ($p=0.06$) (Appendix 18). In order to highlight this difference to a clinically significant level, it is recommended to increase the sample size to approximately 24 subjects for each group.

It has also been identified in the current study that the majority of the reparative process seemed to occur after four weeks of passive retention following heavy force application. However, the time intervals between the retention periods studied were too long to extrapolate the precise reparative process through time. In addition, the experimental period of eight weeks was not long enough to elucidate whether the resorption cavity would fully recover following retention. This may justify the use of heavy forces in some clinical situations. Therefore, future research should be directed towards increasing the experimental period and decreasing the observation intervals.

Due to ethical and practical constraints in human clinical research, this study was built on the assumption that the intra-individual differences in the volumes of the resorption craters between the teeth of the positive control and experimental groups were estimates of the amount of reparative cementum. Histological sectioning

would be the only mean in the current research armamentarium to differentiate between the reparative cementum from existing cementum. However, this would be limited to qualitative analysis which has already been widely documented in previous studies. There are evidently structural and possibly chemical differences between the reparative cementum and the existing cementum. Perhaps with the evolution of the nano-computed tomography and the associated software, it may be possible to quantify the exact amount of reparative cementum. Another method that could possibly detect the amount of reparative cementum with the current technology of the XMT is to stain the experimental teeth with a radioactive dye that could be selectively absorbed by the newly formed cementum matrix. However, to date there is no literature that has indicated the appropriate staining medium. Therefore, more research on the structural differences between the reparative and existing cementum would indicate the appropriate staining method for this type of research.

This experiment created a basis for future research on the reparative process of root resorption craters. Future studies could be directed to investigate:

- The amount of repair following longer force applications e.g. eight or 12 weeks;
- The amount of repair following different force application schemes e.g. intermittent vs. continuous force and different force directions e.g. intrusion vs. torque vs. extrusion;
- The amount of repair with the use of different retention appliances;
- The mineral analysis of the root resorption craters following repair through time;
- The physical hardness test on the repaired resorption craters through time;

- The use of fluoride, calcium or phosphorus following force application to enhance the amount of cementum repaired.

11. Appendix

Appendix 1 – Incidence of Orthodontically Induced Inflammatory Root Resorption

Authors	Type of Study	Number of Subjects	Incidence of Physiological Root Resorption	Incidence of OIIRR
Ketcham 1927 ³⁴	Periapical radiographs	385	1%	21%
Rudolph 1936 ⁴⁰	Radiographs	439	0%	75%
Rudolph 1940 ⁴¹	Radiographs	513 orthodontic 739 non-orthodontic	5%	100%
Becks 1939 ⁴²	Periapical radiographs	145	32%	73.6%
Levander & Malmgren 1988 ⁴⁵	Periapical radiographs	390	33% 1% (severe)	48% (minor), 17% (severe), 1% (extreme)
Mirabella & Artun 1995 ⁴⁶	Periapical radiographs	343 (adults)	-	40% ≥1 teeth with ≥2.5mm
Lupi et al 1996 ⁴⁷	Periapical radiographs	88 (adults)	15% 2% (moderate – severe)	73% 24.5% (moderate – severe)
Sameshima & Sinclair 2001 ⁴⁹	Periapical radiographs	868	-	25% ≥2mm (maxillary anterior teeth)

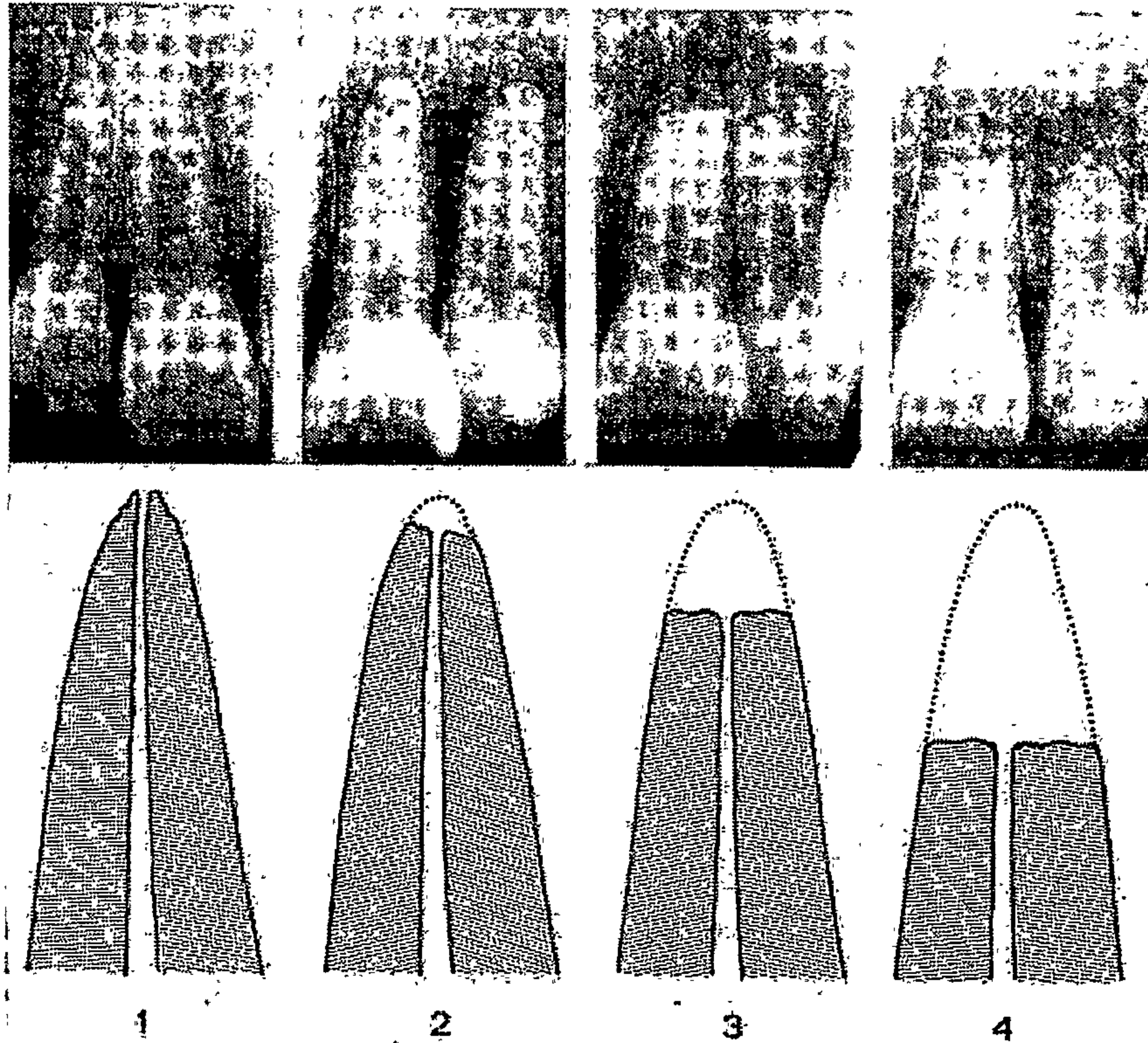
Appendix 2 – Method of Assessing Periapical Root Resorption in Radiographs

(Massler M, Malone AJ. Root resorption in human permanent teeth; a roentgenographic study. Am J Orthod 1954;40:619-633)

Degree or Type of Resorption	Description
0	No evidence of resorption
?	Resorption questionable. Root outline intact but there appear to be minute areas of spotty resorption. Lamina dura is interrupted and the periodontal membrane widened.
1+	Root apex definitely blunted and resorbed for at least 1mm to about 2mm. Lamina dura interrupted and periodontal membrane widened about the periapical area of the root.
2+	Resorption of root apex for at least 2mm to 4mm. lamina dura interrupted and peridontal membrane widened.
3+	Resorption of root 4mm to one-half the root length.
4+	More than one-half the root resorbed.
#5	Root resorption definitely related to root canal therapy (degree not assessed).
#6	Root resorption definitely related to periapical infection (cysts etc.).
8	Not diagnosable (roentgenogram of poor quality).
9	Tooth missing.

Appendix 3 – Root Resorption Index for Qualitative Assessment of Root Resorption

(Malmgren O, Goldson L, Hill C, Orwin A, Petrini L, Lundberg M. Root resorption after orthodontic treatment of traumatized teeth. Am J Orthod 1982;82:487-491)



1. Irregular root contour
2. Root resorption apically, amounting to less than 2mm. Minor resorption.
3. Root resorption apically, from 2mm to one third of the original root length. Severe resorption.
4. Root resorption exceeding one third of the original root length. Extreme resorption.

Appendix 4 – Studies on Orthodontically Induced Inflammatory Root Resorption

Authors	Type of Studies	Type of Subjects	Number of Subjects	Type of Appliance	Duration of Experiment	Type of Findings
Brudvik & Rygh 1993 ^{2,12}	Light microscopy	Wistar rats	21	Springs 50g	6 hours - 5 days	Cellular reactions
Kvam 1972 ⁶⁷	SEM	Human extracted teeth	26 teeth	Springs 50g	5-76 days	Topographic, diameters or craters
Harry & Sims 1982 ³⁹	SEM	Human extracted teeth	18 control 18 experiment 4 RME + 3 mo. retention	Intrusion force 50g, 100g, 200g	14, 35, 70 days	Topographic changes
Barber & Sims 1981 ⁸	SEM	Human extracted teeth	5 control 13 experiment	RME	Retention of 8-9 months after RME	Topographic changes
Mavragani et al 2004 ⁷⁷	SEM & light microscopy	Wistar rats	36	Springs 50g	1-4 days	Morphologic, area of craters
Brudvik & Rygh 1994 ⁶⁹	TEM	Wistar rats	12	Springs 50g	7-10 days	Ultrastructural
Faltin et al 2001 ¹³	TEM	Human extracted teeth	12	NiTi wires 50 or 100 cN	4 weeks	Ultrastructural

Appendix 5 – Cellular Events Associated with the Periphery Zone of Root Resorption Crater

(Brudvik P, Rygh P. The initial phase of orthodontic root resorption incident to local compression of the periodontal ligament. Eur J Orthod 1993;15:249-263

Brudvik P, Rygh P. Root resorption beneath the main hyalinized zone. Eur J Orthod 1994;16:249-263)

2-3 days	The layer of PDL adjacent to the main hyalinised zone seemed necrotic. TRAP negative mono-nucleated cells were detected in the surrounding and close to minor cementum cavities near hyalinised tissue indicating that they were not odontoclasts.
3-4 days	TRAP positive mono- and multi-nucleated cells were observed in the alveolar bone then later in the PDL and near the root surface. TRAP negative mono-nucleated cells were observed at the periphery of the hyalinised zones.
5-7 days	TRAP positive multinucleated cells in the resorption cavities extended its activity into the dentine. Resorption activity continued with its advancing front as an undermining resorption toward the main hyalinised zone.
10-21 days	Reparative cementum like material that was stained relatively dark by H&E appeared in the periphery of the resorption lacunae. TRAP negative mono-nucleated fibroblast like cells were observed close to the newly formed cementum.

Appendix 6 - Cellular Events Associated with the Main Hyalinised Zone of Root Resorption Crater

(Brudvik P, Rygh P. The initial phase of orthodontic root resorption incident to local compression of the periodontal ligament. Eur J Orthod 1993;15:249-263

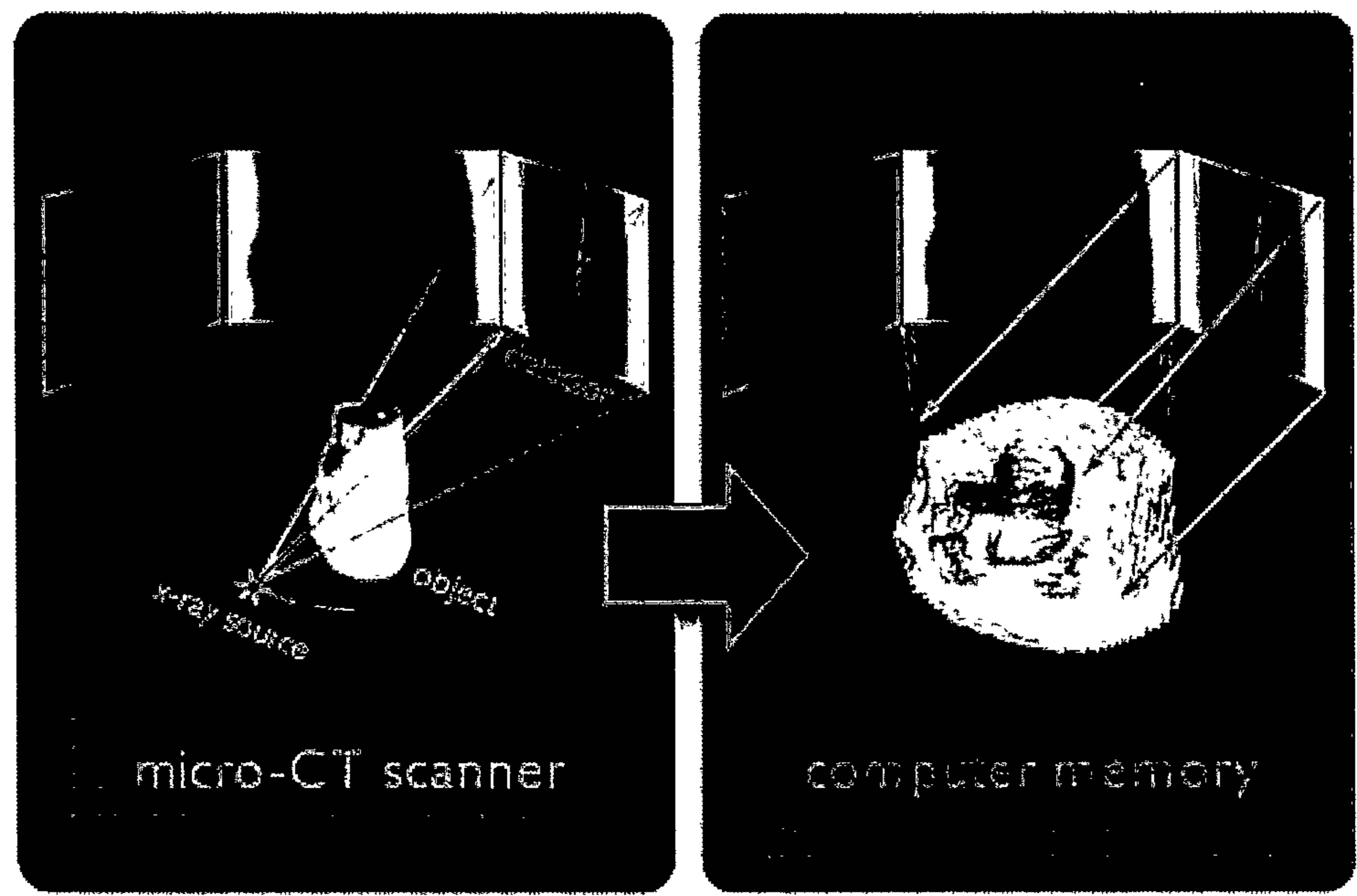
Brudvik P, Rygh P. Root resorption beneath the main hyalinized zone. Eur J Orthod 1994;16:249-263)

2-3 days	No Sharpey's fibres or fibrillar structures were attached to the cementum on the root surface beneath the main hyalinised zone. The cementum in this zone was thinner and stained darker by H&E due to the presence of coagulated necrotic tissue. The width of PDL was compressed to about 1/3 of normal size.
3-7 days	Undermining resorption from all peripheries towards central part of the hyalinised zone. TRAP negative mono-nucleated cells and TRAP positive multinucleated giant cells were responsible for the elimination of the main part of the necrotic tissue and resorb the outer layer of adjacent root cementum.
10-14 days	Most of the hyalinised tissues were eliminated. Extensive root resorption was observed in the peripheral area where the hyalinised tissue used to be located during the first 3 days. TRAP positive multi-nucleated cells with ruffled borders (odontoclast like cells) were infiltrating the remnants of hyalinised tissue in the PDL and the root resorption lacunae. No TRAP positive cells were found in the resorption lacunae in the peripheral hyalinised zone and PDL width was increased.
14-21 days	Deposition of cementum occurred in resorption lacuna near the periphery of hyalinised tissue. No TRAP positive cells in the reparative areas except in the remnants of the hyalinised tissue and on the root surface. Where hyalinised tissue had been completely removed, new PDL cells, fibres, and blood vessels were observed. The PDL width returned to normal.

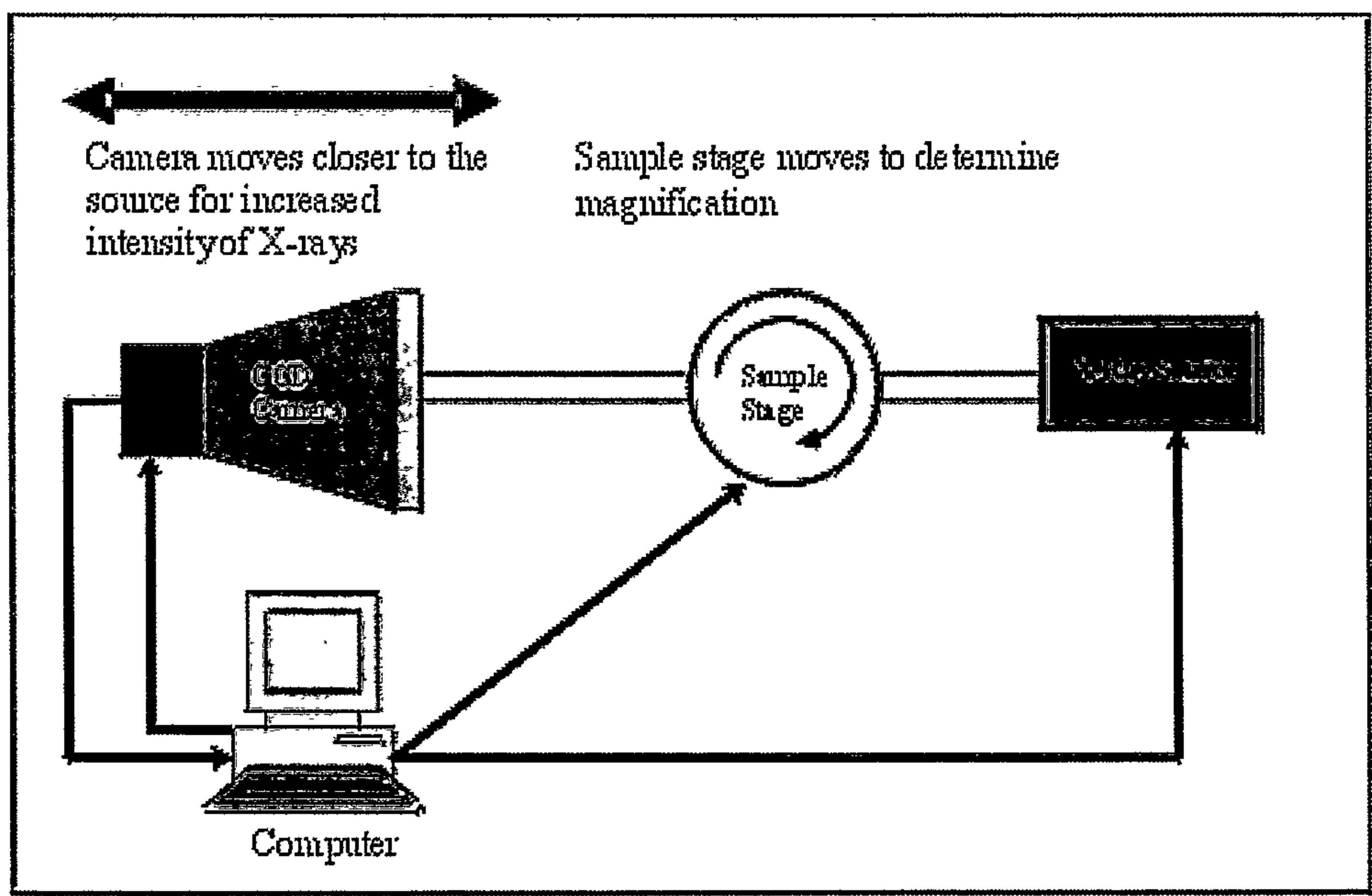
Appendix 7 – Concepts of Desktop X-ray

Microtomography

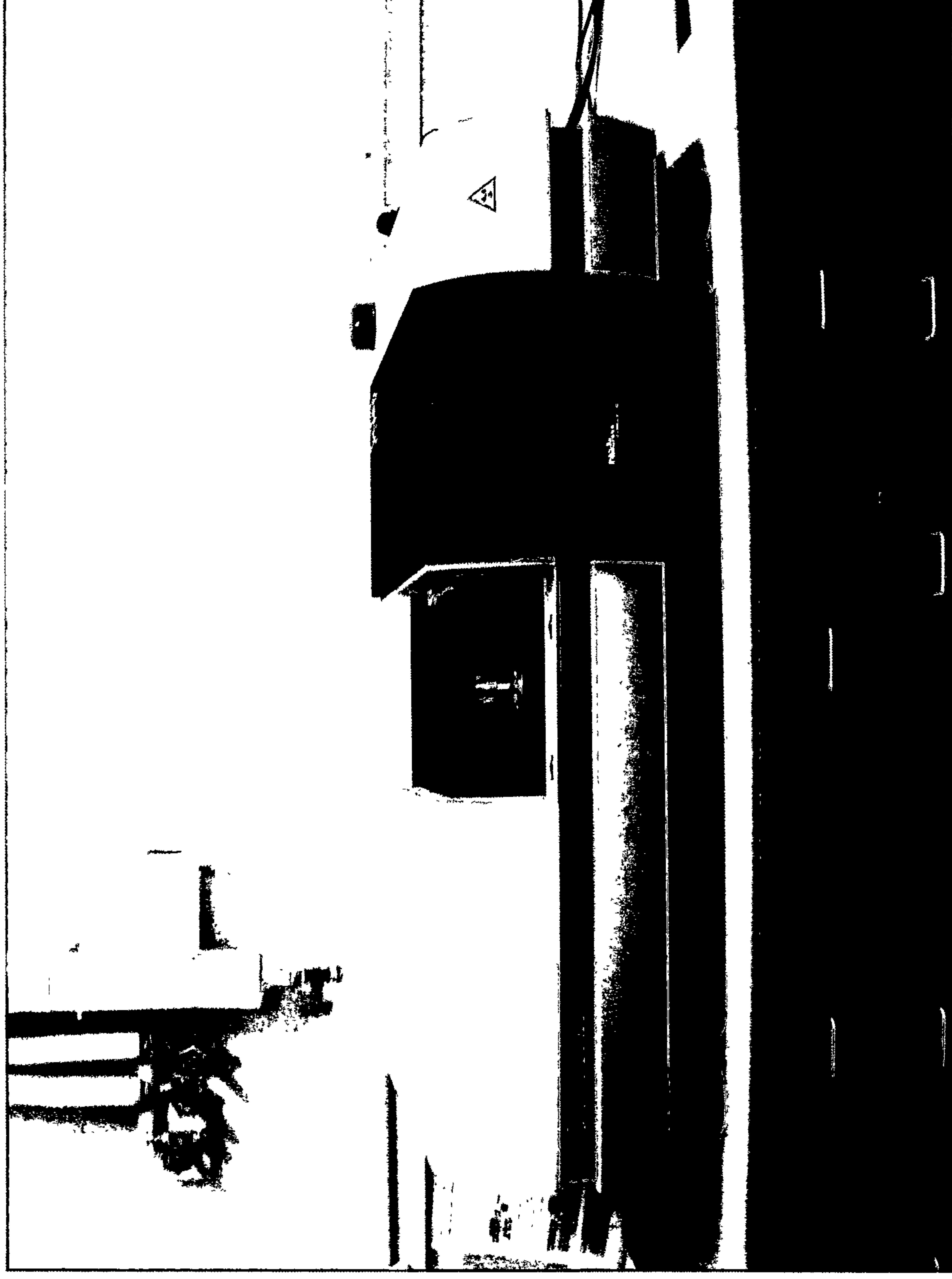
Sasov A. Skyscan. [Online]. 2007 Jun 07 [cited 2007 Jul 16]; Available from: URL: <http://www.skyscan.be/company/methods.htm>



A micro-focus x-ray source illuminates the object and a planar x-ray detector collects magnified projection images. Based on hundreds of angular views acquired while the object rotates, a computer synthesizes a stack of virtual cross section slices through the object.

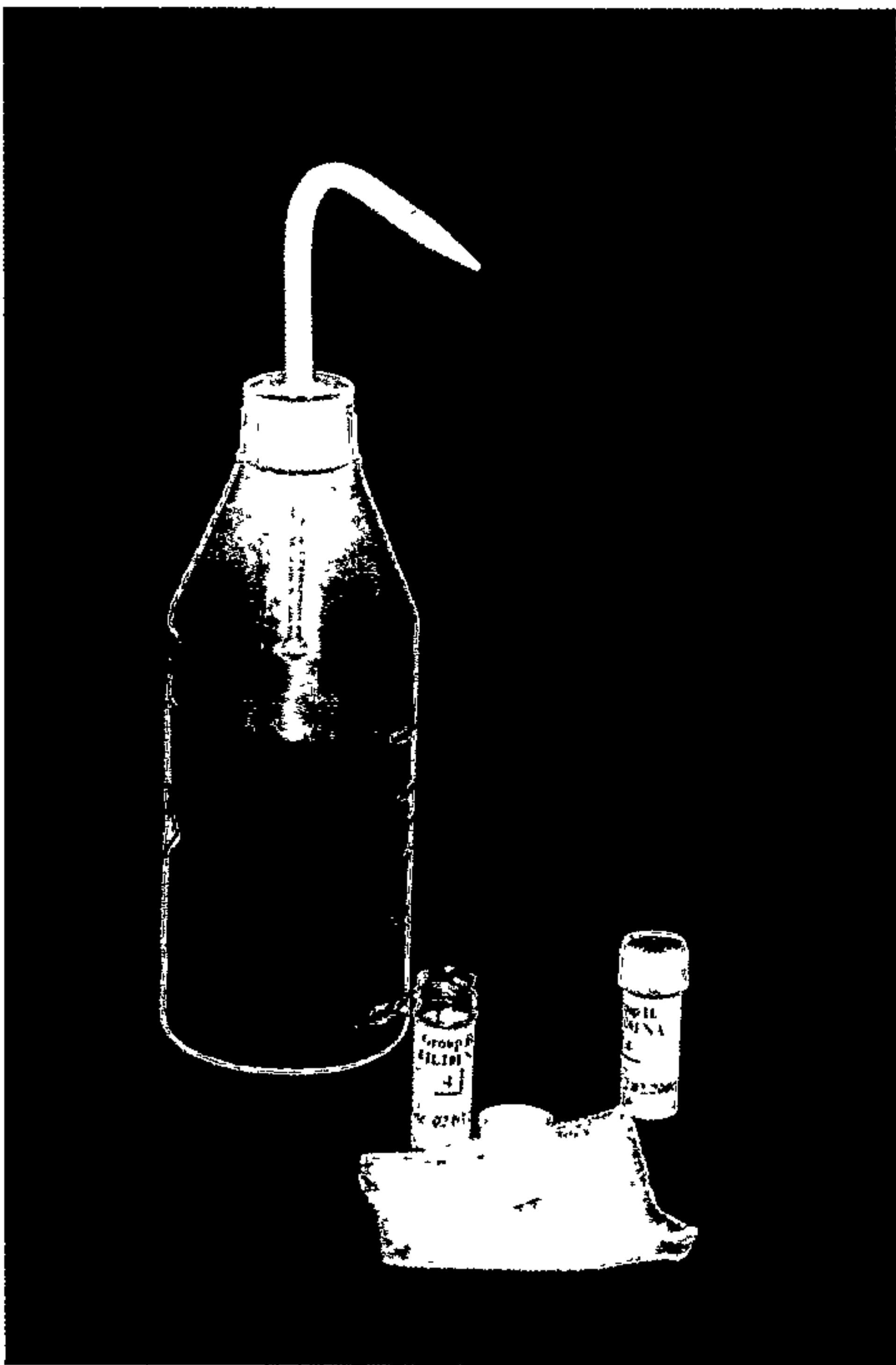
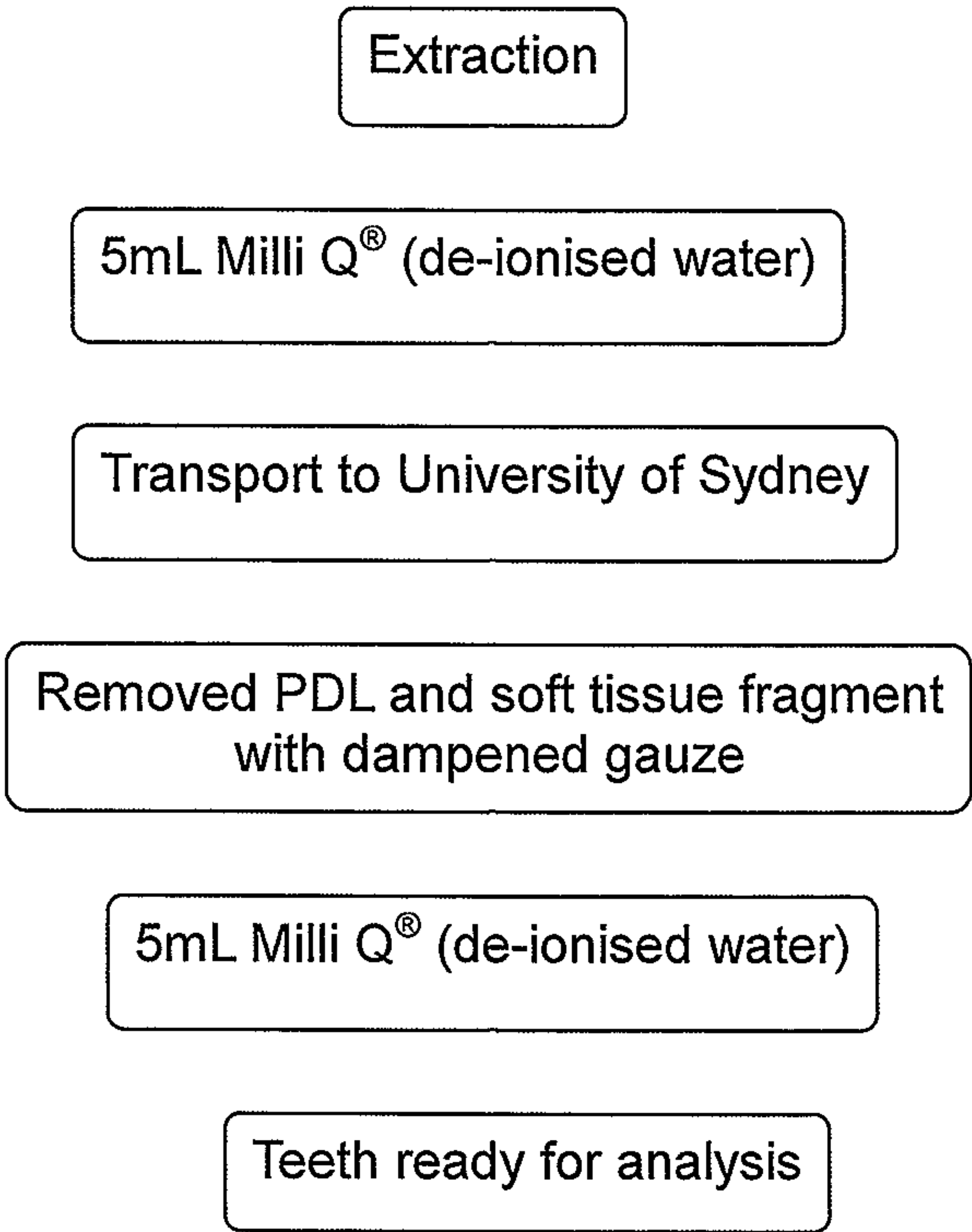


Appendix 8 – Sky Scan 1172 Desktop X-ray Microtomograph



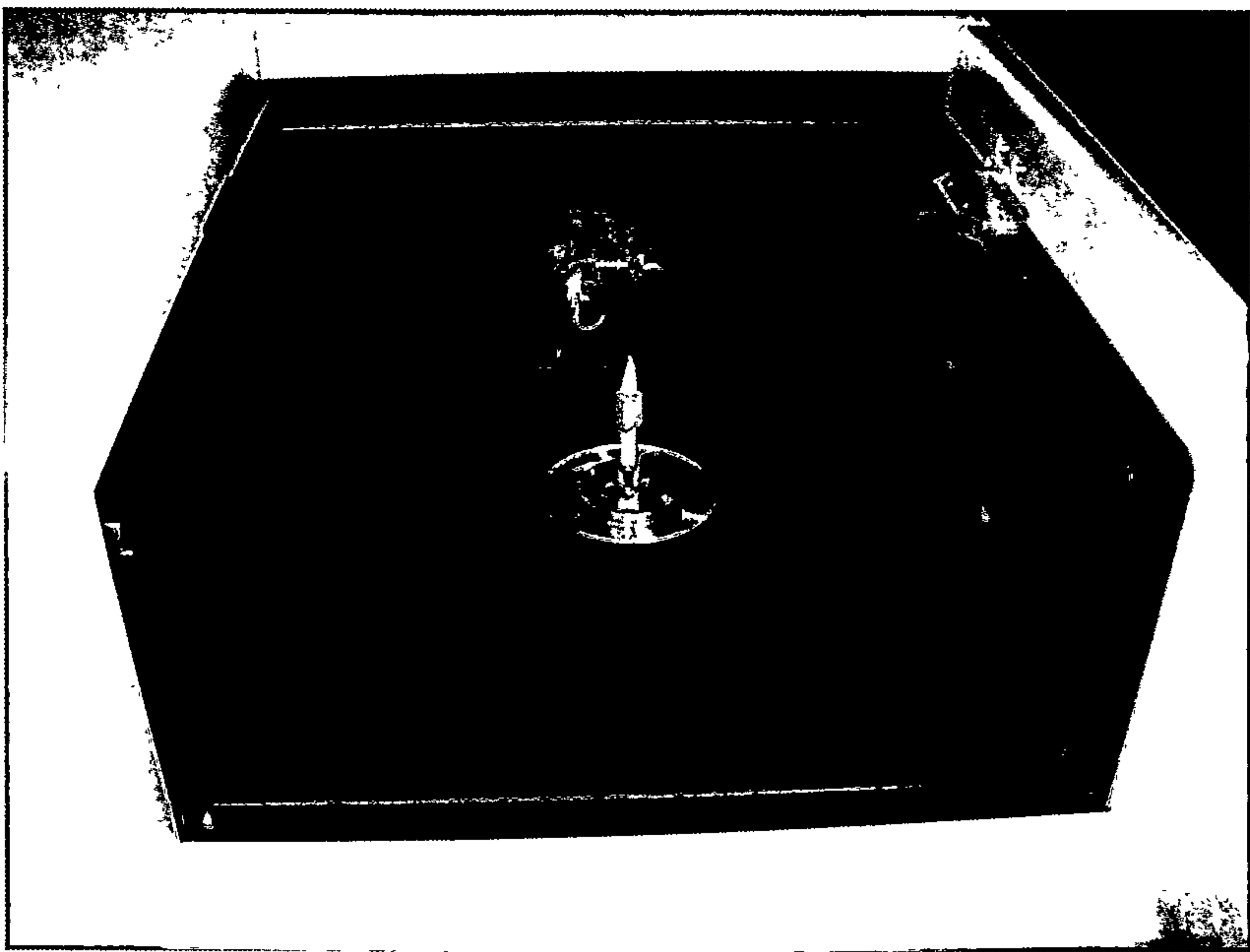
Appendix 9 – Experimental Setup for micro-CT Study

Storage Method

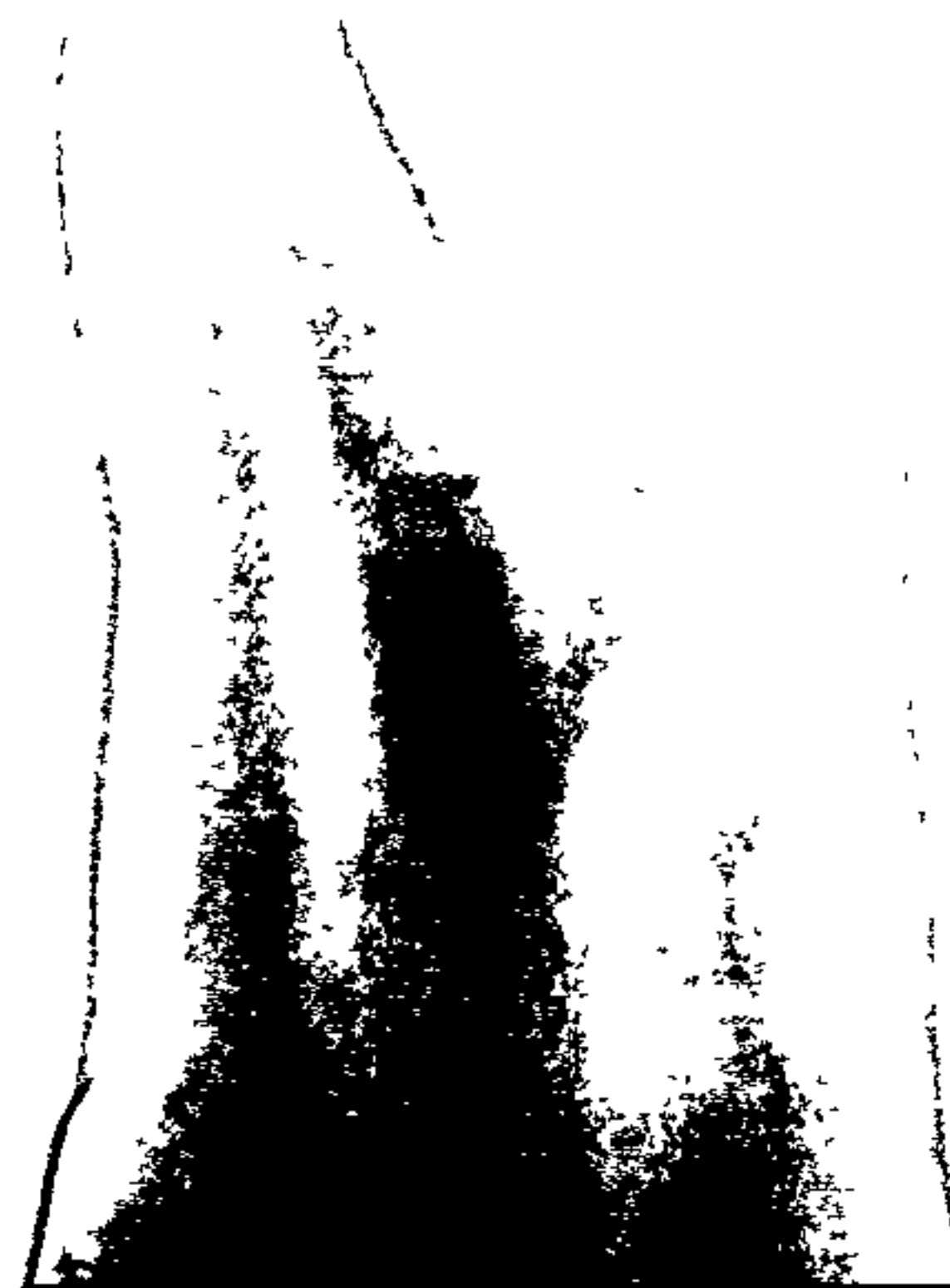
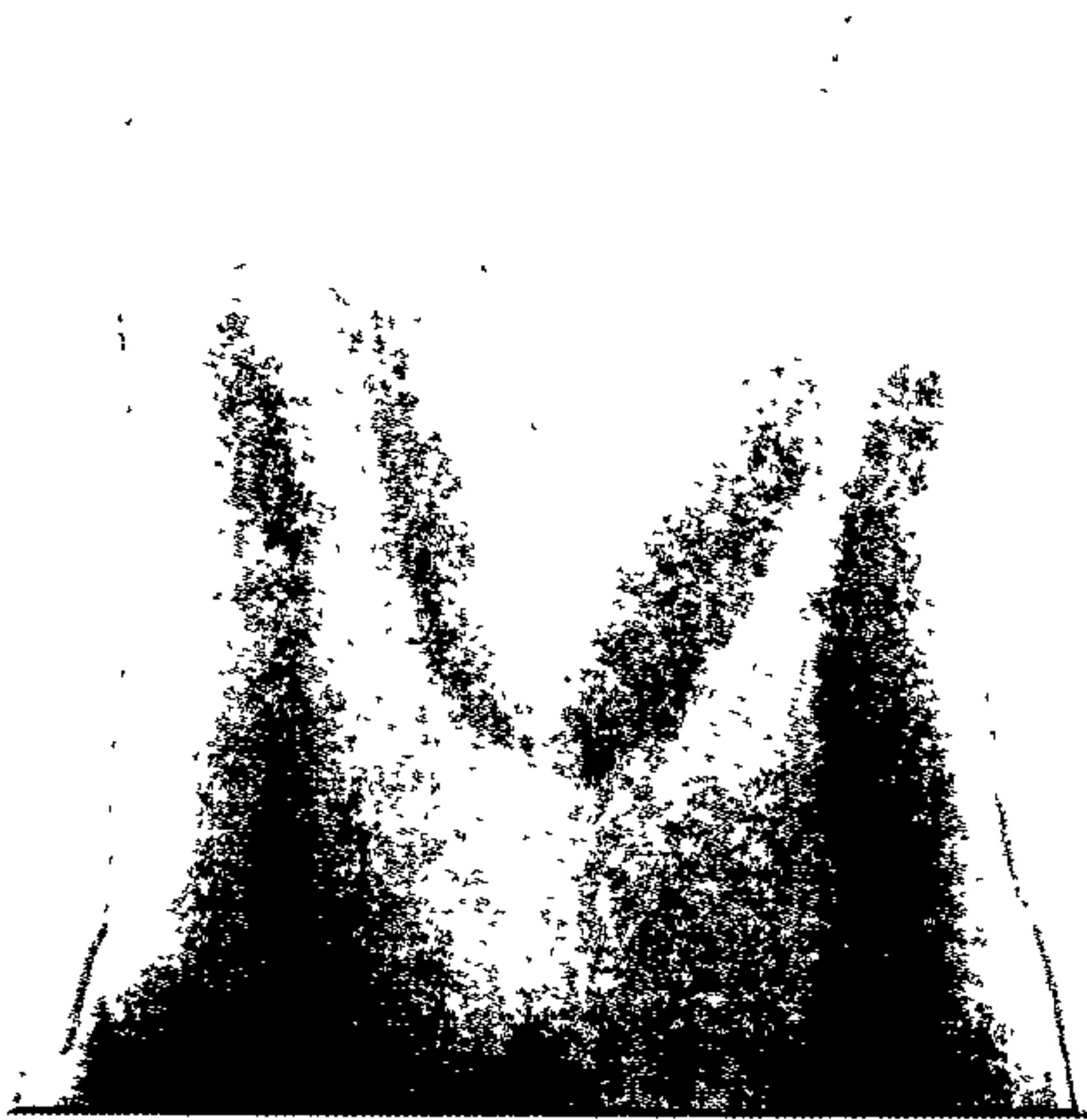


Mounting of Teeth on the SkyScan 1172 Desktop X-ray Microtomograph

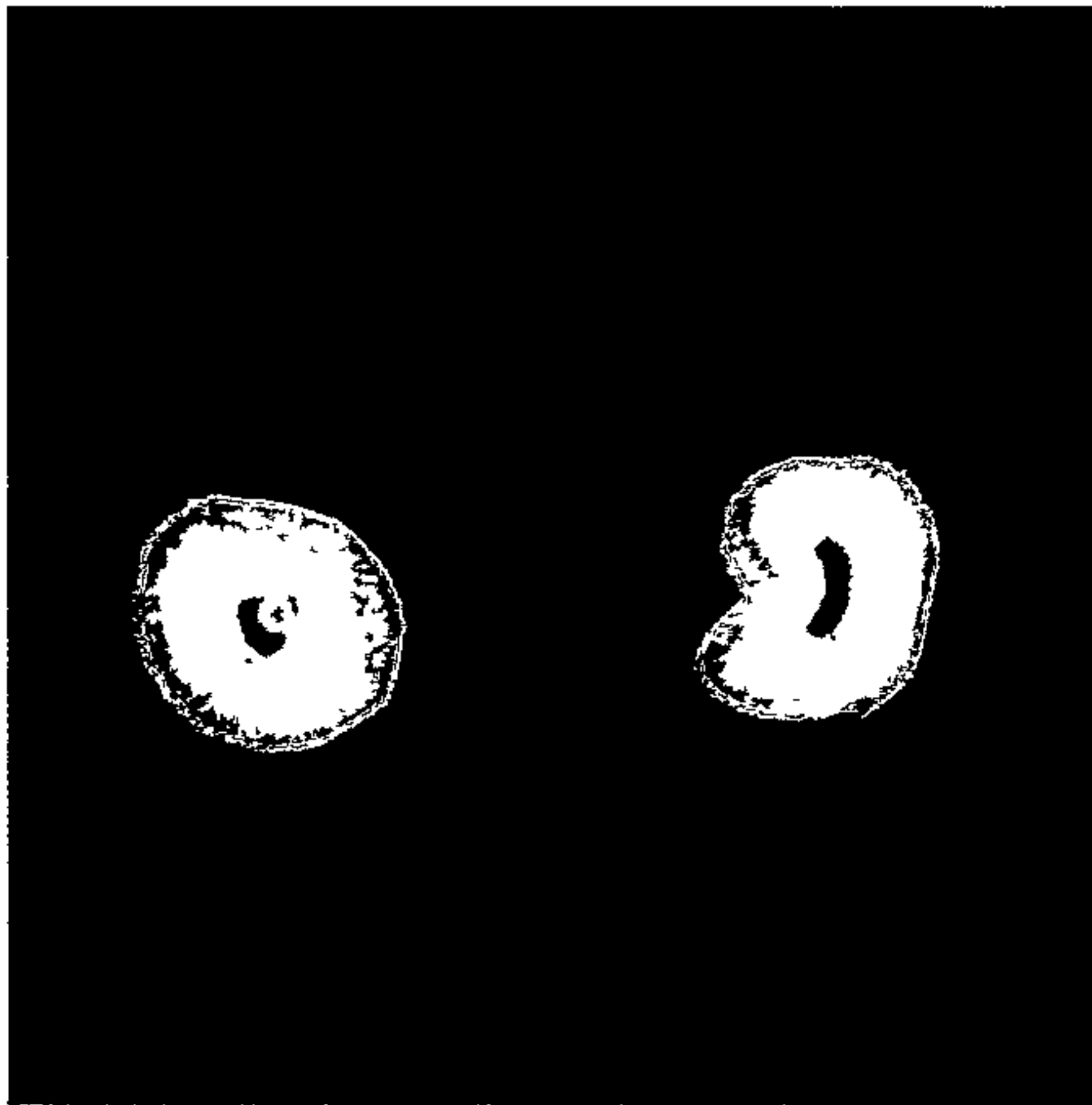
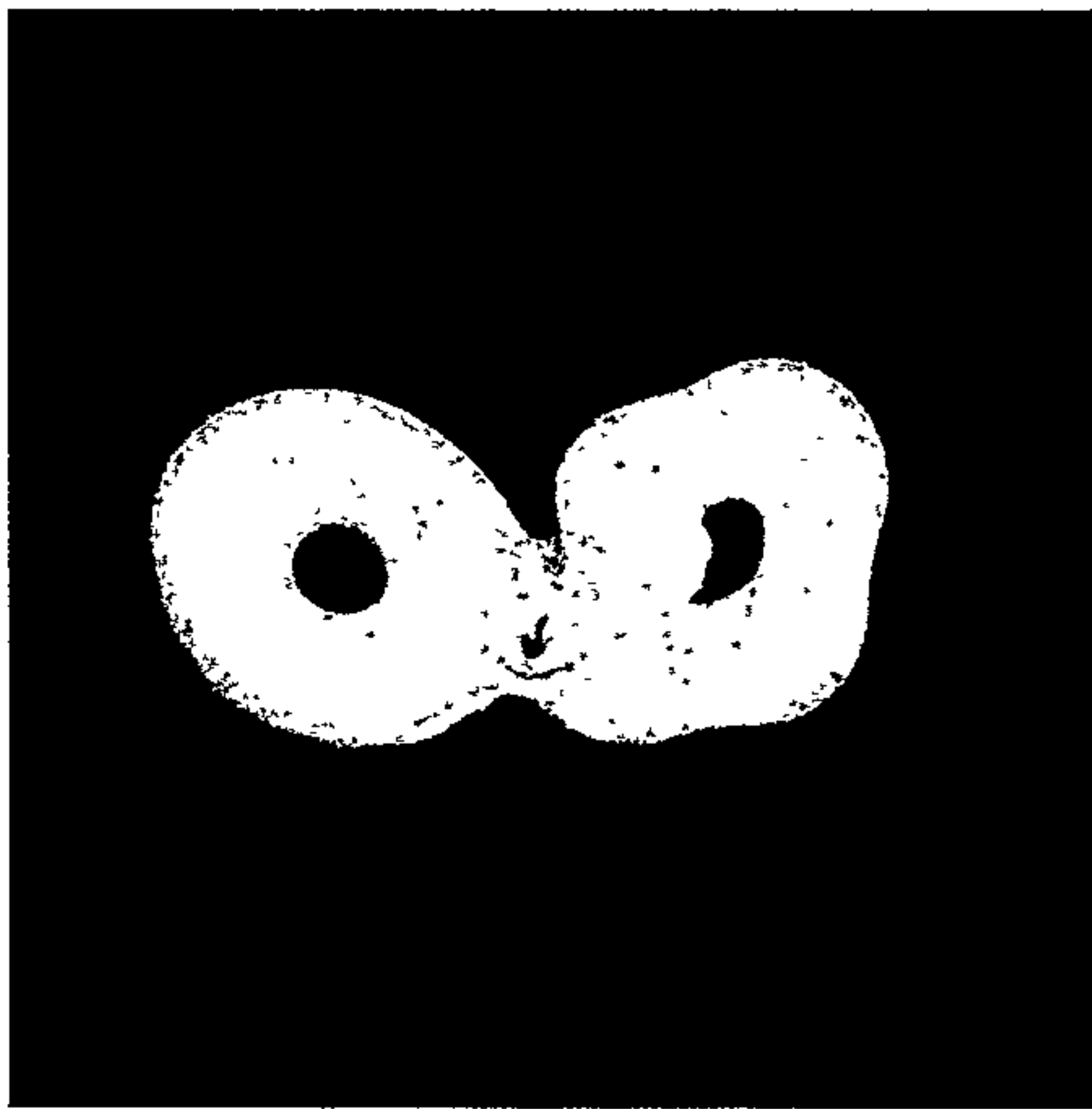
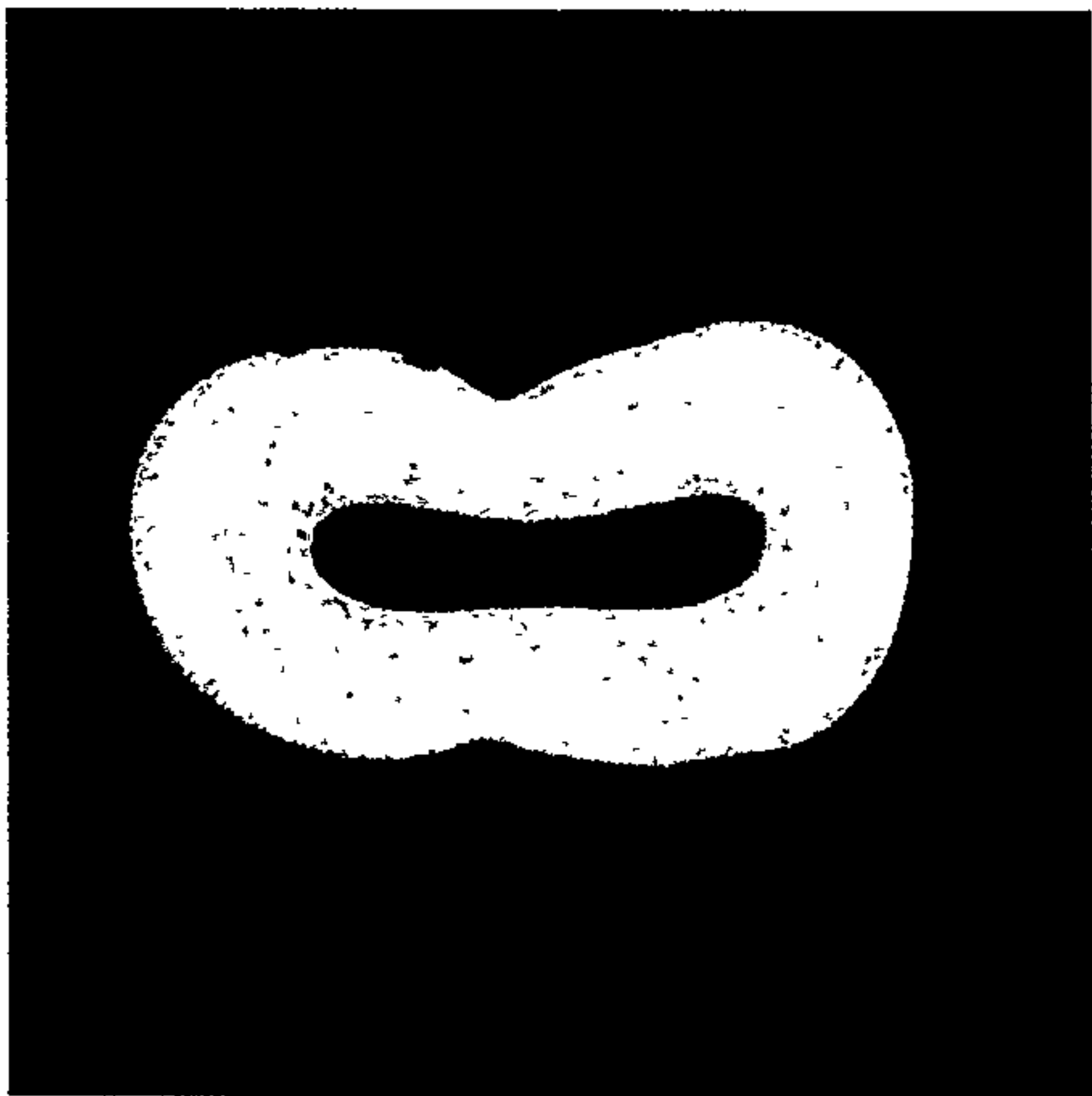
Specimen Mounting in micro-CT Scanner



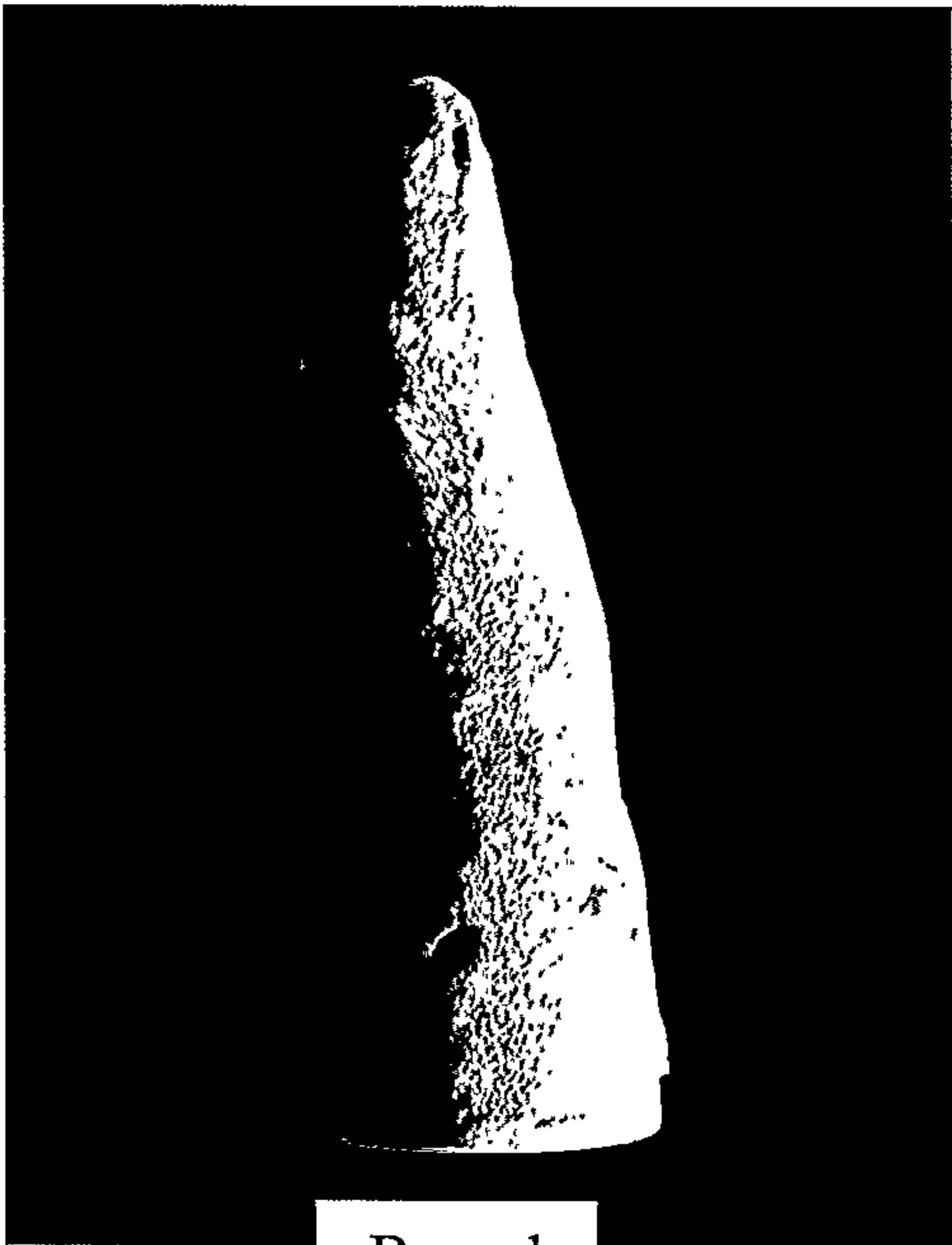
Appendix 10 – Images from Sky Scan 1172 Desktop X-ray Microtomograph



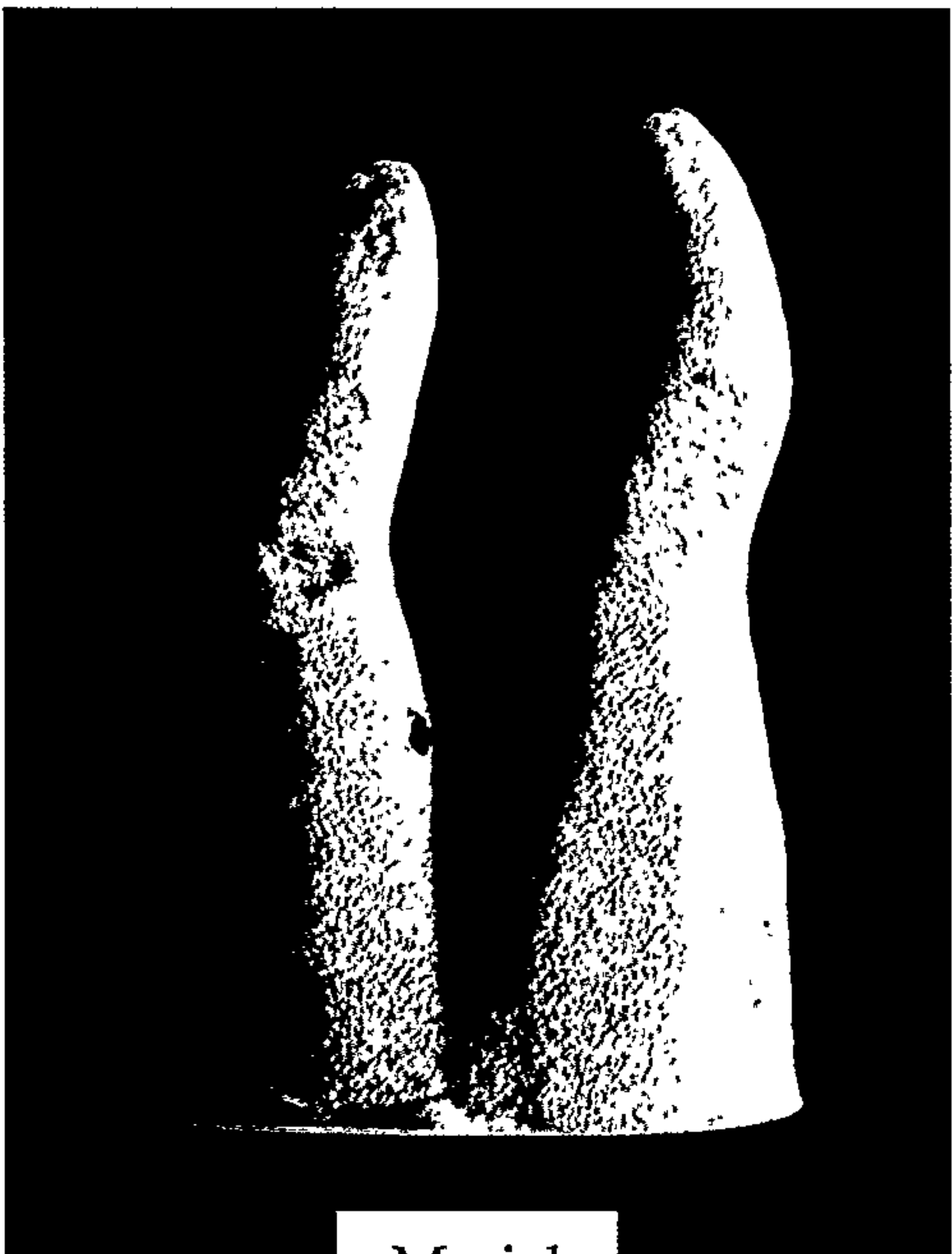
**Appendix 11 – Images from Cone Beam
Reconstruction with NRecon 1.4.2 (Aartsellaar,
Belgium)**



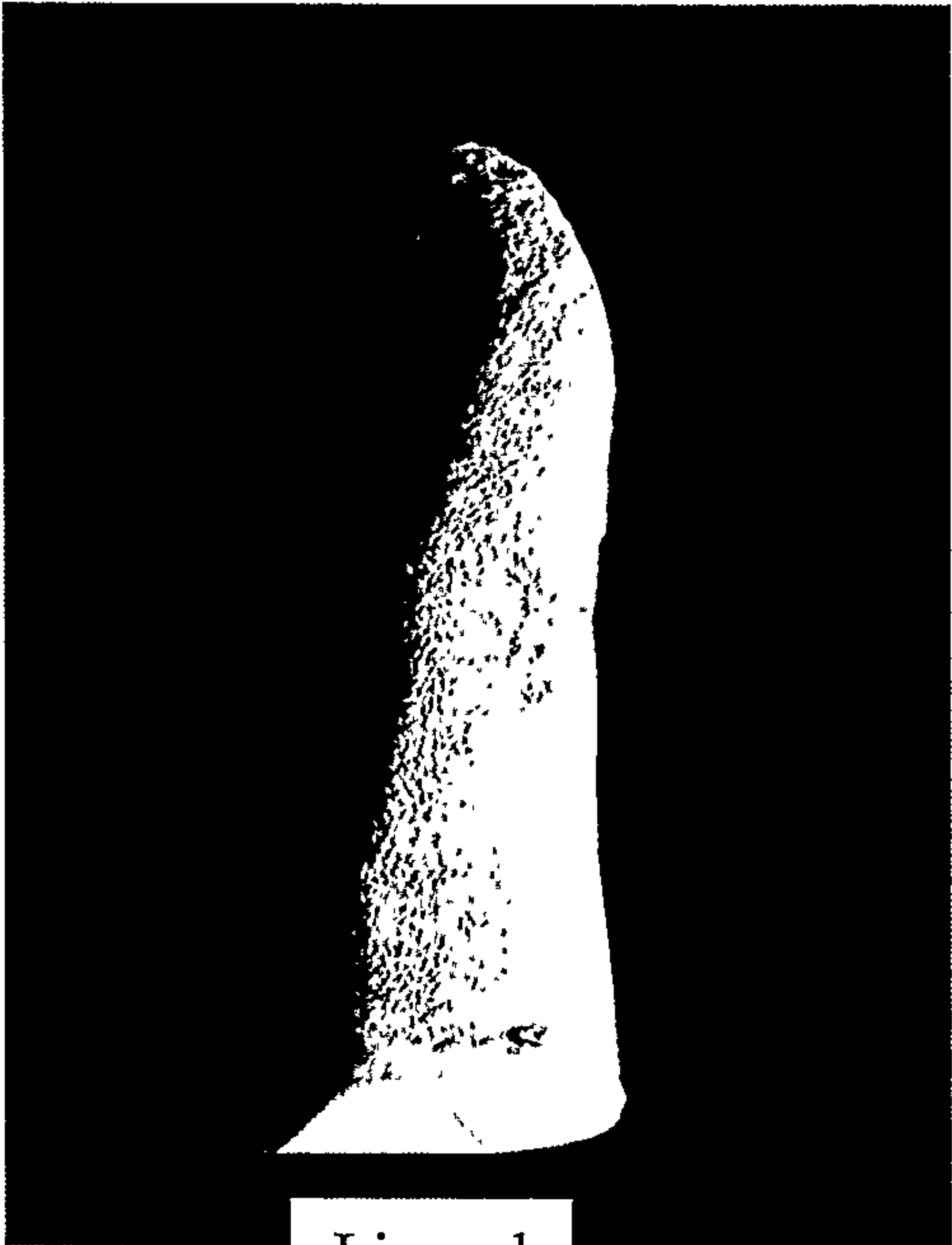
Appendix 12 – Images from 3D reconstruction with VG Studio Max 1.2 (Volume Graphics, GmBH, Heidelberg, Germany)



Buccal



Mesial

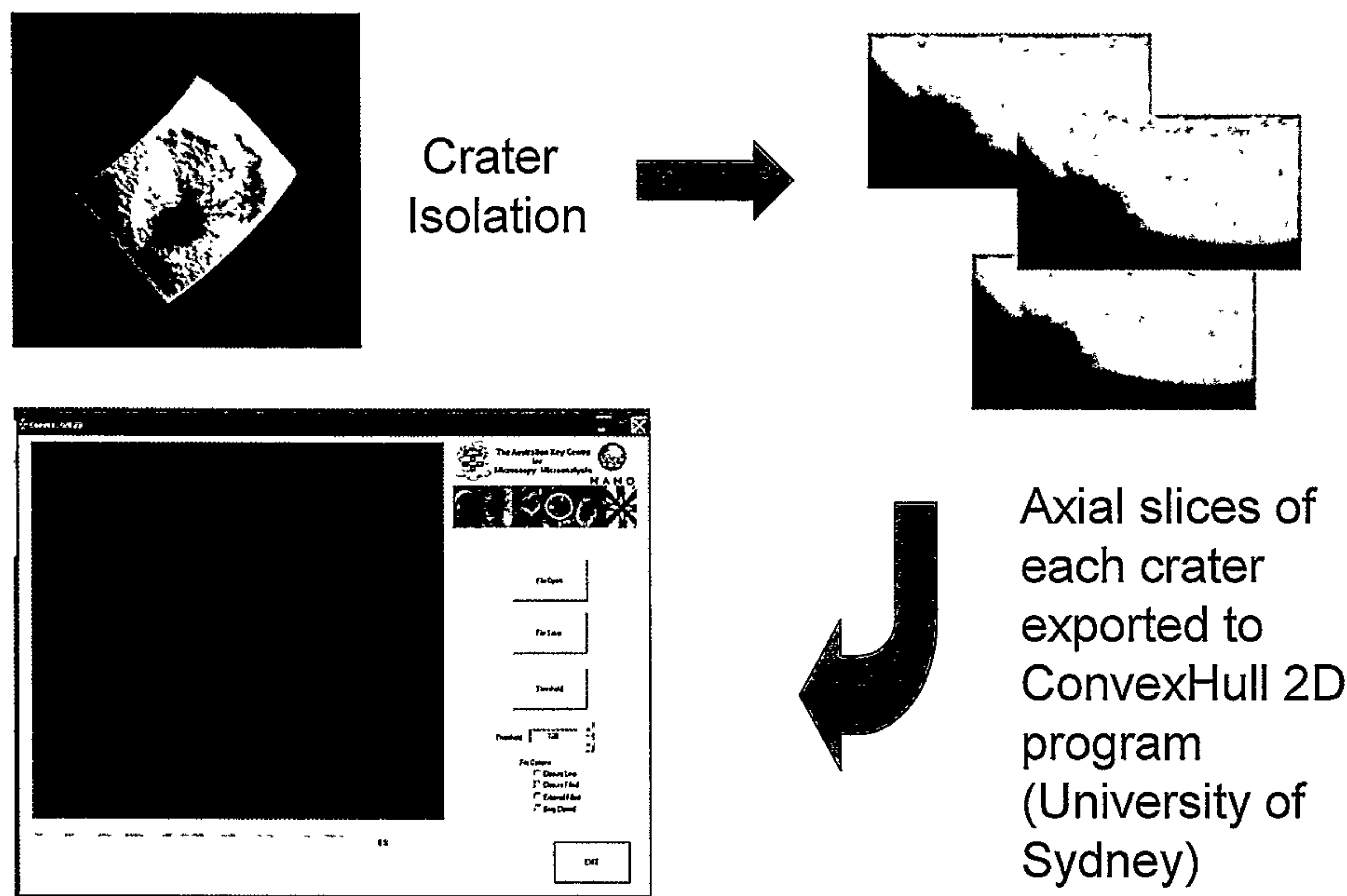


Lingual



Distal

Appendix 13 – Convex Hull 2D



ConvexHull 2D

An imaginary line is drawn to enclose the crater on each slice of the extracted image.

The area bordered by this line and the outline of the resorption crater is calculated by the program.

The result is measured as Voxels. The actual volume of the resorption crater is the product of the Voxels and the scanning resolution.

Appendix 14 – Raw Data for micro-CT Study

Group IL

ID	Age	Fluoride (mg/L)	Positive Control Teeth (mm3)								Experimental Teeth (mm3)							
			Buccal	Lingual	Mesial	Distal	Cervical	Middle	Apical	Total	Buccal	Lingual	Mesial	Distal	Cervical	Middle	Apical	Total
IL1EY	13.83	0.01	0.0021	0.0031	0.0000	0.0028	0.0039	0.0000	0.0042	0.0080	0.1031	0.0000	0.0000	0.0000	0.0816	0.0216	0.0000	0.1031
IL2BO	16.50	0.01	0.0113	0.0000	0.0013	0.0000	0.0127	0.0000	0.0000	0.0127	0.0000	0.0000	0.0017	0.0042	0.0059	0.0000	0.0059	0.0059
IL3AK	18.17	0.01	0.0215	0.0013	0.0014	0.0000	0.0215	0.0000	0.0027	0.0241	0.0059	0.0103	0.0013	0.0012	0.0000	0.0059	0.0128	0.0187
IL4IT	14.08	0.27	0.0560	0.0000	0.0007	0.0127	0.0560	0.0000	0.0133	0.0693	0.0586	0.0000	0.0168	0.0547	0.0557	0.0403	0.0342	0.1301
IL5OK	15.92	0.01	0.0081	0.0000	0.0000	0.0000	0.0065	0.0000	0.0017	0.0081	0.0061	0.0000	0.0000	0.0551	0.0612	0.0000	0.0000	0.0612
IL6SCG	15.67	0.01	0.0703	0.0000	0.0019	0.0000	0.0722	0.0000	0.0000	0.0722	0.1194	0.0348	0.0494	0.0098	0.1200	0.0277	0.0657	0.2134
IL7KG	12.17	0.05	0.0172	0.0109	0.0639	0.0198	0.0187	0.0822	0.0109	0.1118	0.0454	0.0045	0.0003	0.0107	0.0472	0.0003	0.0135	0.0609
IL8GN	16.33	0.08	0.0071	0.0000	0.0590	0.0200	0.0177	0.0586	0.0098	0.0861	0.0243	0.0022	0.0000	0.1046	0.1301	0.0010	0.0000	0.1311
IL9EC	13.83	0.3	0.1259	0.0087	0.0000	0.0000	0.1259	0.0000	0.0087	0.1346	0.0732	0.0547	0.0276	0.0000	0.0732	0.0278	0.0545	0.1555
IL10ENA	14.17	0.01	0.0338	0.0000	0.0000	0.0000	0.0338	0.0000	0.0000	0.0338	0.0126	0.0842	0.0215	0.0597	0.0342	0.0000	0.1438	0.1780

Group II

ID	Age	Repaired Cementum (Control - Experiment) (mm3)							
		Buccal	Lingual	Mesial	Distal	Cervical	Middle	Apical	Total
IL1EY	13.83	-0.1010	0.0031	0.0000	0.0028	-0.0777	-0.0216	0.0042	-0.0951
IL2BO	16.50	0.0113	0.0000	-0.0004	-0.0042	0.0068	0.0000	0.0000	0.0068
IL3AK	18.17	0.0156	-0.0090	0.0000	-0.0012	0.0215	-0.0059	-0.0102	0.0005
IL4IT	14.08	-0.0026	0.0000	-0.0162	-0.0421	0.0003	-0.0403	-0.0208	-0.0608
IL5OK	15.92	0.0020	0.0000	0.0000	-0.0551	-0.0548	0.0000	0.0017	-0.0531
IL6SCG	15.67	-0.0492	-0.0348	-0.0474	-0.0098	-0.0478	-0.0277	-0.0657	-0.1412
IL7KG	12.17	-0.0282	0.0065	0.0636	0.0091	-0.0285	0.0819	-0.0025	0.0509
IL8GN	16.33	-0.0171	0.0022	0.0590	-0.0846	-0.1125	0.0576	0.0098	-0.4500
IL9EC	13.83	0.0527	-0.0460	-0.0276	0.0000	0.0527	-0.0278	-0.0458	-0.0209
IL10ENA	14.17	0.0212	-0.0842	-0.0215	-0.0597	-0.0003	0.0000	-0.1438	-0.1442

Group IIL

ID	Age	Fluoride (mg/L)	Positive Control Teeth (mm3)								Experimental Teeth (mm3)							
			Buccal	Lingual	Mesial	Distal	Cervical	Middle	Apical	Total	Buccal	Lingual	Mesial	Distal	Cervical	Middle	Apical	Total
IIL1OI	15.75	0.1	0.0229	0.0000	0.0201	0.0158	0.0460	0.0128	0.0000	0.0588	0.0000	0.0602	0.0378	0.0545	0.0000	0.0477	0.1047	0.1525
IIL2FNS	14.50	0.05	0.0392	0.0120	0.0000	0.0167	0.0000	0.0355	0.0325	0.0679	0.0243	0.0270	0.0000	0.0000	0.0243	0.0000	0.0270	0.0513
IIL3MO	18.00	0.01	0.0520	0.0035	0.0004	0.0005	0.0512	0.0012	0.0040	0.0564	0.0137	0.0241	0.0000	0.0000	0.0137	0.0000	0.0241	0.0378
IIL4TG	18.25	0.01	0.0445	0.0197	0.0057	0.0028	0.0608	0.0000	0.0120	0.0728	0.0216	0.0222	0.0000	0.0250	0.0284	0.0258	0.0146	0.0688
IIL5CK	13.92	0.01	0.0059	0.0786	0.0000	0.0279	0.0059	0.0000	0.1066	0.1125	0.0633	0.0044	0.0020	0.0000	0.0673	0.0020	0.0004	0.0698
IIL6SD	13.83	0.01	0.0265	0.0102	0.0030	0.0178	0.0333	0.0030	0.0212	0.0575	0.0797	0.0085	0.0024	0.0278	0.0604	0.0293	0.0288	0.1185
IIL7NB	17.42	0.1	0.0348	0.0163	0.0244	0.0000	0.0348	0.0313	0.0094	0.0755	0.1038	0.0137	0.0031	0.0162	0.1036	0.0171	0.0162	0.1368
IIL8TS	15.17	0.01	0.0676	0.0186	0.0607	0.0002	0.0680	0.0092	0.0699	0.1471	0.0583	0.0029	0.0000	0.0000	0.0612	0.0000	0.0000	0.0612
IIL9FI	14.42	0.01	0.1164	0.0000	0.1011	0.0008	0.1364	0.0189	0.0630	0.2183	0.1421	0.0000	0.0162	0.0016	0.1432	0.0151	0.0016	0.1599
IIL10SS	15.08	0.1	0.0240	0.0514	0.0040	0.0284	0.0240	0.0497	0.0341	0.1078	0.0720	0.0000	0.0000	0.0129	0.0727	0.0000	0.0122	0.0848

Group III

ID	Age	Repaired Cementum (Control - Experiment) (mm3)							
		Buccal	Lingual	Mesial	Distal	Cervical	Middle	Apical	Total
III10I	15.75	0.0229	-0.0602	-0.0178	-0.0387	0.0460	-0.0349	-0.1047	-0.0937
III2FNS	14.50	0.0149	-0.0150	0.0000	0.0167	-0.0243	0.0355	0.0055	0.0166
III3MO	18.00	0.0383	-0.0206	0.0004	0.0005	0.0375	0.0012	-0.0201	0.0186
III4TG	18.25	0.0229	-0.0025	0.0057	-0.0222	0.0324	-0.0258	-0.0026	0.0040
III5CK	13.92	-0.0574	0.0742	-0.0020	0.0279	-0.0614	-0.0020	0.1061	0.0427
III6SD	13.83	-0.0532	0.0017	0.0005	-0.0100	-0.0271	-0.0264	-0.0076	-0.0610
III7NB	17.42	-0.0690	0.0026	0.0213	-0.0162	-0.0688	0.0142	-0.0068	-0.0613
III8TS	15.17	0.0093	0.0157	0.0607	0.0002	0.0069	0.0092	0.0699	0.0859
III9FI	14.42	-0.0257	0.0000	0.0849	-0.0007	-0.0068	0.0038	0.0615	0.0584
III10SS	15.08	-0.0480	0.0514	0.0040	0.0155	-0.0487	0.0497	0.0219	0.0229

Group IH

ID	Age	Fluoride (mg/L)	Positive Control Teeth (mm3)								Experimental Teeth (mm3)							
			Buccal	Lingual	Mesial	Distal	Cervical	Middle	Apical	Total	Buccal	Lingual	Mesial	Distal	Cervical	Middle	Apical	Total
IH1ST	17.17	0.01	0.0415	0.0003	0.0000	0.0963	0.1368	0.0011	0.0003	0.1382	0.0706	0.0185	0.0000	0.0000	0.0706	0.0000	0.0185	0.0891
IH2NB	12.50	0.01	0.0375	0.0000	0.0441	0.1188	0.1400	0.0604	0.0000	0.2004	0.0000	0.0000	0.0911	0.0510	0.0510	0.0884	0.0028	0.1421
IH3BB	11.58	0.01	0.1318	0.0491	0.0534	0.1223	0.1557	0.1518	0.0491	0.3565	0.0777	0.0000	0.0317	0.0458	0.0607	0.0945	0.0000	0.1552
IH4MZO	12.75	0.016	0.0417	0.0094	0.0267	0.4052	0.0911	0.2758	0.1160	0.4830	0.1347	0.0978	0.3604	0.7557	0.6214	0.3052	0.4222	1.3487
IH5ZK	12.50	0.01	0.0307	0.0174	0.0327	0.0058	0.0307	0.0352	0.0207	0.0866	0.0857	0.1190	0.0206	0.0477	0.0807	0.0790	0.1132	0.2730
IH6SA	12.92	0.2	0.0456	0.0000	0.0000	0.0899	0.1127	0.0227	0.0000	0.1354	0.0324	0.1045	0.1297	0.0004	0.1297	0.0353	0.1019	0.2670
IH7UY	16.75	0.3	0.0422	0.0000	0.0000	0.0066	0.0488	0.0000	0.0000	0.0488	0.0277	0.0000	0.0082	0.0186	0.0236	0.0227	0.0082	0.0545
IH8BBK	13.00	0.1	0.0938	0.0026	0.0144	0.0003	0.1006	0.0079	0.0026	0.1112	0.0880	0.0055	0.0000	0.0657	0.1394	0.0143	0.0055	0.1592
IH9EK	17.33	0.3	0.0248	0.0000	0.0107	0.0202	0.0381	0.0176	0.0000	0.0557	0.0151	0.1090	0.0487	0.0000	0.0151	0.0487	0.1090	0.1728
IH10AG	17.75	0.08	0.0619	0.0000	0.0000	0.0000	0.0619	0.0000	0.0000	0.0619	0.0633	0.0108	0.0000	0.0000	0.0741	0.0000	0.0000	0.0741

Group IH

ID	Age	Repaired Cementum (Control - Experiment) (mm3)							
		Buccal	Lingual	Mesial	Distal	Cervical	Middle	Apical	Total
IH1ST	17.17	-0.0291	-0.0182	0.0000	0.0963	0.0661	0.0011	-0.0182	0.0490
IH2NB	12.50	0.0375	0.0000	-0.0471	0.0679	0.0891	-0.0280	-0.0028	0.0583
IH3BB	11.58	0.0540	0.0491	0.0217	0.0765	0.0950	0.0572	0.0491	0.2013
IH4MZO	12.75	-0.0930	-0.0885	-0.3337	-0.3505	-0.5303	-0.0293	-0.3062	-0.8658
IH5ZK	12.50	-0.0550	-0.1017	0.0122	-0.0418	-0.0500	-0.0438	-0.0925	-0.1864
IH6SA	12.92	0.0132	-0.1045	-0.1297	0.0894	-0.0170	-0.0126	-0.1019	-0.1315
IH7UY	16.75	0.0145	0.0000	-0.0082	-0.0120	0.0252	-0.0227	-0.0082	-0.0057
IH8BBK	13.00	0.0058	-0.0028	0.0144	-0.0653	-0.0388	-0.0064	-0.0028	-0.0480
IH9EK	17.33	0.0097	-0.1090	-0.0380	0.0202	0.0231	-0.0311	-0.1090	-0.1171
IH10AG	17.75	-0.0014	-0.0108	0.0000	0.0000	-0.0121	0.0000	0.0000	-0.0121
IH10DI	15.25	0.0540	-0.0295	-0.0001	-0.0269	0.0540	-0.0037	-0.0528	-0.0025

Group IIIH

ID	Age	Fluoride (mg/L)	Positive Control Teeth (mm3)								Experimental Teeth (mm3)							
			Buccal	Lingual	Mesial	Distal	Cervical	Middle	Apical	Total	Buccal	Lingual	Mesial	Distal	Cervical	Middle	Apical	Total
IIH1OE	14.00	0.2	0.0708	0.0000	0.0000	0.0509	0.1109	0.0108	0.0000	0.1217	0.1018	0.0003	0.0000	0.1134	0.2069	0.0000	0.0087	0.2156
IIH2ID	14.67	0.01	0.0281	0.0296	0.0000	0.0000	0.0281	0.0000	0.0296	0.0577	0.0447	0.0000	0.0132	0.0486	0.0621	0.0208	0.0235	0.1065
IIH3ZK	18.00	0.01	0.0346	0.0107	0.0055	0.0000	0.0401	0.0000	0.0107	0.0507	0.0210	0.0000	0.0000	0.0022	0.0180	0.0000	0.0052	0.0232
IIH4MA	14.83	0.1	0.0133	0.0000	0.0444	0.0099	0.0220	0.0360	0.0095	0.0676	0.0312	0.0205	0.0543	0.0371	0.0630	0.0186	0.0615	0.1431
IIH5HKC	18.33	0.01	0.0099	0.0009	0.0000	0.0000	0.0093	0.0016	0.0000	0.0109	0.0285	0.0000	0.0076	0.0133	0.0285	0.0089	0.0120	0.0494
IIH6BA	17.08	0.15	0.1024	0.0000	0.0014	0.0253	0.1024	0.0253	0.0014	0.1291	0.0386	0.0088	0.0000	0.0020	0.0434	0.0000	0.0060	0.0494
IIH7AY	13.83	0.15	0.1310	0.0032	0.0000	0.0359	0.1570	0.0000	0.0131	0.1701	0.0250	0.0630	0.0344	0.0106	0.0291	0.0060	0.0978	0.1329
IIH8OY	12.75	0.01	0.0263	0.0009	0.0015	0.0049	0.0263	0.0000	0.0073	0.0336	0.0216	0.0799	0.0060	0.1050	0.1266	0.0000	0.0859	0.2125
IIH9AD	16.17	0.01	0.0209	0.0000	0.0000	0.0068	0.0276	0.0000	0.0000	0.0276	0.0567	0.0239	0.0165	0.0024	0.0590	0.0032	0.0372	0.0994
IIH10DI	15.25	0.01	0.0694	0.0000	0.0000	0.0000	0.0694	0.0000	0.0000	0.0694	0.0153	0.0295	0.0001	0.0269	0.0153	0.0037	0.0528	0.0719

Group IIIH

ID	Age	Repaired Cementum (Control - Experiment) (mm3)							
		Buccal	Lingual	Mesial	Distal	Cervical	Middle	Apical	Total
IIIH1OE	14.00	-0.0311	-0.0003	0.0000	-0.0625	-0.0960	0.0108	-0.0087	-0.0939
IIIH2ID	14.67	-0.0166	0.0296	-0.0132	-0.0486	-0.0340	-0.0208	0.0060	-0.0488
IIIH3ZK	18.00	0.0136	0.0107	0.0055	-0.0022	0.0221	0.0000	0.0054	0.0276
IIIH4MA	14.83	-0.0180	-0.0205	-0.0099	-0.0272	-0.0410	0.0174	-0.0520	-0.0756
IIIH5HKC	18.33	-0.0186	0.0009	-0.0076	-0.0133	-0.0192	-0.0073	-0.0120	-0.0385
IIIH6BA	17.08	0.0638	-0.0088	0.0014	0.0233	0.0590	0.0253	-0.0046	0.0797
IIIH7AY	13.83	0.1060	-0.0598	-0.0344	0.0253	0.1278	-0.0060	-0.0847	0.0372
IIIH8OY	12.75	0.0047	-0.0790	-0.0045	-0.1002	-0.1004	0.0000	-0.0786	-0.1790
IIIH9AD	16.17	-0.0358	-0.0239	-0.0165	0.0044	-0.0314	-0.0032	-0.0372	-0.0718
IIIH10DI	15.25	0.0540	-0.0295	-0.0001	-0.0269	0.0540	-0.0037	-0.0528	-0.0025

Appendix 15 – Descriptive Statistics of the Raw Data

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
cuberoot Ctrl tot vol	IL	.3509	.11693	.03698	.2673	.4346	.20	.51
	IIL	.4495	.07209	.02280	.3979	.5011	.38	.60
	IH	.4879	.10998	.03666	.4034	.5724	.37	.71
	IIH	.3977	.10138	.03206	.3251	.4702	.22	.55
	Total	.4198	.11026	.01766	.3841	.4555	.20	.71
cuberoot Exp tot vol	IL	.4415	.13386	.04233	.3458	.5373	.18	.60
	IIL	.4446	.07221	.02283	.3929	.4962	.34	.54
	IH	.5218	.09305	.03102	.4503	.5934	.38	.65
	IIH	.4602	.10260	.03244	.3868	.5336	.29	.60
	Total	.4656	.10403	.01666	.4319	.4993	.18	.65

Descriptives

Diff tot vol

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
IL	10	-.049720	.0638562	.0201931	-.095400	-.004040	-.1442	.0509
IIL	10	.003310	.0576613	.0182341	-.037938	.044558	-.0937	.0859
IH	9	-.021356	.1174998	.0391666	-.111674	.068963	-.1864	.2013
IIH	10	-.036560	.0750778	.0237417	-.090267	.017147	-.1790	.0797
Total	39	-.026203	.0800208	.0128136	-.052142	-.000263	-.1864	.2013

Appendix 16 – Analysis of Variance

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
cuberoot Ctrl tot vol	Between Groups	3	.034	3.343	.030
	Within Groups	35	.010		
	Total	38			
cuberoot Exp tot vol	Between Groups	3	.013	1.222	.316
	Within Groups	35	.011		
	Total	38			

ANOVA

Diff tot vol

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.016	3	.005	.795	.505
Within Groups	.228	35	.007		
Total	.243	38			

Appendix 17 – Independent T-Test between Groups IIL and IH

Group Statistics

		grp	N	Mean	Std. Deviation	Std. Error Mean
cuberoot Exp tot vol	IIL		10	.4446	.07221	.02283
	IH		9	.5218	.09305	.03102

Independent Samples Test

		t-test for Equality of Means					
		Levene's Test for Equality of Variances					
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference Std. Error Difference
cuberoot Exp tot vol	Equal variances assumed	.241	.630	-2.035	17	.058	-.07729 .03799
	Equal variances not assumed			-2.007	15.083	.063	-.07729 .03852
						95% Confidence Interval of the Difference	
						Lower	Upper
						-.15744	.00285
						-.15935	.00476

Appendix 18 – Raw Data for micro-CT Study (Re-measurements)

Groups IL & IIL

ID	Positive Control Teeth (mm3)	Experimental Teeth (mm3)	Repaired Cementum (mm3)
IL1EY	0.008	0.104	-0.096
IL2BO	0.013	0.007	0.006
IL3AK	0.110	0.018	0.092
IL4IT	0.114	0.146	-0.032
IL5OK	0.010	0.063	-0.053
IL6SCG	0.074	0.211	-0.137
IL7KG	0.093	0.207	-0.114
IL8GN	0.176	0.116	0.060
IL9EC	0.141	0.145	-0.004
IL10ENA	0.065	0.171	-0.106
IIL1OI	0.055	0.130	-0.076
IIL2FNS	0.081	0.042	0.040
IIL3MO	0.047	0.039	0.008
IIL4TG	0.082	0.119	-0.037
IIL5CK	0.116	0.057	0.059
IIL6SD	0.113	0.115	-0.002
IIL7NB	0.069	0.164	-0.095
IIL8TS	0.169	0.069	0.099
IIL9FI	0.239	0.227	0.013
IIL10SS	0.107	0.063	0.043

Groups IH & IIH

ID	Positive Control Teeth (mm3)	Experimental Teeth (mm3)	Repaired Cementum (mm3)
IH1ST	0.177	0.109	0.069
IH2NB	0.211	0.065	0.147
IH3BB	0.425	0.166	0.259
IH4MZO	0.541	1.488	-0.946
IH5ZK	0.068	0.256	-0.189
IH6SA	0.192	0.299	-0.107
IH7UY	0.029	0.162	-0.133
IH8BBK	0.106	0.185	-0.079
IH9EK	0.337	0.339	-0.002
IH10AG	0.066	0.105	-0.039
IIH1OE	0.628	0.266	0.362
IIH2ID	0.081	0.195	-0.115
IIH3ZK	0.063	0.022	0.041
IIH4MA	0.066	0.131	-0.065
IIH5HKC	0.008	0.018	-0.010
IIH6BA	0.195	0.070	0.125
IIH7AY	0.213	0.144	0.069
IIH8OY	0.032	0.191	-0.159
IIH9AD	0.031	0.075	-0.044
IIH10DI	0.073	0.113	-0.040

Appendix 19 – Raw Data for Tooth Movement

Groups IL & IIL

	Positive Control Teeth			Experimental Teeth					
ID	PMD T1 (mm)	PMD T2 (mm)	TM T2-T1 (mm)	PMD T1 (mm)	PMD T2 (mm)	PMD T3 (mm)	TM T2-T1 (mm)	TM T3-T2 (mm)	TM T3-T1 (mm)
IL1EY	17.25	17.62	-0.56	14.25	14.6	14.81	0.35	0.21	0.56
IL2BO	16.41	16.13	-0.43	13.47	13.94	13.90	0.47	-0.04	0.43
IL3AK	13.30	14.65	0.95	12.43	12.59	11.48	0.16	-1.11	-0.95
IL4IT	17.29	16.37	-0.20	13.00	13	13.20	0	0.20	0.20
IL5OK	12.22	12.72	-0.14	13.33	13.54	13.47	0.21	-0.07	0.14
IL6SCG	15.75	16.85	0	15.68	15.68	15.68	0	0	0
IL7KG	11.25	11.63	-1.24	11.09	12.33	12.33	1.24	0	1.24
IL8GN	14.32	15.68	-0.55	12.33	12.66	12.88	0.33	0.22	0.55
IL9EC	13.12	14.52	-0.49	16.34	17.03	16.83	0.69	-0.20	0.49
IL10ENA	14.66	15.11	-1.18	14.4	15.91	15.58	1.51	-0.33	1.18
IIL1OI	15.18	16.13	-0.54	15.33	16.09	15.87	0.76	-0.22	0.54
IIL2FNS	15.96	16.47	-0.78	15.15	15.95	15.93	0.80	-0.02	0.78
IIL3MO	13.69	12.62	-1.09	13.19	14.96	14.28	1.77	-0.68	1.09
IIL4TG	14.09	14.26	-0.17	12.59	12.75	12.76	0.16	0.01	0.17
IIL5CK	13.80	14.58	-0.77	13.39	14.55	14.16	1.16	-0.39	0.77
IIL6SD	12.55	13.63	0.53	13.44	13.84	12.91	0.40	-0.93	-0.53
IIL7NB	15.40	15.01	-0.90	9.75	10.93	10.65	1.18	-0.28	0.90
IIL8TS	13.70	14.52	-1.14	12.94	13.46	14.08	0.52	0.62	1.14
IIL9FI	10.53	11.80	-0.09	13.04	13.52	13.13	0.48	-0.39	0.09
IIL10SS	12.92	14.38	-0.21	17.12	18.24	17.33	1.12	-0.91	0.21

Note: PMD = Premolar distance, TM = Amount of tooth movement

Groups IH & IIH

	Positive Control Teeth			Experimental Teeth					
ID	PMD T1 (mm)	PMD T2 (mm)	TM T2-T1 (mm)	PMD T1 (mm)	PMD T2 (mm)	PMD T3 (mm)	TM T2-T1 (mm)	TM T3-T2 (mm)	TM T3-T1 (mm)
IH1ST	14.01	14.28	-0.63	13.81	14.77	14.44	0.96	-0.33	0.63
IH2NB	13.15	14.79	0.17	13.76	13.99	13.59	0.23	-0.40	-0.17
IH3BB	13.07	13.73	-1.63	13.90	15.81	15.53	1.91	-0.28	1.63
IH4MZO	11.66	14.44	-1.40	15.32	17.90	16.72	2.58	-1.18	1.40
IH5ZK	14.46	15.48	-0.79	13.62	15.06	14.41	1.44	-0.65	0.79
IH6SA	10.06	11.72	-0.51	12.03	12.83	12.54	0.80	-0.29	0.51
IH7UY	18.95	19.31	-0.10	13.81	14.09	13.91	0.28	-0.18	0.10
IH8BBK	14.62	15.52	-0.49	14.14	15.88	14.63	1.74	-1.25	0.49
IH9EK	11.90	12.67	-1.49	10.46	11.52	11.95	1.06	0.43	1.49
IH10AG	12.76	12.86	0.16	17.22	18.78	17.06	1.56	-1.72	-0.16
IIH1OE	15.35	17.06	-0.25	14.26	14.49	14.51	0.23	0.02	0.25
IIH2ID	12.92	12.78	-1.20	8.95	10.24	10.15	1.29	-0.09	1.20
IIH3ZK	15.03	15.42	-0.47	13.90	14.62	14.37	0.72	-0.25	0.47
IIH4MA	13.15	14.35	0.57	15.56	16.46	14.99	0.90	-1.47	-0.57
IIH5HKC	15.86	16.42	-0.41	13.72	14.48	14.13	0.76	-0.35	0.41
IIH6BA	13.51	14.39	-0.91	12.81	14.44	13.72	1.63	-0.72	0.91
IIH7AY	14.04	15.07	-0.35	12.85	13.00	13.20	0.15	0.20	0.35
IIH8OY	17.49	18.16	-1.25	16.44	17.80	17.69	1.36	-0.11	1.25
IIH9AD	12.75	13.08	-0.07	13.02	13.86	13.09	0.84	-0.77	0.07
IIH10DI	16.35	16.74	-0.15	14.60	15.04	14.75	0.44	-0.29	0.15

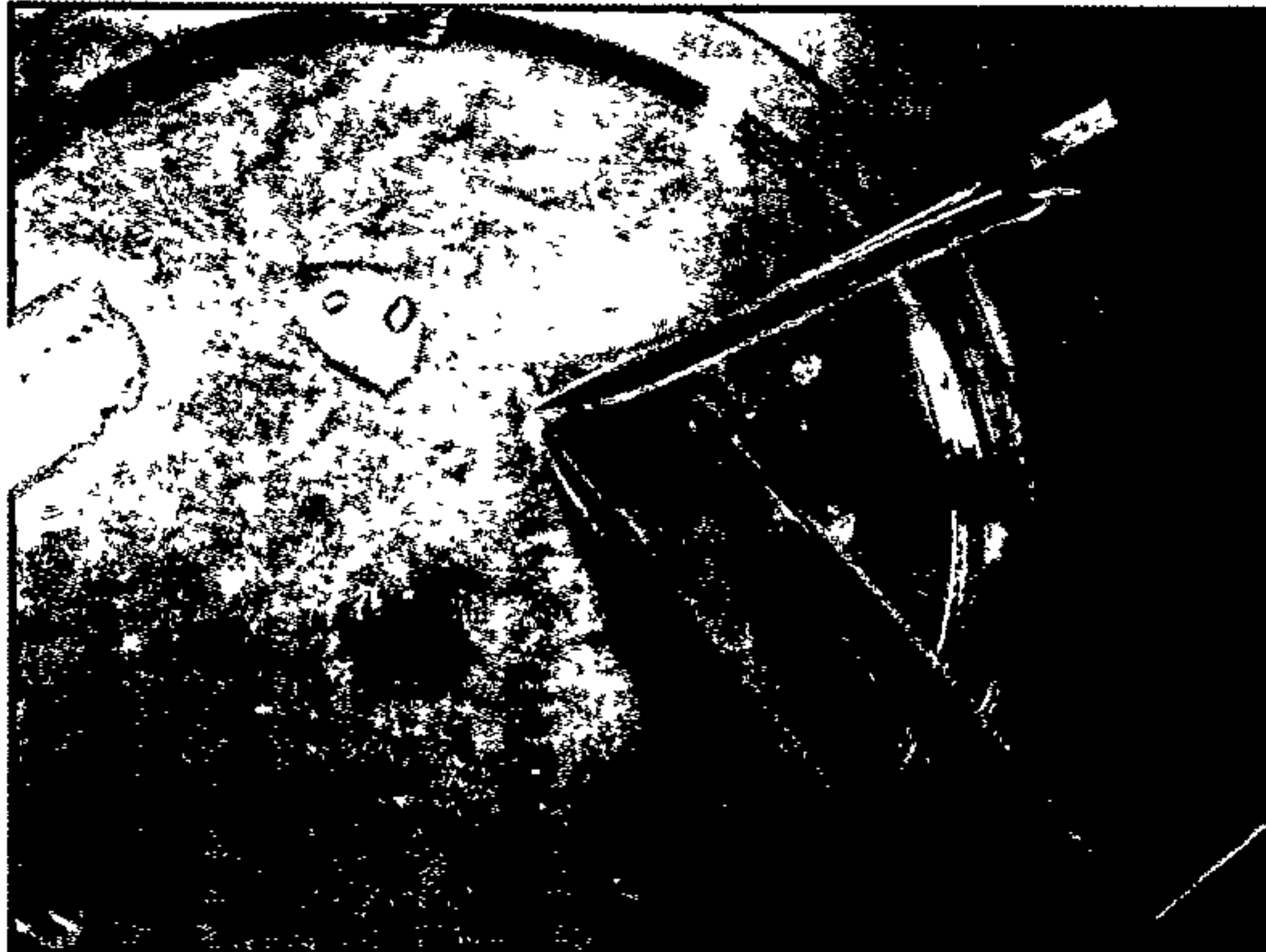
Note: PMD = Premolar distance, TM = Amount of tooth movement

Appendix 20 – Tooth Sectioning for Histology Preparation

1. After decalcification, the crown of the tooth was sectioned using a scalpel blade no.10.



2. Two rooted-teeth were sectioned into buccal and lingual roots.



3. Each root was sectioned para-sagittally from the buccal surface to the lingual surface of the root. The sectioned tooth material was then put into a cassette and embedded in paraffin wax.



*“I can do everything through him who gives me strength”
Philippians 4:13*