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A COMPARISON OF THE SUPRA-ALVEOLAR PULP SIZE OF IMPEDED AND UNIMPEDED MANDIBULAR RAT INCISORS USING MICRO-COMPUTED TOMOGRAPHY

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TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Materials and methods
  3.1. Operative procedure
  3.2. Micro-computed tomography
  3.3. Measurements of dentine widths and pulp volume
4. Results
  4.1. Measurements of dentine width
  4.2. Supra-alveolar level of the incisor
  4.3. Volume of the pulp
5. Discussion
6. Acknowledgements
7. References

1. ABSTRACT

The pulp space is almost totally obliterated in the incisal end of a mandibular rat incisor. Repeated shortening of the incisor has been shown to increase the pulp space below the alveolar bone level; however, little is known about that above the alveolar bone level. This study investigated the changes in the pulp space superior to the bone level after rendering the incisor unimpeded for a period of three weeks. A desktop micro-computed tomography scanning unit, which is non-invasive, non-destructive, accurate, less technique sensitive and less time consuming than conventional methods, was used to calculate the volume of the pulp space. The results showed that the pulp size increased 140% at a level 3mm below the alveolar bone margin and more than 700% at a level 2.5mm above the alveolar bone. Moreover, the bone level of the unimpeded incisor was found to move incisally 0.4-0.5mm; while deposition of dentine on the mesial and distal aspects of the pulp space were affected differently to that on the buccal and lingual sides.

2. INTRODUCTION

The ability of the rat incisor to grow, calcify and erupt continuously at an extremely rapid rate during the life of the animal makes it almost ideal for the study of tissue interaction, odontogenesis, amelogenesis, tooth eruption, dental material testing and tissue regeneration. Tissue regeneration is becoming a reality especially in the fields of bone induction (1,2) and periodontal tissue regeneration (3-5). The progress in the field of dentine regeneration is much slower and the need for an experimental model against which induced dentinogenesis can be measured is becoming more urgent. The rat incisor provides an almost ideal model because, unlike bone remodelling, dentine formation is not coupled with a phase of resorption.

The daily eruption rate of rat incisor is reported to be 0.4 to 0.6mm/day and is affected by a number of factors such as: mastication, diet, hormones, salivary glands, nervous system, irradiation, vascularity of the pulp, pathological conditions, surgical procedures and repeated shortening (6,7). Repeated shortening of the incisor renders the eruption of the tooth free of occlusal stress; so the tooth becomes “unimpeded”, a term coined by Bryer (8). Besides being able to significantly increase the eruption rate (9,10), repeated shortening affects the morphology of the incisor socket and its location within the mandible (11), it increases the volume of the periodontal ligament (9), influences the mechanical properties of the periodontium (12), decreases the width of the dentine (9,13) and increases the pulp size (9,14). Nevertheless, it does not affect the final thickness of the enamel (10,15,16).

Several histomorphometric studies, using serial sections of the rat incisor, have estimated the dentine thickness, dentine area, pulp area and pulp circumstance of impeded and unimpeded mandibular incisors, of that portion of the tooth that extends from the posterior limit of the alveolar socket to the alveolar bone level (9,14,17-19). However, these studies focused on the portion of the tooth below the bone level. No detailed information on the size of the pulp above the alveolar bone level is available.

Since the introduction, by Hounsfield in 1973 (20), computerized tomography has become widely used in medicine to obtain three-dimensional images of the body with a resolution of 1-2mm. Although it has been used in the detection of root fractures (21) and root resorption (22), it is not widely used in the *in vivo* study of dental tissues because of inadequate resolution. However, the development of micro-computed tomography provides an excellent tool for the *in vitro* study of odontogenic tissues.

Following its introduction (23-25), micro-computed tomography has been used extensively in the study of bone architecture (26-31). Later it was used in the study of caries progression (32) and mineral distribution within teeth (33-35). More recently it was used in the study of the pulp morphology of human teeth (36-40). Therefore, this technique should be employed to gather the information that is required to develop an experimental model for studying the effects of different agents on the genesis of different odontogenic tissues.

The objectives of this study were to compare, using micro-computed tomography, the position of the alveolar level and the supra-alveolar pulp size of unimpeded mandibular rat incisors, after regular shortening, for a period of three weeks; to impeded incisors.

3. MATERIALS AND METHODS

Seventeen Sprague-Dawley rats, seven to eight weeks old, with an average weight of 190.9g (175.6g-206.6g) were divided into two groups. Six rats were assigned to the control group and 11 rats to the experimental group. All of the rats were placed in a windowless room which was illuminated artificially from 0700hrs to 1900hrs and was otherwise dark. The temperature and humidity were controlled to be $21^0C \pm 1^0C$ and from 55% to 73% respectively. The rats were caged in groups of two to three, fed on laboratory autoclavable pellet rodent diet (PMI Nutrition International Inc.) and able to take water *ad libitum*. The experimental procedure was approved by the Committee on the Use of Live Animals in Teaching and Research, University of Hong Kong.

3.1. Operative procedure

Every morning on Monday, Wednesday and Friday between 0900hrs and 1400hrs, the rats were weighed and then anaesthetised with an intra-peritoneal injection of Ketamine (67mg/kg) and Xylazine (6.7mg/kg). The experiment was always started promptly at 0900 hrs and the rats were routinely anaesthetised in the same sequence in order to ensure the accuracy of the 48hrs or 72hrs periods. For the experimental group, the right incisor was shortened at the level of interdental papilla with a diamond disc. Marks were cut, below the interdental papilla, on the incisors of both the control and experimental group animals. The eruption rate was measured as being the distance the cut mark traveled with the reference to the lowest point of the labial gingiva of the unshortened left incisor in the experimental group (41,42) and of the corresponding incisors in the control group. The measurements were taken using a graticule in the eyepiece of a stereomicroscope with one unit of scale equivalent to 100µm. After three weeks, all of the rats were sacrificed and their mandibles were dissected out and fixed in 2.5% glutaraldehyde, or 10% neutral buffered formaldehyde.

3.2. Micro-computed tomography

The right side of the mandible was fixed to an acrylic plate with sticky wax and put into a 17mm diameter cylindrical sample holder. Because of the curvature of the mandibular incisor, the incisal edge of the tooth and the buccal gingival margin were aligned so as to be on a vertical axis. As the length of the incisor had already been measured *in vivo* under the stereomicroscope, the incisal edge and buccal gingival margins were orientated so as to be on the same plane. This measurement of the length of the incisor was then used to determine the level of the gingival margin prior to calculating the dentine width of the tooth at the level of the gingival margin.

For the micro-tomographic imaging, the specimens were scanned with a desktop fan beam type micro-computed tomography system (microCT20, Scano Medical AG, Bassersdorf, Switzerland), following the technique of Rüegsegger and co-workers (43). An initial step consisted of a “scout view” which facilitated the identification of the position and length of the specimen prior to scanning. Then the incisal 10mm to 12mm portion of the tooth was scanned in a multi-slice, high resolution mode with a size of 1024x1024 pixels. The pixel size was 17µm while the slice thickness was 50µm, which resulted in a voxel size of 17x17x50µm. The scanning time ranged from 12hrs to 14.5hrs and the number of slices from 198 to 239.

3.3. Measurements of dentine widths and pulp volume

After scanning, the image of each slice was examined working from the incisal tip towards the apex. The slices taken at the levels where the alveolar bone just appeared to surround the tooth and where the circumference of the incisor became completely surrounded by bone, were identified. Thus, the length of the incisor from the gingival margin to these two levels could be calculated. Then the slice equivalent to the level of the buccal gingival margin was identified and the image was magnified. A line was drawn across the maximum bucco-lingual dimension of the pulp and the widths of the dentine on the buccal and lingual aspects were measured from the two ends of the line to the surface of the tooth (figure 1). Another two lines, passing through the mid-point of the first line and at right angles to the mesial and distal tooth surfaces were drawn. The widths of the dentine on the mesial and distal aspects of the section were then measured. After recording the widths of the dentine on all four sides, the measurements were repeated to test for measurement errors.

Because the contrasts of the bone and pulp were

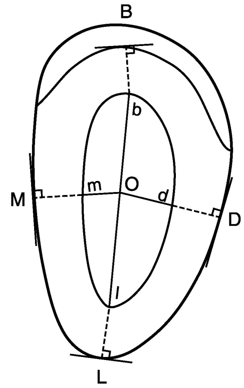

Figure 1. Measurements of the widths of dentine at the buccal, lingual, mesial and distal sides. A line "bl" was drawn across the maximum bucco-lingual dimension of the pulp and "O" was the mid-point of the line. Dotted lines "Bb", "Ll", "Mm"and "Dd" represent the dentine width at buccal, lingual, mesial and distal sides.

very high on the computer images of the specimens, the pulp was segmented from the dentine with the help of a thresholding procedure. All samples were binarized using the same parameters for the filter width (1.0), the filter support (2) and the threshold (140). The value of the threshold was determined to be when the pulp size became constant in spite of increasing the threshold. Therefore, the pulp area of each 50μm thick slice was generated by the computer.

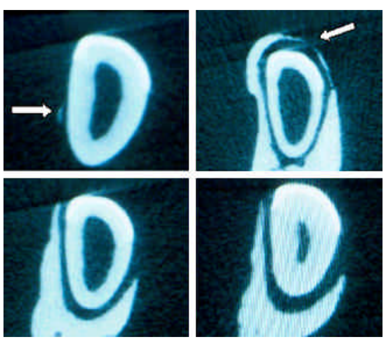

Figure 2. Micro-computed tomographic images shown by the computer. The image at the bottom right is from the control group and the other three are from the experimental group.Top left: bone starts to appear at the mesial side.Top right: the surrounding bone just starts to disappear at the disto-buccal side.Bottom left: bone surrounds the mesial and lingual surface at the level of buccal gingival margin.Bottom right: image from the control group at the gingival level, the same level as the one on the left, showing near obliteration of the pulp space.

In the specimens from the experimental group, the pulp space was larger; so the area was measured from 3mm below the gingival margin to at least 4mm above the gingival margin while, for the specimens in the control group, because the pulp space was almost obliterated above the gingival margin, the area was measured from 3mm below the gingival margin to at least 2.45mm above the gingival margin. Subsequently, the pulp volume of each slice was calculated by multiplying the thickness (50μm) of the slice by the area of the pulp in each slice.

4. RESULTS

All 17 of the rats survived the 3-week experimental period. Although the average weight gain for the experimental group (39.4g) was approximately 6% less than that of the control group (41.8g), it was not statistically significant.

The eruption rates of the left and right incisors in the control group on all nine of the measuring sessions were not statistically significant; therefore, the eruption rate of the right incisor in the control group was compared to that of the right incisor in the experimental group. The eruption rates of the right incisor in the control group were not significantly different at the nine measuring sessions and the mean was 0.60mm/day. For the experimental group, the unimpeded eruption rate varied from 0.93mm/day on Day 7 to 1.16mm/day on Day 11 with a mean of 1.02±0.12mm/day; indicating that the unimpeded eruption rate of the teeth in the experimental group increased by 170% of the impeded eruption rate of the teeth in the control group.

4.1. Measurements of dentine width

For each dentine width measurement, two readings were taken and the T test showed there was not statistically significant difference between the two readings; the mean readings are shown in table 1. The mean dentine widths on all four sides were significantly different ($p<0.001$) in the control and experimental groups. One way ANOVA showed a statistically significant difference ($p<0.001$) for the dentine widths on the four sides in the control group teeth. Further analysis using the Student-Newman-Keuls test showed that the dentine width of the lingual side was significantly different from that of the buccal, mesial and distal sides. However, in the experimental group, the width of the dentine on the buccal side was significantly different from that on the lingual, mesial and distal sides. When the dentine width on each side of the teeth, in the experimental group, were compared to that of the corresponding side in the control group, the percentages of the mesial, distal, lingual and buccal sides were 64.4%, 63.0%, 53.2% and 53.9% respectively. When the percentages were tested with one way ANOVA, there was significant difference ($p<0.001$) between the four readings. Further analysis with the Student-Newman-Keuls test showed that the readings for the buccal and lingual sides were statistically significant different from those for the mesial and distal sides.

4.2. Supra-alveolar level of the incisor

When the images were scanned from the incisal tip towards the apex, the alveolar bone on the mesial side always appeared first. When moving further apically, the lingual bone appeared, then the distal and buccal bone; the last piece of bone to surround the whole circumstance of the tooth was always on the disto-buccal surface (figure 2). The distances from the level of gingival margin to the points where the circumference of the tooth just became

Table 1. Widths of the dentine at the mesial, distal, lingual and buccal sides of the incisor at the level of gingival margin in the experimental and control groups

Side	Dentine width (mm)		p value	% of E/C
	Experimental group (E)	Control group (C)		
mesial	0.2990	0.4644	<0.001	64.4%
distal	0.2935	0.4657	<0.001	63.0%
lingual	0.3047	0.5724 *	<0.001	53.2%
buccal	0.2615 *	0.4853	<0.001	53.9%

* statistically significant different (p<0.001) from the widths of the other three sides in that group

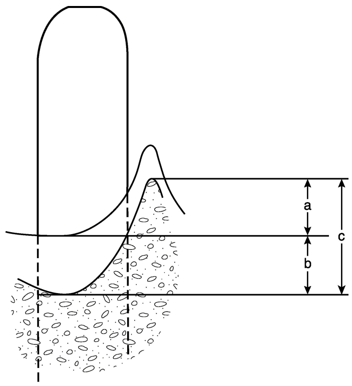

Figure 3. Diagram of a rat incisor showing the level of the lowest point of the buccal gingival margin and two levels of the supporting bone. (a,b,c refer to the lengths defined in table 2).

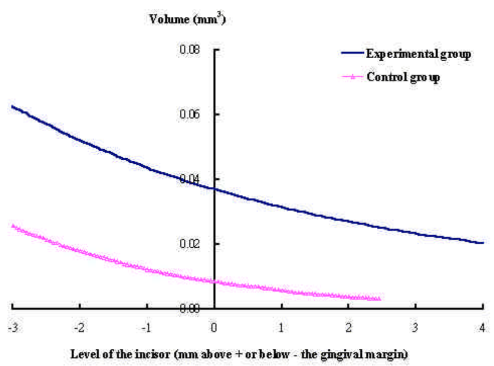

Figure 4. The pulp volume (mm^3) of 0.05mm lengths of the mandibular incisor of the experimental and control groups in relation to the length of the incisor above and below the buccal gingival margin.

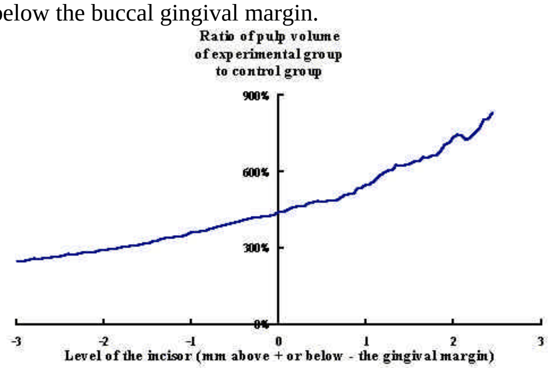

Figure 5. The ratio of the pulp volumes of the experimental group to control group in relation to the length of the incisor above and below the buccal gingival margin.

completely surrounded by bone and where all the surrounding bone just disappeared, were measured (figure 3). The mean values for the control and experimental groups are shown in table 2. The length of the tooth from the point where all of the surrounding bone just disappeared to the buccal gingival margin was statistically significant (p=0.033) in the experimental and control groups, so was the length of the tooth from the level where the circumference of the tooth just becomes completely surrounded by bone to the buccal gingival margin (p=0.008). However, when the experimental and control groups were compared, the sum of the two lengths which were the length of the tooth where the circumstance of the tooth was partially surrounded by bone, was not statistically significant (p=0.428).

4.3. Volume of the pulp

In four of the six rats in the control group, the pulp area at the level 2.5mm above the buccal gingival margin was very small (<0.06mm^2); hence no measurements were taken above this level. For the rats in the experimental group, the lingual dentine of the incisal 1-2mm of the tooth was always removed by attrition, causing an abrupt change in the pulp size. Therefore, the taking of measurement of the pulp area was ceased 4mm above the buccal gingival margin. When the mean volume of the pulp of each 0.05mm slice was plotted in relation to the length of the tooth near the gingival margin, it resulted in parabolic curves for both the control and experimental groups (figure 4). Using regression analysis, the data from the experimental group could be fitted to a third-order polynomial, $y = 0.036894 - 0.006102 + 0.000669x^2 – 0.000048x^3$, where y represents the area parameter and x represents the distance measured from the gingival margin. The value of R^2 was 0.99994, which represented an excellent curve fit. The data from the control group could also be fitted to a third-order polynomial, $y = 0.008414 - 0.003208x + 0.000600x^2 – 0.000077x^3$ and the value of R^2 was 0.99967. When the ratio of the pulp volumes of the experimental group to the control group were plotted against the length of the tooth near the gingival margin, it produced a curve that could be fitted to a third-order polynomial, $y = 4.354115 + 0.955824x + 0.175945x^2 + 0.024705x^3$, where y represents the ratio of pulp volume and x represents the distance measured from the gingival margin; the value of R^2 was 0.99732 (figure 5). If the volume of a 1mm length of the incisor, instead of the scanned thickness of 0.05mm, was used to calculate the ratio of the pulp volume, the result was almost the same (table 3).

Table 2. The lengths of the tooth as measured from different reference points of the incisor in the experimental and control group

Reference point	Experimental group		Control group		p value
	Mean	SD	Mean	SD	
a	2.55	0.38	2.14	0.23	0.033
b	2.43	0.37	2.94	0.22	0.008
c	4.98	0.30	5.08	0.15	0.428

Reference point:a = length of the tooth (mm) from the level where all the surrounding bone just disappears to the buccal gingival margin b= length of the tooth (mm) from the level where the circumference of the tooth just becomes completely surrounded by bone to the buccal gingival margin c= length of the tooth (mm) where the circumference of the tooth is partially surrounded by bone

Table 3. The ratio of pulp volumes, expressed as percentages, at the level below and above the gingival margin of the mandibular incisor, and the experimental group to the control group measured from 0.05mm and 1mm lengths of tooth

0.05mm length of incisor		1mm length of the incisor	
level of the incisor	Ratio	Level of the incisor	Ratio
2.50mm-2.55mm below gingival margin	265%	2.05mm-3.00mm below gingival margin	265%
1.50mm-1.55mm below gingival margin	318%	1.05mm-2.00mm below gingival margin	317%
0.50mm-0.55mm below gingival margin	394%	0.05mm-1.00mm below gingival margin	394%
0.45mm-0.50mm above gingival margin	483%	0mm-0.95mm above gingival margin	479%
1.45mm-1.50mm above gingival margin	624%	1.00mm-1.95mm above gingival margin	622%

5. DISCUSSION

Micro-computed tomography, used in this study, is a useful experimental tool to measure the volume of the pulp and is more reliable, accurate, less time-consuming and less destructive. The specimen remains intact for future histological or mechanical testing; it also provides a high quality three-dimensional image which is independent of the planes of sectioning and is more reproducible (26,28,30,44). When compared with tissue processing for histological examination, micro-computed tomography is less technique sensitive and less prone to human error (43,45).

Although the micro-computed tomography scanning unit allowed for a smaller slice thickness to be utilized, a slice thickness of 50µm was used in the present study because the root canal anatomy changed only gradually along the length of the tooth (40). Therefore, the measurements of different bone levels by the micro-computed tomography in this study could be measured to an accuracy of 0.05mm, much is a level of sensitivity which is difficult to achieve using conventional sectioning methods. The length of the tooth where the circumference of the tooth was partially surrounded by bone was not significantly different in the experimental and control groups (table 2 and figure 3). However, the length of the tooth from the level where all the surrounding bone just disappeared to the buccal gingival margin and the length from the level where the circumference of the tooth just became completely surrounded by bone to the buccal gingival margin in the two groups were significantly different. The bone level where the surrounding bone just disappeared in the unimpeded incisor was 0.41mm higher than that in the impeded incisor while the bone level where the circumference of the tooth was completely surrounded by bone was 0.51mm higher than that in the impeded incisor. In other words, the bone levels of an unimpeded incisor were higher than those of an impeded incisor; the same phenomenon was found in the level of the buccal gingival margin. This has important implications if it is decided to measure the eruption rate of the rat incisor by using the inter-incisal bony crest as the reference point (46-49).

For the dentine widths in the control group, the measurements of the mesial, distal and buccal sides were significantly smaller than those of the lingual side and for the dentine widths in the experimental group, the measurements of the buccal side were significantly smaller than those of the mesial, distal and lingual sides. Nevertheless, in our study using micro-computed tomography, the scanning, which was equivalent to histological sectioning, was in one direction and was directed perpendicular to the mid-point between the incisal edge and the gingival margin. Due to the antero-posterior curvature of the incisor, higher readings for the buccal and lingual sides would have been expected for the levels beyond the reference point, when compared to the readings obtained by sectioning perpendicular to these points on the buccal surface. Moreover, the further away from this reference point, the higher the measurements will be. However, it had a minimal effect on the mesial and distal sides because the measurements were taken in the middle of the sectioning plane when the planes were tilted. Therefore, taking into consideration the degree of curvature and the clinical length of the tooth, the dentine widths of the buccal and lingual sides were calculated to be about 10% more than the correct value. Nevertheless, the significantly smaller dentine width on the buccal side in the experimental group, whether uncorrected, or calculated using the corrected value, was different from the findings of Michaeli and co-workers, who found the dentine widths of all the four sides to be the same (17).

Although there were some limitations to the values of the measurements of the tooth outside the chosen reference point, they could still be used for comparisons between the control and the experimental groups because they were scanned into the computer in the same way. Further analysis of the data, by calculating the ratio of the dentine widths of the experimental group to those of the control group, the lingual (53.2%) and buccal (53.9%) sides showed similar figures as did the mesial (64.4%) and distal (63.0%) sides; there was a significant difference between these two groups.

The eruption rate of the unimpeded incisor had increased about 170%, so during the 3-week experimental period, the whole tooth structure would have been totally renewed (17). If the deposition of dentine was unaffected by the increased eruption rate, as it has been suggested (10,13), the dentine width should be about 58.8% of that of the impeded incisor. The issue is complicated by the previously reported finding that the deposition of dentine by the odontoblasts is not constant along the whole length of the incisor (14,17,19,50,51). By the use of a simple mathematical formula, it has been found that the daily deposition of dentine in impeded and unimpeded incisor are similar during the first few days of life; then the daily deposition rate of the unimpeded incisor increases towards the incisal tip while that of the impeded incisor decreases (17,19). Therefore, the dentine width of an unimpeded incisor, though thinner, should be thicker than when it is calculated as an inverse proportion to the increased eruption rate. In this study, the dentine widths of the mesial (64.4%) and distal (63.0%) sides were higher than the expected value of 58.8% while those of the lingual (53.2%) and buccal (53.9%) sides were lower. In other words, these findings showed that the odontoblasts on the buccal and lingual sides of an unimpeded incisor deposited dentine slower than that of the impeded incisor, while the odontoblasts on the mesial and distal sides deposited dentine faster. In the present investigation, it was not possible to determine the daily change of dentine along the tooth and further experiments would be needed to confirm this new finding.

For the measurement of the cross sectional area of the pulp, the same graph (figure 4) could be used to compare the pulp volume, or the cross sectional area of the control group to those of the experimental group, with only the Y-axis being different. The curves in figures 4 & 5 can be fitted into third-order polynomials with a $R^2 > 0.99$, which produces an excellent fit to the curve. The cross sectional area of the pulp in the control group at the level of the buccal gingival margin was $0.169 mm^2$, which coincided with the finding of $0.1 mm^2$ in an earlier study by Steigman and co-workers (18), in which the lingual bone level was used as the reference point. For the measurement of the cross sectional area of the pulp, same errors, as occurred with the measurements of the dentine width, would have occurred due to the curvature of the tooth. Nevertheless, accurate results can still be achieved when the pulp areas or volumes in the two groups are compared. At the 3mm level below the buccal gingival margin, or about 0.5mm below the level of where the circumference of the tooth became completely surrounded by bone, the pulp volume of the experimental group was 244% that of the control group. At the level of the buccal gingival margin, the ratio was 439%, while at the level where all the surrounding bone just disappeared, the ratio was more than 800%. If the pulp volume is calculated for a larger thickness, such as 1mm, instead of 0.05mm, a similar result was obtained (table 3). This means that any point on the curve of figure 5 can be used to compare volumes of 1mm lengths, which may be of more significance to studies involving pulp volume.

This study determined the change in the supra-alveolar pulp size when the mandibular rat incisor was rendered impeded three times a week for a period of three weeks using micro-computed tomography. To the best of the authors' knowledge, this area has not been investigated according to the published literature. Because of the curvature of the rat incisor and the unidirectional scanning of the micro-computed tomography, the measurements of the dentine width and the cross sectional area may have some methodological errors. However, the pulp volume can be measured extremely accurately by the thresholding procedures and subsequently three-dimensional images can be presented.

In conclusion, the findings of this study were that the dentine deposition on the buccal and lingual sides was affected differently from that of the mesial and distal sides when the mandibular rat incisor was unimpeded for three weeks. Moreover, the bone level of the unimpeded incisor moved incisally 0.4-0.5mm. The pulp size increased by 140% at a point 3mm below buccal gingival margin, by 340% at the level of the buccal gingival margin and by more than 700% at the point 2.5mm above the buccal gingival margin when the incisors were rendered unimpeded for three weeks. Moreover, micro-computed tomography is a more reliable, accurate, less time-consuming and less destructive experimental tool to measure the volume of the pulp.

6. ACKNOWLEDGEMENTS

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