ORIGINAL ARTICLE Open Access

Assessing the feasibility of micro-computed tomography in comparing mineral densities and volume values of enamel and dentine in permanent premolars which were extracted teeth for orthodontic and periodontal treatment

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Abstract

The objective of our study was to show that the volumes of enamel-dentin tissues and mineral tissue densities of the teeth of young and adult individuals extracted for orthodontic and periodontal purposes could be measured using micro-computerized tomography. Non-decayed teeth extracted due to orthodontic and periodontal reasons were used. The teeth were scanned using a micro-CT (Skyscan 1172, Bruker, Belgium) device. The image data of the samples scanned with micro-CT were used in computer settings through the CTAn program for the calculation of the volumes of enamel and dentin tissues and their mineral densities. Comparisons between groups showed that there is no statistically significant difference between occlusal, middle, or apical zone mineral density values of the enamel and dentin tissues of the teeth in group 1 and group 2 ($p>0,05$). In addition, no statistically significant difference was detected between the mineral density values of average enamel and dentin tissues. Comparison between groups themselves showed a statistically significant difference between percentage ratios of enamel, dentin, and pulp volume compared to crown volume ($p<0.05$). We believe that the micro-CT technique is an imaging method that can perform accurate and sensitive measurements meant of volume changes observed in tooth tissues with time. In addition, we concluded that with micro-CT, the densities in enamel and dentin tissues in study groups could be measured reliably.

Keywords: *dentin, enamel, micro-computerized tomography, mineral density, volume*

Received:
22 March 2022
Revised:
1 July 2022
Accepted:
9 July 2022
Published Online:
30 July 2022
How to cite this article:
Yildiz, S., Yavuz, I., Kamalak, H., & Akkus, Z. Assessing the feasibility of micro-computed tomography in comparing mineral densities and volume values of enamel and dentine in permanent premolars which were extracted teeth for orthodontic and periodontal treatment. *IIUM Journal of Orofacial and Health Sciences*, 3(2), 172–180. https://doi.org/10.31436/ijohs.v3i2.138
Article DOI:
https://doi.org/10.31436/ijohs.v3i2.138
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Introduction

Recent developments in digital technologies ensured that micro-computerized tomography (micro-CT) in the experimental studies of dentistry could be used in vitro studies in a variety of areas such as root canal morphological analysis, evaluation of root canal structuring, examination of the remaining filling materials in root canal after retreatment, examination of the development of head and face skeleton, evaluation of the microstructure of the bone around implant and root, measurement of enamel thickness and determination of the mineral concentration of teeth (Sahin &

Topuz, 2014; Kurt & Orhan,2016; Keles & Alcin, 2015).

The crown of the tooth consists of enamel, dentin, and pulp. Matured enamel is the hardest structure of the body, and its organic matrix is negligible. Enamel tissue does not include any veins and neural packages, which means that it lacks renovation features. In addition, it is a tissue that cannot remain stable. The enamel is exposed to change in a lifetime due to certain reasons (Bath-Balogh & Fehrenbach, 2006; Nanci, 2003). With aging, abrasion, erosion, attrition, and abfraction can be witnessed on the surface of the enamel with the impact of chewing (Nanci, 2003; Karaaslan *et al., 2008*).

Dentin is the most volumetric mineralized tissue of the tooth, which can be accepted as connective tissue. After the formation of primary dentin, in the later stages of life, physiological secondary dentin is formed as a relatively slow apposition without an apparent external stimulant (Bath-Balogh & Fehrenbach, 2006).

Mineral density distribution in the structure of bones and teeth affects the mechanical features of these structures (Farah *et al.*, 2010). Thus, the distribution of mineral density in tooth tissue is essential for clinicians and researchers (Zou *et al*., 2011). Researchers accept two methods as a golden standard for measuring the mineral density of hard tissues such as teeth and bones. These are histological / histo-morphometric analysis and the micro-CT method (Kim & Henkin, 2015; Dogan *et al.,* 2018).

Materials and Methods

The tooth samples used in this study were classified into the following groups: Group 1: young permanent upper first premolar teeth extracted due to orthodontic reasons (between 13-15 years old), and Group 2: permanent upper first premolar teeth extracted for periodontal treatment (over 40 years old). For this effect, a total of 30 teeth were collected. Permanent, non-decayed teeth which were not subjected to root canal treatment, restoration, and crown were used in this research.

The soft tissue scraps on the roots of extracted teeth were removed using a scaler and cleaned using pumice and bleaching; they were then left in distilled water at chamber temperature.

Micro-CT (Skyscan 1172, Bruker, Belgium) device was used for examining the samples. The teeth were placed in their holder. Samples were fixed on the sample bedding with the sample holder. The cover was closed. Camera values were 9 µm for pixel size, 0.9983 for camera angle, 85 kV for voltage, 118 milliampere, 360 degrees for re-constructor rotating angle, and 2 degrees for rotating angle, and each sample was scanned for 1 hour, and 1000-1200 image section data on average were obtained. Then, the image data collected from the samples were reconstructed. While the samples in the groups were rotating with half-angle, projection images are received until 180 degrees were completed. The images were recorded using 16-bit TİFF (Tagged İmage File Format) format (N.V., 2005).

After the scanning was completed, a series of x-ray images were formed. The number of the included images showed differences according to the chosen rotating speed of the device and the total number of rotations. Reconstruction was started after all these procedures. A raw data section was created during reconstruction. CT- volumetric images of sample models for which micro-CT scanning was made were created by CTAn (computer tomography Analyzer). Using this program, three-dimensional volumetric images were formed on the scanning image data obtained from the samples.

The sections obtained from the materials scanned with a micro-CT device were examined using the Data Viewer Software program (Version 1.6.6.0; Bruker micro-CT, Belgium). Dispersions were identified and corrected in scanned areas due to deficiency, error, or scanning.

Based on the sectional view data of the samples which were reconstructed and

analyzed using the Data Viewer Software program, crown volumes were calculated to the Cementoenamel junction using the CTAn software program (Version 1.16.4.1; Bruker micro-CT, Belgium). CTAn Software program was used to segregate the crown parts of the samples belonging to all study groups, and three-dimensional volumetric image data were obtained using the CTvol program similarly, the pulp, enamel, and dentin tissues of the samples used in our study were segregated using the CTvol program with the help of density differences and three-dimensional volumetric data the volumes of pulp, enamel, and enamel dentin tissues were measured and obtained for comparisons.

The densities of enamel and dentin tissues of the teeth in the groups were obtained by measuring with the CTAn program from different zones such as occlusal, median, and apical. In order to get the densities of the materials, calibration was made by scanning two phantom sticks with different densities using a micro-CT.

When the material is exposed to X-ray, an attenuation coefficient is determined related to the density of the material. Calibration phantoms are used in determining the material density in micro-CT. The concentrations of these calibration phantoms vary between 0.25 and 0.75 g.cm^3. For 0.25 calibration phantom, the attenuation coefficient was 0,00905. Then, for the 0.75 calibration phantom, the attenuation coefficient was found as 0,01948. These values were recorded in the program system. After choosing with the program the zone for which we wanted to see the density value, the density of the relevant zone was identified using attenuation coefficients. Finally, the observed values were loaded onto the system.

In the marked occlusal triple-zone, the target points in the enamel and dentin sections were marked, and densities were identified. In the occlusal triple, the zones were marked so that the entire coronal zone for which enamel volume was calculated was marked. The white areas in the figure were taken as reference points for measurement. Densities were examined by descending from occlusal to apical. These processes were applied separately for an occlusal, apical, and medium triple.

Results

Among the samples used in our study, 15 were young first premolar teeth and 15 were mature maxillary first premolar teeth, making up 30 samples in total.

During the evaluation of the findings obtained in the study, the convenience of parameters to normal distribution was tested using the Shapiro-Wilk test. According to these results, as the data showed normal distribution, the comparison between groups was carried out using the Student t-test, a parametric test that tested the mean values of two independent groups. Paired comparisons between study groups did not reveal any statistically significant difference between the occlusal, medium, and apical mineral density values of enamel and dentin tissues of group 1 teeth and the occlusal, middle and apical mineral density values of enamel and dentin tissues of group 2 teeth ($p>0,05$). At the same time, no statistically significant difference was revealed by comparing mineral density values of mean enamel and dentin tissues between groups (Table 1). Although a statistically significant difference was not found, the density values of occlusal, apical third, and dentine occlusal third of the enamel were higher in group 1 teeth. Meanwhile, the middle third density of enamel tissue and, the middle and apical third density of dentin tissue were higher in group 2 teeth. In all samples, the mineral density of enamel and dentin tissue was higher in the occlusal section compared to the apical section.

A comparison of the mineral density values of the enamel and dentin tissues of all teeth included in our study (Table 2) showed that there was a statistically significant difference ($p<0,05$) between the mean dentin density (1,309 ±0,200 g.cm^{-3}) and

mean enamel density (2,164± 0,195 g.cm^{-3}) of all teeth in the groups.

In the paired comparisons between groups, statistically significant differences ($p<0.05$) were found in the percentage of enamel, dentin, and pulp volume as per crown volume. For example, the percentage ratio of enamel and pulp volume of group 1 teeth was higher than Group 2 teeth; however, the dentin volume percentage per crown volume was lower (Table 3).

Table 1. Density and standard deviation values obtained from teeth.

	Groups	N	Mean (g.cm^{-3})	Std. Deviation
Enamel occlusal third density	1	15	2,2619	,19246
	2	15	2,1950	,25167
Enamel median third density	1	15	2,0690	,24933
	2	15	2,1716	,23602
Enamel apical third density	1	15	2,1711	,09025
	2	15	2,1177	,14463
Dentin occlusal third density	1	15	1,3594	,06980
	2	15	1,3251	,15194
Dentin medium third density	1	15	1,2861	,11187
	2	15	1,4064	,41150
Dentin apical third density	1	15	1,2358	,03896
	2	15	1,2468	,14524
Entire crown enamel density	1	15	2,1740	,10747
	2	15	2,1614	,10101
Entire crown dentin density	1	15	1,2938	,05461
	2	15	1,3423	,20614

**There was no statistically significant difference between the groups in terms of all variables, $p>0.05$.*

Table 2. Mean dentin and enamel density and standard deviation values of all teeth in the groups.

	N	Mean (g.cm^{-3})	Std. Deviation
Mean dentin density of all teeth in groups	90	1,309	,200
Mean enamel density of all teeth in groups	90	2,164	,195

**There was a difference between the mean dentin and enamel density and standard deviation values of all teeth in the groups, $p<0.05$.*

Table 3. The percentage volume and standard deviation values of the mean pulp, enamel and dentin volume compared to the crown volume.

	Groups	N	Mean	Std. Deviation
Percentage of pulp volume	1	15	% 1,690 mm^3	,423
compared to crown volume	2	15	%1,322 mm^3	,573
Percentage of enamel volume	1	15	%48,81 mm^3	3,068
compared to crown volume	2	15	%45,25 mm^3	3,760
Percentage of dentin volume	1	15	% 49,49 mm^3	2,888
compared to crown volume	2	15	% 53,42 mm^3	3,919
Crown volume	1	15	284,2 mm^3	40,65
	2	15	253,8 mm3	42,18

**There was a difference between all groups between the percentage volume and standard deviation values of the mean pulp, enamel and dentin volume compared to the crown volume, p<0.05.*

Discussion

Philippas (1961) conducted the first radiological studies to provide quantifiable data on the size of pulp chambers. The response of pulp and dentin to the abrasion and attrition occurs as a result of occlusal forces in forming secondary dentin. Although more primitive compared to the three-dimensional techniques of the present day, it is essential for being the first study that is parallel to our research.

One of the most essential advantages of micro-CT is that the structures of tissues can be viewed as three-dimensional without making any change in the makeup of the examined sample. Therefore, the sample can be used for other studies as no invasive processes are performed for scanning (Sahin & Topuz, 2014; Kurt & Orhan, 2016; Keles & Alcin, 2011; Marciano *et al.,* 2012; Davis & Wong, 1996). In addition, micro-CT is less time-consuming and less costly compared to other analyses (Uchiyama *et al.,* 1997).

In this study, we used micro-CT to calculate volumetric measures of the tissues that form the teeth due to such superior features.

Gant D. *et al*., (2006), P. Hofmann *et al.,* (2009), and Anthony J. O. *et al.,* (2008) examined the teeth of fossils with micro-CT. They calculated both the thickness and volumes of enamel dentin tissues. For this purpose, they stated that measuring the area covered by enamel and dentin tissue and its amount with micro-CT, which is a non-destructive method, is an accurate choice.

Similar to the studies indicated above, our study separates the young and adult maxillary 1. premolar teeth from cementoenamel junction using micro-CT and compares the volume measurement values obtained from the tissues.

The literature indicates that pulp chamber volume decreases due to increased secondary dentin formation with aging. Agematsu *et al.,* (2010) examined the change in secondary dentin accumulation and pulp chamber volume based on age and sex using micro-computerized tomographic images. Iwaka (2006) examined lower permanent first molar tooth on micro-computerized tomography and reported that the pulp volume decreased with age.

Similarly, the study conducted by Oi *et al.* (2004) using micro-computerized tomography analyzed the pulp chamber of the upper permanent first premolar tooth three-dimensional and reported that the size of the pulp chamber and canal openings

decreased with age. They argued for the reliability of micro-CT for volumetric measurements.

Aboshi *et al.* (2010) calculated using micro-CT the ratio of pulp volume of lower permanent premolar teeth to the entire tooth volume for age determination. Someda *et al.,* (2009) examined the enamel, dentin, and pulp volume of mandibular teeth for age determination and compared age with sex. They concluded that due to the abrasions in enamel with age, enamel should not be taken as a reference in age determination, and the volume of the pulp canal was affected by secondary dentin, which is formed with age. Colour settings were made at different tissues using micro-CT and pulp room, and hard tooth tissues were observed relatively well (Ma *et al*., 2013).

In their study, Kim I. *et al.* (2007) found that micro-CT was a more reliable method in the *in vitro* measurement of structures and volumes of teeth. This result is a study that supports our study in terms of reliability.

Ketterl (1983) compared the pulp volumes of permanent mandibular first molar extracted from individuals between the ages of 20 and 40 and found out that the volume of pulp chamber decreased in 40 years period.

Ma *et al.,* (2013) calculated the crown volume of mandibular central incisors of 5- and 6-years old children using micro-CT. Changes in enamel, dentin and pulp volumes were examined concerning age and sex. The ratio of pulp volume to the volume of the entire tooth crown is higher in 5 years old children (6.035%±1.568) compared to 6 years old children (5.106%±1.323).

In our study, the percentage ratio of pulp volume to crown volume is calculated as (1,690% mm^3 ± 0,423) in group 1 and (1,322% mm^3 ± 0,573) in Group 2. The difference between these groups was statistically significant, for which reason it was found that significant changes were made in pulp volume with age.

In our study, the volumetric calculations we made with micro-CT showed that the volume of enamel tissue decreased with age due to the abrasions in the enamel. In addition, secondary dentin formation increased the volume of dentin tissue, and the volume of pulp tissue decreased. This showed that the volume of enamel, dentin, and pulp tissues could be measured reliably using micro-CT. The conclusion we reached in the study was parallel to the volumetric measurements of Agemetsu *et al.* (2010), Oi *et al.,* Orhan *et al.* (2004), Someda *et al.* (2009), Ketterl *et al.* (1983), and Ma *et al.,* (2013) in the literature. Kinney *et al.* (1994) examined the mineral distribution in decayed canine teeth using the 3-D technique for the first time. Mineral concentration was found as 1,29 $g.cm^{-3}$ in healthy dentin and demineralized dentin was found as 0,55 $g.cm^{-3}$.

Djomehri *et al.* (2015) compared mineral densities of normal and diseased teeth hard teeth using micro-CT. They showed that mineral density distribution could be sectioned three-dimensional.

Dowker *et al.* (2006) reported that mineral density distribution was important in developing and identifying decays. For this purpose, they 3-D visualized the fissures of premolar teeth using micro-CT, examined the distribution of mineral density of the enamel, and showed the method's usefulness.

Microtomography and CTAn computer software used in our study helped determine that enamel tissue was more mineralized compared to dentin tissue in quantitative terms. These data were parallel to the information in the literature. Thus, it was concluded that enamel and dentin tissue could be measured reliably using micro -CT. Farah *et al.* (2010) compared the mineral density distribution in the teeth of MIH individuals (molar incisor hypo-mineralization) to individuals with healthy teeth using micro-CT. They reported that at the enamel-cement level, mineral density increased towards occlusal and reached its high at cusp/incisor tops. Also, they reported that as teeth could be dehydrated due to the solution, which was used for sterilization,

mineral density values could show higher figures. In addition, they stated that the thickness of the section taken from the samples could affect the measurement of mineral density distribution. Clementino – Luedemann & Kunzelman (2006) found mean enamel density value as 2,47 – 2,7 $g.cm^{-3}$.

Weidmann *et al.* (1967) took a section at the micron level from the samples and examined the entire mineral content of a mature enamel using a chemical method for the first time.

Hayashi-Sakai, S *et al.* (2018) concluded that the distribution of mineral density in sound enamel and dentin and attempted to determine the standard mineral density for each tooth type using micro-CT. The mineral density distributions found in this study contribute to our understanding of the mechanical properties of enamel and dentin. A positive correlation suggests that the systemic bone mineral density could be predicted based on the analysis of exfoliated teeth, such as in patients with hypophosphatasia. The present results may be useful in establishing a numerical standard for the mechanism involved in root fracture and for early detection of root fracture risk.

Schmitz *et al*. (2014) analyzed the mineral change during enamel formation using micro-CT and compared its reliability with the chemical method. However, measuring the mineral content and enamel volume is extremely difficult as dentin is connected to the enamel and the dental crown to create a two-layered bio-mineral. For this purpose, invasive and non-invasive methods were developed.

Nakata *et al*. (2012) observed that mineral content could be measured reliably using micro-CT. Similar values were found with the chemical method the density changes in minerals at the four layers (surface layer, lesion layer, medium layer, deep layer) of the enamel affected from decay longitudinally during remineralization of the enamel process. This approach reported that micro-CT was a reliable method to create a mineral density profile.

Wong *et al.* (2004) examined the mineral density of baby teeth enamels and found that mineral density at the occlusal zone was higher than in the cervical area.

Our study showed similar features to the studies conducted by Clementino – Luedemann & Kunzelman (2006), Farah *et al.* (2010), and Wong *et al.* (2004). We found out that the mineral density of enamel tissue increased from cervical to occlusal and from the enamel-dentin border to the outer enamel. In addition, it has been determined that the mineral densities of dentin tissue increased from the collum dentis layer to the occlusal layer of dentin tissue.

It is well-known that the mineral density of dentin tissue increases with age (Bath-Balogh & Fehrenbach, 2006; Nanci, 2003; Wong *et al.*, 2004). Our study's values are parallel to the information in the literature. Thus, the reliability and usefulness of our research conducted using micro-CT have been supported.

In addition, in our study, the mineral density of enamel tissue slightly decreased with age, but this finding is not statistically significant.

Conclusion

As a result, it was thought that the volumetric changes observed in teeth tissues in time could be accurately and delicately measured using the micro-CT technique as an imaging method. Parallel to the findings of previous studies, our research found that the mineral density of dentin tissue increased with age. Furthermore, it was seen that the density distribution of enamel tissue increased from cervical to occlusal. In addition, in our study, the mineral density of enamel tissue slightly decreased with age, but this finding is not statistically significant. It was concluded that mineral density distribution in enamel and dentin tissue could be accurately measured with micro-CT.

As the samples were not subjected to any procedure before being scanned, it was believed that it was a more favorable method for measuring the mineral density of hard tissues of teeth compared to other densities. The scanning speed and features of micro-CT are still in development. As a result, the researchers believed that micro-CT could develop more and provide images from samples with higher resolution and quality, thus serving as an essential *in vitro* research method in dentistry. Furthermore, it was also believed that the progress in this technology could reduce the radiation dose and increase its usefulness for *in vivo* studies in the future, as a result of which breakthroughs could be witnessed in scientific studies.

Researchers will enjoy broader perspectives in dentistry studies with micro-CT technologies.

This study will guide future studies on broader and different sample groups such as other racial and gender groups.

Acknowledgement

This study was supported by Dicle University Scientific Research Project Foundation Department, Diyarbakır, Turkey, with the project number DİŞ.16.006.

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