Repair of root resorption 2 to 16 weeks following the application of continuous force on upper first molars in rats; a 2 and 3D quantitative evaluation.

## Carmen Gonzales,<sup>a</sup> Hitoshi Hotokezaka,<sup>b</sup> M. Ali Darendeliler,<sup>c</sup> Noriaki Yoshida<sup>d</sup>

<sup>a</sup> PhD student, Division of Orthodontics and Dentofacial Orthopedics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan.

<sup>b</sup> Senior Assistant Professor, Division of Orthodontics and Dentofacial Orthopedics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan.

<sup>c</sup> Professor, Department of Orthodontics, University of Sydney,

Sydney Dental Hospital, South Western Sydney Area Health Service, Sydney, Australia.

<sup>d</sup> Professor, Division of Orthodontics and Dentofacial Orthopedics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan.

Corresponding author: Dr Hitoshi Hotokezaka, Division of Orthodontics and Dentofacial Orthopedics, Nagasaki University Graduate School of Biomedical Sciences, Sakamoto 1-7-1, Nagasaki, Nagasaki 852-8588, Japan

E-mail: hotoke@nagasaki-u.ac.jp

## ABSTRACT

Root resorption is an inevitable side-effect of orthodontic treatment which occurs in association with the removal of hyalinised tissue. Several studies have shown that a reparative process in the periodontium commences when the applied orthodontic force is discontinued or reduced below a certain level. However there is no study on quantitative 3D evaluation of root resorption repair. **Objective:** The aim of this study was to evaluate the amount of repair by quantitative assessment of the two and three dimensional changes of the root resorption craters after two to sixteen weeks retention periods following two weeks of continuous mesially applied orthodontic forces of 50 grams on rat molars using scanning electron and laser microscopes.

Materials and Methods: 50 g mesial force was applied to move the upper left first molars of sixty Wistar male rats (10-week old) by using nickel titanium closed-coil springs for 2 weeks. Rats in one of the six randomly divided groups were sacrificed following two weeks of force application. These rats constituted the zero-week retention group. In the remaining five groups, the interdental space between the upper first and second molars was filled with resin to retain the molars. The molars were extracted after periods of retention varying between 2 and 16 weeks. Upper right molars were used as controls. Mesial and distal roots (disto-buccal and disto-palatal) were examined using scanning electron and 3D scanning laser microscopes. The surface area, depth, volume, and roughness of the root resorption craters were measured. Results: The examination showed that all the area, depth and volume of the craters decreased gradually and showed similar trends over retention time approaching a plateau at 12<sup>th</sup> week. After 16 weeks of retention, volume of the resorption craters of the disto-buccal and disto-palatal roots reached a recovery peak of 69.5% and 66.7%, respectively. Small pits on the mesial root showed a recovery of 62.5% at 12<sup>th</sup> week. The healing pattern in the distal roots with severe resorption and mesial roots with shallow resorption did not show significant differences. The results of this study suggest that the resorption and repair processes during the early stages of retention are balanced and the majority of the reparative process occurs after four weeks of passive retention following the

application of orthodontic force and that frequent orthodontic re-activations should be avoided so that recovery and repair of the root surface damage can happen.

## INTRODUCTION

Root resorption is a common side effect of orthodontic treatment and it may occur during and at the end of the treatment. When severe root resorption occurs, it is recommended to stop the application of force to avoid the worsening of iatrogenic sequelae.<sup>1</sup> It is believed that, the healing process of a resorption cavity starts as early as the first week of retention following orthodontic treatment when the orthodontic force is discontinued <sup>2,3,4</sup> or reduced to below a certain level.<sup>5,6</sup> Many studies have demonstrated that the resorptive defects are repaired by deposition of new cementum and re-establishment of a new periodontal ligament.<sup>2,5,7,8</sup> Schwarz,<sup>9</sup> considered the possibility that fibers of the periodontal ligament incorporated in the new cementum during repair to some extent may compensate for the loss of root substance. Correspondingly, Langford and Sims,<sup>7</sup> found that a considerable amount of new cementum can be deposited in the resorption crater under repair.

Several qualitative studies have been undertaken to evaluate resorption repair using light microscopy, scanning electron microscopy and transmission electron microscopy.<sup>10-13</sup> Radiographic, two dimensional, evaluation studies were limited to measuring only the amount of root apex loss and were highly inaccurate because of the magnification error and low reproducibility.<sup>14</sup> Histological studies were technique sensitive and quantitative measurement of root resorption was not reliable as loss of material occurs during histological sectioning. Three dimensional reconstruction of small specimens such as the root resorption crater with high spatial resolution were recently created using micro-computed tomography (micro-CT) and used in quantifying the amount of root resorption in human and animal samples.<sup>15-16</sup> However, these studies did not quantify the process of root resorption healing.

The aim of the present investigation was to evaluate the amount of repair by

quantitative assessment of the dimensional and volumetric changes of the root resorption craters after two to sixteen weeks retention periods following two weeks of continuous 50 grams mesially applied orthodontic forces on rat molars using scanning electron and laser microscopes.

#### Materials and Methods

Sixty 10-week old young adult Wistar male rats were used (ethics approval, Animal Welfare Committee of Nagasaki University, No. 0603170498). The rats (SLC, Shizuoka, Japan. Body weight, 230-250 g) were allowed one week to acclimatize to their new laboratory environment. The appliance was set under anesthesia using intraperitoneal injection of pentobarbital with a dosage of 60 mg/kg body weight. The posterior end of the coil spring was tied around the first molar with a 0.01 mm diameter stainless steel ligature wire. The anterior end of the coil spring was fixed to a hole drilled through the incisors at the alveolar bone level with a 0.09 inch diameter ligature wire (Fig. 1). The appliance was activated by pulling to a triple deflection (9 mm) to produce a nearly constant force. The force magnitude was measured with a tension gauge (DTN-150, Teclock, Japan) in a water bath at 37.5°C. The procedure was accomplished when the appliance was set and at the end of the experimental time. The measurements before and after the experiments were  $49.1 \pm 2.4$  g and  $53.6 \pm 5.8$  g, respectively (n =20). Contralateral molars served as controls. After 2 weeks of orthodontic force application, the amount of tooth movement was measured on cephalometric radiographs, as previously reported.<sup>17</sup> Briefly, a cephalostat was specially constructed to standardize the rat's head position. The distance between the X-ray tube and film was 50cm. The cephalometric radiographs were digitized with a film scanner at 600 dpi. Tooth movement was measured on the digitized images with Scion Imaging software (Scion Corp, Frederick, Md). The appliance was removed and self cured resin (Super Bond, Sun Medical Co. Ltd, Shiga, Japan) was placed in the interdental space between the left first and second molars in order to maintain the obtained tooth movement until the day of sacrifice. The rats were then randomly divided into 6 groups (10 animals each) according

to the retention time. One randomly selected group of rats was sacrificed at the end of the 2 weeks force application period and served as zero-week retention group (0W). The other 5 groups of rats were sacrificed after 2, 4, 8, 12, and 16 weeks of retention (Fig 2). The left (experimental) and right (control) upper first molar including its surrounding bone were cut as a block, followed by delicately removing the alveolar bone to avoid any root surface damage. The molars were submerged in 1% sodium hypochlorite for more than 10 minutes to eliminate remaining periodontal ligament remnants. Molars were kept in separate and labeled storage containers without storage media. The molars were then sectioned bucco-lingually through the crown near the cemento-enamel junction with a thin diamond disc. Root resorption craters on the apical region were not evaluated due to anatomical variations and difficulties in delimitating the craters. The mesial and distal surfaces of disto-buccal, disto-palatal and mesial roots were evaluated with a scanning electron microscope (TM-1000, Hitachi, Tokyo, Japan) and three-dimensional (3D) laser scanning microscope (VK-9500, Keyence, Kyoto, Japan). Since resorption craters in the distal surfaces of the roots were scarcely detectable they were also excluded from the study. All craters scattered on the cervical and middle thirds of the roots (mesial side) were digitally obtained and stored as BMP images. Surface area was measured by means of commercial software (Mimics program, DICO, Tokyo, Japan). The deepest point and the surface roughness of the resorption craters were calculated with the laser microscope program (VK-9500). The average depth was verified by the software. With a resolution of 0.01 µm, the microscope enabled the root surface profile measurement as well as observation. It performed non-contact and nondestructive measurement without leaving any marks on the surface of the roots. The crater edges were determined by a numerical value of roughness over 10 µm and confirmed by exploring the surface roughness of the root by observing the images obtained by the SEM. The cervical edge of a root was determined at the cemento-enamel junction. The measured value of crater area was multiplied by the average depth to give the volume. The same investigator performed all measurements, and every measurement was repeated three times. The mean value was used as the final measurement. To assess measurement reproducibility, serial measurements of area, depth, surface roughness and volume were performed ten times in one randomly selected disto-buccal root. Each value of mean and standard deviation was  $28.1 \pm 0.4 \mu m$  for the area;  $147.5 \pm 0.37 \mu m$  for the depth;  $18.7 \pm 0.5 \mu m$  for surface roughness; and  $10.9 \pm 0.05 \times 10^6 \mu m^3$  for the volume.

#### **Statistical Methods**

Statistical analysis was performed with SPSS version 16.0 (SPSS, Chicago, III). Univariate analysis of variance (ANOVA) and pairwise comparisons between the groups were performed. Bonferroni adjustments were made for multiple comparisons. Pearson's correlation coefficient was used to calculate the correlations between retention duration and root resorption crater volume.

#### Results

After 2 weeks of tooth movement, the value of rat tooth movement was  $0.24 \pm 0.07$  mm. Most of the control roots exhibited areas covered by undamaged cementum with a characteristic smooth surface. The apical third of the control roots was covered with thick cementum with a rough and irregular surface that, occasionally, contained resorption craters. The zero-week group showed resorption craters with well-defined margins. Three different types of craters were clearly identified: isolated lacunae, wide shallow resorption pit, and deep resorption craters. Small isolated lacunae were mainly seen scattered on the mesial roots (cervical half of its mesial surface). Wide shallow and deep resorption craters were observed on the distal roots covering cervical and middle portions of the root. In the mesial roots, small resorption pits were distributed mostly on the cervical portion of the root. In the retention groups' molars, the healing process was evaluated after 2, 4, 8, 12, and 16 weeks of retention. Examination of root surfaces in retention groups using SEM revealed that the resorption craters gradually became smaller, even and smooth with time (Fig. 3).

The surface area recovered noticeably after 8 weeks. The tendency of the resorption craters to diminish its area continued until the end of the experiments at 16 weeks (Table I). Resorption area of the distal roots at 16<sup>th</sup> weeks was not significantly smaller than that at 12<sup>th</sup> weeks (Fig. 4B). At that point, light depressions, remaining as scars of root resorption, were still present as evidenced by the SEM (Fig. 3, 16W). The depth of resorption craters in the distal roots also showed a gradual and significant decrease from the 4th week of retention (Table II) (Fig. 4C). The area and depth measurements were consistent with the volumes, which is also reasoned by high correlation coefficient between the values and retention time (Table III). The disto-buccal roots presented a gradual reduction in volume: 12.7 percent after 2 weeks, 33.2 percent after 4 weeks, 44.1 percent after 8 weeks, 57.7 percent after 12 weeks and 69.5 percent after 16 weeks (calculation from Table I, Fig. 4D). A significant volume recovery was observed from the 4<sup>th</sup> week of retention until the end of the experiments. In the disto-palatal roots, volume recovery rates were 55.4 percent and 66.7 percent after 12 and 16 weeks, respectively. With regards to surface roughness, the bottom of the root resorption cavities revealed an extensive, irregular, disorganized, and rough layer with irregular borders at the zero-week group. The surface roughness in the disto-palatal roots showed a significant decrease after 4 weeks of retention continuing until the end of the experiments. In the disto-buccal roots, a clear smoothing of the bottom of the craters was observed from the 8<sup>th</sup> week of retention. At this point of time, as evidenced by SEM, reparative cementum surface showed an even and smooth structure. The borders of the resorption craters became round and less sharp (Fig 4E).

Compared with the distal roots, the mesial roots suffered much less resorption reflected in the small size of the resorption pits. In order to study the difference of the healing process between large craters and small pits, we also analyzed the mesial roots. Resorption pits scattered mainly in the cervical portion of the roots became 47.6 percent smaller after 12 weeks of retention. Furthermore, depth, volumes, and surface roughness decreased, respectively, 61.6, 62.5, and 56.9 percent. These results may indicate that healing in the mesial and distal roots

exhibited a similar pattern.

#### DISCUSSION

We previously investigated the effects of force magnitude on root resorption in the rat molar and observed that resorption craters at the mesial surfaces of the distal roots showed more severe damage than the mesial roots.<sup>17</sup> For this reason we evaluated the healing process on the distal and mesial roots as representatives of severe and shallow root resorption, respectively. The amount of healing was investigated by measuring the changes in the surface area, depth, volume, and roughness of the resorption craters during a 16-week retention period. It has been stated that early repair starts from the centre to the outside,<sup>2</sup> or in the periphery of the resorption lacunae in association with the invasion of fibroblast-like cells from the surrounding periodontal ligament.<sup>5,13,12</sup> Our results revealed that the area, volume, and the depth of the craters were reduced over retention time, which suggests that the healing seems to occur in all directions. Brudvik and Rygh's histological findings<sup>13</sup> suggested that small, shallow resorption lacunae may be completely filled with new cementum. However, the retention periods were not clearly specified. The results in the present study showed that, following a retention period of 16 weeks, even the smallest resorption craters scattered on the cervical portion of the mesial roots did not heal completely. After a 12-week retention period a maximum of 62.5 percent of resorption healing was observed. Similar to our results, Owman-Moll<sup>4</sup> reported that repair of resorptive areas ranged from 28 percent after 1 week of retention to 75 percent after 8 weeks. Langford<sup>7</sup> investigated root surface resorption repair following rapid maxillary expansion for periods varying between 14 and 52 weeks. He found that repair was generally further advanced on specimens retained for longer periods. However, the data were not quantified. From our results, healing of the distal roots showed a remarkably decreasing tendency that may continue beyond the experimental time set in the study.

When clinicians select a force delivery system during orthodontic treatment with

fixed appliances, exactly how much active force will exist between force applications is very difficult to predict. It depends on the procedure, wire size, material, clinical expertise, etc. The applied force may be capable enough to move teeth without causing damage to the teeth and periodontal structures. With this in mind, light forces are the choice for most of the clinicians. However, heavy forces may be applied during space closing mechanics. Koga et al<sup>18</sup> calculated the force systems acting on brackets, and demonstrated that a vertical loop fabricated from 0.017 x 0.025 (inches) stainless steel wire gabled to 30° placed within 7 mm interbracket distance could produce a force of 1300 g. Khambay and McHugh<sup>19</sup> found forces up to 1100 g during laceback placement. Rock and Wilson<sup>20</sup> reported forces of 830 g exerted by orthodontic aligning archwires. During rapid maxillary expansion, expansion screws may produce 1300-4500 g by a single activation.<sup>21</sup> In the present investigation, we used coil springs of 50 g to move mesially the rat's molar. This force should correspond to 1,000 g in a human molar<sup>22</sup> and may be comparable to the force applied in some orthodontic procedures. Previous studies proved that heavy orthodontic forces (225g) cause more root resorption than light orthodontic forces (25g) during buccal tipping and intrusion movements in human first premolars.<sup>15,23</sup> If, in a similar study design, lighter forces are applied on rats molars, one may expect less amount of root resorption and maybe complete repair of the craters following similar amount of retention periods.

Clinically, during conventional orthodontic treatment, the process of resorption and healing may occur between appointments. Proffit<sup>24</sup> suggested that activating an appliance too frequently, short circuiting the repair process, can produce damage to the teeth or bone that a longer appointment cycle would have prevented or at least minimized. According to previous studies reparative process increases over the first four weeks of retention then slows down in the fifth and sixth weeks and finally reaches a plateau phase<sup>4,5</sup> However, the present study showed there was no significant difference in resorption area during the retention periods of 2 to 4 weeks, in the volume of the craters during the first two weeks of retention. One explanation was that resorption and repair processes occurred simultaneously and resorption diminished the amount of repair during the initial phases of the retention. This may also indicate that the repair process might have started to reach a steady rate from two to four weeks which was earlier than suggested in a previous study.<sup>4</sup> The lesser area and volume of resorption craters found on the roots seemed to suggest that the majority of the reparative process occurred after four weeks of retention. It was demonstrated in this study that a significant resorption healing rate increased with time, from 12.7 percent after 2 weeks of retention to 69.5 percent after 16 weeks (disto-buccal root). In the disto-palatal root the healing ranged from 6.8 percent after 2 weeks to 66.7 percent after 16 weeks. In addition, small resorption pits on the mesial root, which may resemble resorption pits occurring during orthodontic treatment, showed a similar tendency. As a matter of fact, root resorption at the end of treatment might be the result of a series of repetitive resorption-healing processes occurring throughout the treatment period. Time spam between each orthodontic adjustment may allow for biological healing to occur. Frequent re-activation of the orthodontic appliances causing re-establishment of new/additional mechanical loading done earlier than the repair process overcomes the resorption process, may provoke severe root resorption. Thus, the longer the time intervals between orthodontic re-activations, the more healing will take place. For these reasons, our results suggest that when heavy orthodontic forces are applied, more than 12 weeks may be recommended between each force application. However, the difference in the healing process of rats and humans deserve to be studied in more detail.

## CONCLUSION

During retention periods of 2, 4, 6, 8, 12 and 16 weeks following two weeks of mesial-directed heavy (50g) force application on upper first molars in rats, the following conclusions were drawn:

1. Application of 50g of mesialising force during two weeks caused significant amount of root resorption on mesial surfaces of the

disto-buccal, disto-palatal and mesial roots of the upper first molars of Wistar rats.

- The majority of the reparative process occurred after four weeks of passive retention following the application of two weeks of heavy (50g) orthodontic force suggesting that the resorption and repair processes during the early stages of retention were balanced.
- 3. After 16 weeks of passive retention following the application of two weeks of heavy (50g) orthodontic force, 62.5 to 69.5 percent of resorption craters were repaired.
- 4. The decrease in the area, depth and volume of the resorption craters showed similar trends over retention periods of 2 to 16 weeks.
- 5. The results of the present study suggest that frequent orthodontic re-activations should be avoided so that recovery and repair of the root surface damage can happen.

## ACKNOWLEDGMENTS

We thank all the staff of the Animal Center of Nagasaki University for their support during the experiments and the members of the Faculty of Mechanical Systems Engineering for their technical support of laser scanning microscope. Thanks are also extended to my colleagues in the Department of Orthodontics for their valuable help and assistance in this study. This work was supported by a grant-in-aid for scientific research from the Ministry of Education, Science, Sports, and Culture of Japan.

## REFERENCES

1. Rygh P. Orthodontic root resorption studied by electron microscopy. Angle Orthod 1977;47:1-16.

2. Barber AF, Sims MR. Rapid maxillary expansion and external root resorption in man: a scanning electron microscope study. Am J Orthod 1981;79:630-652.

3. Langford SR, Sims MR. Root surface resorption, repair, and periodontal attachment following rapid maxillary expansion in man. Am J Orthod 1982;81:108-115.

4. Owman Moll P, Kurol J, Lundgren D. Repair of orthodontically induced root resorption in adolescents. Angle Orthod 1995;65:403-408.

5. Vardimon AD, Graber TM, Pitaru S. Repair process of external root resorption subsequent to palatal expansion treatment. Am J Orthod Dentofac Orthop. 1993; 103: 120-130.

 Reitan K. Biomedical principals and reactions. In: Vanarsdall RL, Graber TM, editors. Orthodontics: current principals and techniques. Saint Louis, Philadelphia: CV Mosby, 1985:101-92.

7. Langford SR. Root resorption extremes resulting from clinical RME. Am J Orthod. 1982; 81: 371-377.

8. Reitan K. 1974: Initial tissue behavior during apical root resorption. Angle Orthod. 1974; 44(1):68–82.

9. Schwarz, A.M. Ligatur oder Feder? Bemerkungen zu A. Oppeinheims "Die Krisis in der Orthodontie" Z.F.Stomat., 31:1344, 1933.

10. 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.

11. Brudvik P, Rygh P. Multi-nucleated cells remove the main hyalinized tissue and start resorption of adjacent root surfaces. Eur J Orthod 1994;16:265-273.

12. Brudvik P, Rygh P. The repair of orthodontic root resorption: an ultrastructural study. Eur J Orthod 1995;17:189-198.

13. Brudvik P, Rygh P. Transition and determinants of orthodontic root resorption-repair sequence. Eur J Orthod 1995;17:177-188.

14. Chan EK, Darendeliler MA. Exploring the third dimension in root resorption. Orthod Craniofac Res 2004;7:64-70.

15. Harris DA, Jones AS, Darendeliler MA. Physical properties of root cementum: part 8. Volumetric analysis of root resorption craters after application of controlled intrusive light and heavy orthodontic forces: a microcomputed tomography scan study. Am J Orthod Dentofacial Orthop 2006;130:639-647.

16. Foo M, Jones A, Darendeliler MA. Physical properties of root cementum: Part 9. The Effect of Systemic Fluoride Intake on Root Resorption in Wistar Rats, Am J Orthod Dentofacial Orthop. 2007; 131:34-43.

17. Gonzales C, Hotokezaka H, Yoshimatsu M, Yozgatian J, Darendeliler MA, Yoshida N. Effects of force magnitude and duration on amount of tooth movement and resorption in the rat molar. Angle Orthod. 2008; 78: 502-509. (In press)

18. Koga Y, Yoshida N, Nakashima M, Naofumi M, Kumiko T, Kobayashi K, Obiya H. Structural Analysis of force systems acting on brackets with a closing arch wire. Orthod. Waves 2001; 60(2): 75-87.

19. Khambay BS, McHugh S. Magnitude and reproducibility of forces generated by clinicians during laceback placement. J. Orthod. 2006; 33:270-275.

20. Rock WP, Wilson HJ. Forces exerted by orthodontic aligning archwires. Br J Orthod. 1988; 15:255-59.

21. Isaacson RJ, Ingram AH. Forces produced by rapid maxillary expansion 2. Forces present during treatment. Angle Orthod. 1964; 34: 261-270.

22. Ren Y, Maltha JC, Kuijpers-Jagtman AM. The rat as a model for orthodontic tooth movement- a critical review and a proposed solution. Eur J Orthod. 2004; 25:483-490.

23. Chan E, Darendeliler MA. Physical properties of root cementum: Part 5. Volumetric analysis of root resorption craters after application of light and heavy orthodontic forces. Am J Orthod Dentofacial Orthop 2005;127:186-195.

24. Proffit, WR. And Fields, HW. Contemporary orthodontics, C.V. Mosby, St. Louis, 2007:342-343.

## LEGENDS

Fig 1. A, occlusal view of the appliance *in situ*; B, lateral view from right side.

**Fig 2.** Study design of force application and retention for rats. Each group consists of 10 rats.

**Fig 3.** Scanning electron micrographs (x60) of the upper left distal roots (mesial view), are shown. Retention time is expressed in weeks (W). 0, 2, 4, 8, 12, 16W of retention. DB, indicates disto-buccal root; DP, disto-palatal root.

**Fig 4. A,** Schematic illustration of the upper right rat molar showing the force applied in the mesial direction and the distal roots. DB, indicates disto-buccal root; DB, disto-palatal root. **B**, Resorption area of the distal roots. (Percentage of the crater area in relation to the whole root two-dimensional area in scanning electron microscope image) **C**, **D**, **E**; Resorption depth, volume, and roughness, respectively. 0W, 2W, 4W, 8W, 12W, 16W indicates the retention time in weeks. \* *P*<.05, \*\**P*<.01 compared with 0 weeks.

**Fig 5. A,** Scanning electron micrograph (x60) of the upper right mesial root, mesial view. M, indicates mesial root. White arrow indicates resorption craters. (Upper). Schematic illustration of the upper rat molar showing the mesial root and the force applied in the mesial direction. **B**, Resorption area of the mesial root. (Percentage of the crater area in relation to the whole root two-dimensional area in scanning electron microscope image). **C, D, E;** Resorption depth, volume, and roughness, respectively. 0W, 12W indicates the retention time in weeks. \* *P*<.05, \*\**P*<.01 compared with 0 weeks.





# Fig 1





Control

0 W

2 W



4 W



8 W



12 W



DB DP 16W







 Table I. Descriptive statistics of disto-buccal and disto-palatal roots.

	Control (n = 10)		0W (n = 10)		2W (n = 10)		4W (n = 10)		8W (n = 10)		12W (n = 10)		16W (n = 10)	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
Disto-buccal root														
Area (%) <i>Recovery rate</i>	1.2	0.0	35.6 0.0	4.0	34.5 2.8	4.6	33.2 6.8	5.4	29.4 17.4	3.4	27.6 22.5	2.5	24.0 32.6	1.3
Depth (µm) <i>Recovery rate</i>	2.5	0.4	123.7 0.0	13.3	120.4 2.7	9.9	96.3 22.2	12.3	82.3 33.5	3.6	64.1 48.2	4.2	53.4 56.8	4.1
Volume (x10 <sup>6</sup> µm <sup>3</sup> ) <i>Recovery rate</i>	0.9	0.1	22.0 0.0	3.8	19.2 12.7	2.0	14.7 33.2	2.2	12.3 44.1	0.9	9.3 57.7	0.7	6.7 69.5	0.7
Roughness (µm) <i>Recovery rate</i>	7.2	0.6	22.1 0.0	5.4	20.8 5.9	1.4	20.5 7.2	3.0	20.1 9.1	4.1	15.0 32.1	4.5	15.0 32.1	1.2
<u>Disto-palatal root</u>														
Area (%) <i>Recovery rate</i>	1.1	0.2	32.8 0.0	5.1	29.7 9.5	9.6	28.5 13.1	5.2	20.7 36.9	4.5	20.0 39.0	2.9	16.8 48.8	0.6
Depth (µm) <i>Recovery rate</i>	2.3	0.4	77.1 0.0	5.2	74.4 3.5	8.3	71.4 7.4	3.9	61.2 20.6	6.2	54.1 29.8	4.7	52.8 31.5	6.5
Volume (x10 <sup>6</sup> µm <sup>3</sup> ) <i>Recovery rate</i>	0.8	0.1	13.2 0.0	2.3	12.3 6.8	1.7	11.2 15.2	1.1	8.4 36.4	0.7	5.9 55.4	0.5	4.4 66.7	0.7
Roughness (µm) <i>Recovery rate</i>	7.4	0.6	18.5 0.0	3.1	18.2 1.6	2.4	14.5 21.6	2.1	13.9 24.9	4.2	11.2 39.5	3.4	11.3 38.9	2.1
<u>Mesial root</u>														
Area (%) <i>Recovery rate</i>	0.3	0.2	4.2 0.0	1.4							2.2 47.6	1.3		
Depth (µm) <i>Recovery rate</i>	2.2	0.4	23.2 0.0	8.4							8.9 61.6	9.3		
Volume (x10 <sup>6</sup> µm <sup>3</sup> ) <i>Recovery rate</i>	0.3	0.1	0.8 0.0	0.4							0.3 62.5	0.1		
Roughness (µm) <i>Recovery rate</i>	1.4	0.3	7.2 0.0	1.1							3.1 56.9	0.7		

*W*, Weeks; *n*, Number; *SD*, standard deviation.

(I) Control Group         (J) Experimental Group         Mean difference (I-J)         S E         Significance <sup>†</sup> Lower bound Upper bound           Disto-buccal root Area (%) <th colspan="3">95% CI for difference<sup>†</sup></th>	95% CI for difference <sup>†</sup>		
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	<sup>.</sup> boun		
Area (%) $0W$ $2W$ $1.16$ $1.46$ $1.00$ $-3.31$ $5.0$ $4W$ $3.24$ $1.46$ $0.45$ $-1.23$ $7.7$ $8W$ $6.20^{**}$ $1.46$ $0.00$ $1.74$ $10.0$ $12W$ $8.09^{**}$ $1.46$ $0.00$ $3.62$ $12.9$ $16W$ $11.73^{**}$ $1.46$ $0.00$ $7.26$ $16.2$ OutputOw $2W$ $3.36$ $3.98$ $1.00$ $-8.86$ $15.9$ $4W$ $27.42^{**}$ $3.98$ $0.00$ $15.20$ $39.6$ $8W$ $41.39^{**}$ $3.98$ $0.00$ $29.17$ $53.6$			
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$			
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	5.64		
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	7.71		
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	10.68		
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	12.56		
Depth (μm)         2 W         3.36         3.98         1.00         -8.86         15.9           4 W         27.42**         3.98         0.00         15.20         39.6           8 W         41.39**         3.98         0.00         29.17         53.6	16.20		
OW         2 W         3.36         3.98         1.00         -8.86         15.4           4 W         27.42**         3.98         0.00         15.20         39.0           8 W         41.39**         3.98         0.00         29.17         53.6			
4 W         27.42**         3.98         0.00         15.20         39.0           8 W         41.39**         3.98         0.00         29.17         53.0	15.59		
8 W 41.39** 3.98 0.00 29.17 53.0	39.65		
	53.62		
12 W 59.61** 3.98 0.00 47.38 71.8	71.84		
16 W 70.30** 3.98 0.00 58.08 82.8	82.53		
Volume (x10°µm³)			
0W 2W 1.68 0.91 1.00 -1.12 4.4	4.48		
4 W 5.35** 0.91 0.00 2.55 8.1	8.16		
8 W 7.91** 0.91 0.00 5.11 10.	10.71		
12 W 11.03** 0.91 0.00 8.24 13.8	13.84		
16 W 12.99** 0.91 0.00 10.19 15.	15.79		
Roughness (µm)			
0W 2W 1.32 1.40 1.00 -2.97 5.6	5.61		
4 W 1.58 1.40 1.00 -2.71 5.8	5.86		
8 W 1.44 1.40 1.00 -2.85 5.	5.72		
12 W 7.04** 1.40 0.00 2.76 11.3	11.33		
16 W 6.99** 1.40 0.00 2.71 11.2	11.28		
Disto-palatal root			
Area (%)			
0W 2W 3.07 2.07 1.00 -3.28 9.4	9.43		
4 W 4.78 2.07 0.37 -1.58 11.1	11.13		
8 W 12.07** 2.07 0.00 5.72 18.4	18.43		
12 W 15.78** 2.07 0.00 9.43 22.1	22.14		
16 W 15.96** 2.07 0.00 9.61 22.3	22.32		
Depth (μm)			
0 W 2 W 2.75 2.67 1.00 -5.45 10.9	10.95		
4 W 5.74 2.67 0.54 -2.46 13.9	13.95		
8 W 15.97** 2.67 0.00 7.77 24.1	24.18		
12 W 23.03** 2.67 0.00 14.83 31.2	31.24		
16 W 24.32* 2.67 0.00 16.12 32.4	32.53		
Volume (x10 <sup>6</sup> µm <sup>3</sup> )			
0W 2W 1.27 0.59 0.54 -0.54 3.0	3.08		
4 W 1.81* 0.59 0.05 0.01 3.0	3.63		
8 W 4.17** 0.59 0.00 2.36 5.9	5.98		
12 W 6.79** 0.59 0.00 4.98 8.0	8.60		
16 W 8.23** 0.59 0.00 6.43 10.0	10.05		
Roughness (µm)			
0W 2W 0.20 1.15 1.00 -3.31 3.	3.72		
4 W 4.12** 1.15 0.01 0.61 7.0	7.64		
8 W 4.09** 1.15 0.01 0.58 7.0	7.61		
12 W 7.30** 1.15 0.00 3.79 10.8	10.82		
16 W 7.18** 1.15 0.00 3.67 10. <sup>-</sup>	10.71		

**Table II.** ANOVA of groups of rats (pairwise comparisons) with area, depth, volume, and surface roughness as dependent variables.

Based on estimated marginal means.

\* Mean difference significant at .05 level.

\*\* Mean difference significant at .01 level.

**†** Bonferroni adjustment for multiple comparisons.

W, weeks.

-	Ρ	n
-0.788*	0.000	60
-0.942*	0.000	60
-0.921*	0.000	60
-0.618*	0.000	60
-0.788*	0.000	60
-0.841*	0.000	60
-0.906*	0.000	60
-0.734*	0.000	60
-0.690*	0.001	60
-0.866*	0.000	60
-0.628*	0.003	60
-0.938*	0.000	60
	-0.788* -0.942* -0.921* -0.618* -0.788* -0.841* -0.906* -0.734* -0.690* -0.866* -0.628* -0.938*	-0.788*       0.000         -0.942*       0.000         -0.921*       0.000         -0.618*       0.000         -0.788*       0.000         -0.841*       0.000         -0.734*       0.000         -0.734*       0.000         -0.690*       0.001         -0.866*       0.000         -0.628*       0.003         -0.938*       0.000

**Table III.** Pearson correlations between time andresorption crater measurements.

\_\_\_\_

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