Mineral Concentration of Natural Human Teeth by a Commercial Micro-CT

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This study aimed to evaluate a commercial micro-CT system (μCT 20) for quantitative analysis of mineral concentration in human enamel and dentin using different methodologies, and thereby compare the obtained results with established data from published literature. A micro-CT device set at 50 kVp (160 μA) was used to scan five whole molars (G1) and five molars ground to 6-mm thickness (G2), as well as evaluate the mineral concentration of the samples. Mean mineral contents for enamel and dentin were 2.57 ($\pm$0.12) and 1.53 ($\pm$0.12) g/cm^3 for G1, and 2.76 ($\pm$0.03) and 1.45 ($\pm$0.02) g/cm^3 for G2. Difference between the groups was significant for enamel. For dentin, there was a clear although not significant tendency towards higher values with G1. The equipment could identify and differentiate a higher mineral content of the tooth enamel and dentin from the external to the inner tissue. Further, the absolute mean values of mineral concentration were lower in whole tooth samples than in sectioned samples due to beam hardening. In conclusion, the equipment is well suited for quantifying the mineral content of teeth. However, it is necessary to consider the limited acceleration voltage of the μCT 20 system and to limit sample evaluation to 6-mm thickness.

Key words : Micro-computed tomography (micro-CT), teeth, mineral concentration

INTRODUCTION

A developing research field in medicine and dentistry is the inspection of specimens by means of non-invasive and non-destructive 3D analytical techniques. These new, innovative techniques boast of a few advantages. First, they do not require the time-consuming preparation of serial sections — which means that these new approaches help to save time. Secondly, they do not require specific staining of the object — which can affect the organization of the investigated structure. Amongst this array of non-invasive imaging tools, micro-computer tomography (micro-CT) emerges as a potential key tool especially in *in vitro* caries research. This is because it allows image recording of inner structures with high spatial resolution — three-dimensionally and without destruction of samples[4].

Micro-CT is a miniaturized form of CT scanning. It was developed in the beginning of the 1980s predominantly for laboratory purposes on small samples or material experiments, and used frequently in the studies of trabecular bone structure and mineral analysis[5−7]. The principle of absorption of micro-CT consists in reconstructing the linear attenuation coefficient, within an object, from the attenuation measurements of an X-ray beam passing through the sample at different viewing angles. Differences in linear attenuation coefficient among tissues are responsible for X-ray image contrast, which allows quantitative analyses to be made[6].

Due to its small size and high X-ray intensity demand, micro-CT is utilized only in laboratorial experiments. Nevertheless, there are new systems of digital volume tomography for dental clinical diagnostics. However, the image quality and resolution of the existing digital dental systems is inferior to that of micro-CT.

To date, commercial micro-CT devices have been used successfully in dental research for qualitative analysis and three-dimensional evaluation of materials as well as in dental treatment procedures[8−11]. To our knowledge, quantitative evaluation of the mineral concentration (MC) of dental tissues has not yet been investigated on a commercial device. The aim of this study was to evaluate a commercial micro-CT system for the quantitative analysis of MC at human enamel and dentin using different methodologies, and then compare the obtained results with established data from published literature. In addition, this study tested the null hypothesis that the equipment was sensitive enough to evaluate image data without the need of any specimen preparation, such as the physical cutting of specimens into thin sections. Our second hypothesis was that the equipment was sensitive enough to measure the MCs of enamel and dentin after cutting the samples to 6-mm dentin thickness — which according to a published report was the critical thickness for radiographic evaluations at an acceleration voltage of 32 keV[5].

MATERIALS AND METHODS

A total of 10 extracted third molars, extracted for orthodontic reasons, were selected from a pool of teeth stored in thymol 0.1%. They were divided into

two experimental groups: G1 (five whole permanent molars) and G2 (five permanent molars which were cut and ground to 6-mm dentin thickness (Leco VP100, LECO, Kirchheim, Germany)). Enamel tissue from vestibular and buccal sides was equally eliminated to reduce the very dense areas and to allow better X-ray absorption through the samples.

X-ray microtomography system
A commercial polychromatic fan-beam microtomographic system (μCT 20, SCANCO Medical AG, Bassersdorf, Switzerland) (Fig. 1), with a spot size of 7 μm and a tungsten target using an acceleration voltage of 50 kVp (160 μA), was used. According to the manufacturer, this energy was sufficient to penetrate objects up to 17 mm in diameter. A 0.3-mm aluminum filter was installed in the beam path to cut off the softest X-rays, so as to achieve a detector response close to 32 keV. This was necessary so as to increase the accuracy of the beam hardening correction, because in contrast to synchrotron illumination, the use of polychromatic X-rays creates a problem of beam hardening.

The object was mounted on a computer-controlled turntable, which synchronized rotation and axial shift. A 50-μm thick amorphous scintillator transformed the X-rays into visible light. The image was projected onto a CCD chip where the signal was digitized by means of an analogue digital converter (ADC) and stored in computer hard-drive for further evaluation[1]. In this study, a CCD array detector with 1024 elements and 25 μm pitch was used.

Nominal isotropic resolution was set to 30 μm and integration time was set to 250 ms and 350 ms

(G1 and G2). Image reconstruction was carried out by the implemented standard convolution back-projection algorithm.

Specimen preparation
In both G1 and G2 groups, 1-mm pure aluminum wire (99,999% purity, Alpha Aesar, Johnson Matthey GmbH, Karlsruhe, Germany) was longitudinally attached with adhesive to the side of each tooth as reference material. The apex of each root was cut to allow the tooth to be positioned into the center of a transparent polyacrylic cylindrical sample holder of 15.3 mm diameter. As no longitudinal experiment was conducted, it was not necessary to reposition the specimens in the sample holder. The long axis of G1 samples was positioned longitudinally in the sample holder, while that of G2 samples was positioned transversally. For the G1 group, this arrangement resulted in a complete circle of very dense tissue (enamel), which probably influenced the hardening of the beam in its path. On the other hand in the G2 group, enamel had only a small influence on X-ray attenuation (Fig. 2).

To avoid drying artifacts, wet foam was positioned on top of the sample holder, which was sealed on the upper side with parafilm (Parafilm M, Pechiney Plastic Packaging, Chicago, USA), thereby maintaining a humid environment. The foam also mechanically fixed the specimen in the sample holder to prevent any small movement — which is also a source of artifact in micro-CT images — during the scan procedure.
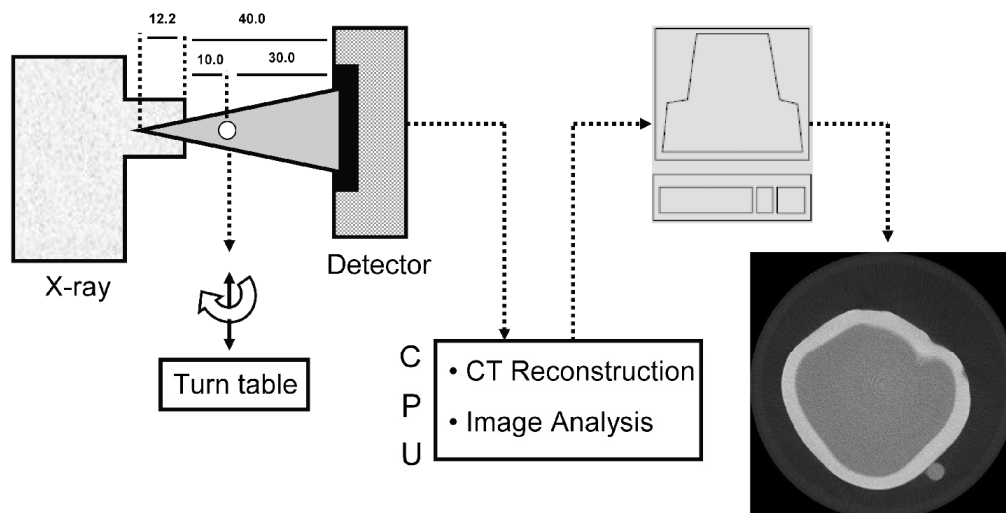

Fig. 1 Schematic picture for the geometry of the fan beam radiation system and the setup distances: between X-ray beam and its collimation lens is 12.2 cm, from collimation lens to the sample is 10.0 cm, and from sample to the detector is 30.0 cm. Scanner on the left side sends the raw information to the computer, where the data will be reconstructed and images will be analyzed.
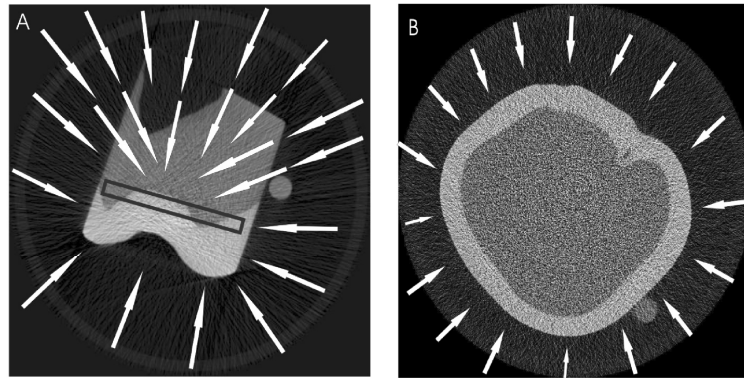
Fig. 2 Image of a reconstructed slice showing the influence of dense areas on the results. "A" represents G2 and visualizes how beams easily reach enamel, even at its internal area (black rectangle). In "B" (G1), it is visualized that the beam has more difficulty going through the whole sample due to a mantle of enamel, which is very dense and attenuates the X-ray more than dentin. The circle-shaped structure besides the tooth is a 1-mm pure aluminum wire.

Mineral concentration evaluation by micro-CT

Mean mineral concentration was measured using an image analysis software (ImageJ 1.32j, Wayne Rasband, National Institute of Health, Bethesda, USA), which is a public domain Java image processing computer program. Besides a large number of native functions, it enables two-dimensional measurements of mineral content through selected regions of interest. Each image data set consisted of a stack of 15 micro-CT slices (1024 × 1024 pixels). At G1, 10 circular regions of interest (ROIs) (±64 pixels each) were drawn for each of the following tooth surfaces: external enamel, middle enamel, internal enamel (near amelodentinal junction, ADJ), external dentin (near ADJ), middle dentin, internal dentin (Fig. 3).
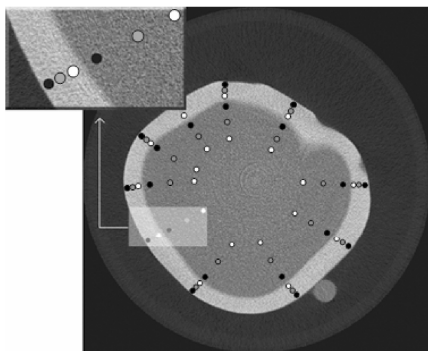

Fig. 3 Two-dimensional micro-CT reconstruction image and selected ROIs in enamel and dentin. Rectangle on the upper left side is a magnified image with detailed view of the ROIs. Black, gray, and white dots are the external, middle and internal selection for each tooth tissue. The circle-shaped structure beside the tooth is a 1-mm pure aluminum wire.

The ROIs were selected from the area between the end of the fissure region and the pulp chamber. Each selection excluded edges to preclude micro-CT partial volume effect[5].

At G2, the mean linear attenuation coefficient (LAC) for enamel and dentin were measured by histogram analysis after the application of two-means cluster algorithm (Isodata) for segmentation of tissues. To evaluate the gradient of MC through enamel and dentin surfaces, ROIs were also drawn.

Through first order calibration, LAC corrections were made based on published values for pure aluminum, which were obtained from the XCOM Photon Cross-Section database program (National Institute of Standards and Technology, Gaithersburg, USA)[12].

Assuming the mineral content in enamel to be pure hydroxyapatite with a density of 3.15 g/cm³ [13], measured LAC for enamel (LAC$_e$) was converted to MC (C$_e$)[14] by the following formula:

$$C_e = \frac{\mu_m \times \mu_{al(pub)}}{\mu_{al} \times \mu_{mhap}} \qquad (3)$$

where μ_m is the LAC of mineral tissue, $\mu_{(pub)}$ the LAC of aluminum wire from published data, μ_{al} the measured LAC of the aluminum wire, and μ_{mhap} the mass LAC of hydroxyapatite (1.74 cm²/g at 32 keV) from published data.

Since the images were previously calibrated and that published data and measured data for aluminum wire were the same, Equation (3) can be simplified to:

$$C_e = \frac{\mu_m}{\mu_{mhap}} \qquad (4)$$

Statistical analysis

After the mean (± standard deviation, SD) MC value for every surface of the tooth was determined, one-way analysis of variance followed by Tukey's multiple comparison post hoc test were used to calculate the differences between the measuring sites. Overall significance level was set at $P < 0.05$. Student's t-test was used to evaluate the significance of the MC differences in enamel and dentin between G1 and G2. All statistical tests were performed with SPSS version 12.0.1 (SPSS Inc. Headquarters, Chicago, Illinois, USA).

RESULTS

Table 1 shows the mean MC values of enamel and dentin for G1 and G2. With the 6-mm dentin samples, the mean MCs of enamel showed a higher value as compared to the whole tooth sample. In G1, the MC for enamel among all teeth varied between 2.43 (±0.28) g/cm³ and 2.75 (±0.45) g/cm³. To obtain the mineral content of the same area of interest from one slice to another, a z-profile was plotted. This z-profile contained additional information within the thickness of each slice, which in the case of traditional microradiograms is integrated to only one value. Differences in mineral content in the same ROI from one slice to the next (30 μm in z-axis) showed a non-smooth mineralization pattern throughout the thickness (Fig. 4). In G2, the mineral content ranged from 2.73 (±0.30) g/cm³ to 2.81 (±0.35) g/cm³. As opposed to G1, the variation in G2 from
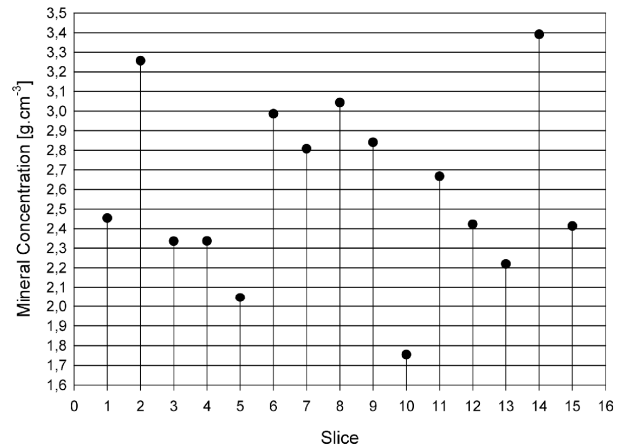

Fig. 4 Mean mineral concentration of ROI throughout the slices along the z-axis of G1, showing a high variation of the mineral concentration between the slices with a 30-μm linear resolution.
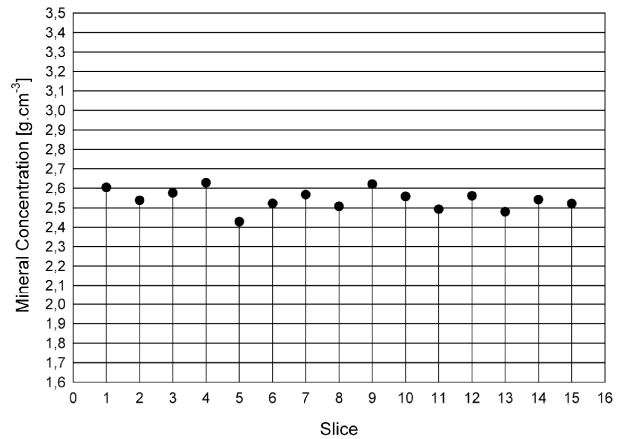

Fig. 5 Mean mineral concentration of ROI throughout the slices along the z-axis of G2, showing little variation of the mineral concentration between the slices with a 30-μm linear resolution.

Table 1 Mean mineral concentration values and standard deviations for each group (G1 and G2)

Group	Mineral Concentration (g/cm³)	
	Enamel	Dentin
Mean G1	2.57 (±0.12) *	1.53 (±0.12) NS
Mean G2	2.76 (±0.03) *	1.45 (±0.02) NS

Significant differences of the same tissue between G1 and G2 are indicated by '*' corresponding to $P < 0.05$.

Table 2 Mean mineral concentration values and standard deviations in different tooth areas of enamel and dentin

Group	Mineral Concentration (g/cm³)		
	External	Middle	Internal
G1 enamel	2.57 (±0.29)	2.58 (±0.31)	2.52 (±0.33) *
G1 dentin	1.51 (±0.27)	1.51 (±0.22)	1.45 (±0.27) *
G2 enamel	2.74 (±0.11)	2.73 (±0.11)	2.64 (±0.14) *
G2 dentin	1.49 (±0.07)	1.46 (±0.06)	1.36 (±0.08) *

Significant differences between areas of the same tissue and group are indicated by '*' corresponding to $P < 0.05$.

voxel to voxel of mineral content throughout the thickness did not show abrupt differences, whereby the differences were mostly around 0.05 g/cm³ and rarely above 0.2 g/cm³ (Fig. 5).

When analyzing the different enamel regions for both G1 and G2 (Table 2), the most external part and the middle part did not show any statistical significant differences. On the other hand, the internal part − which was near to the amelodentinal junction (ADJ) − had a lower MC with a statistically significant difference when compared to the other two enamel regions (P<0.001). The difference between external enamel and the enamel near ADJ was about 1.94% for G1 and 3.64% for G2. Student's t-test showed a statistically significant difference in overall MC among G1 and G2 for the enamel tissue (P< 0.001).

For dentin, results among teeth in G1 ranged from 1.36 (±0.30) g/cm³ to 1.69 (±0.22) g/cm³. For G2, the minimum MC value was 1.43 (±0.23) g/cm³ and the maximum was 1.49 (±0.23) g/cm³. Just like enamel, two distinct groups of dentin were defined: one consisted of the most external and middle dentin with no statistical differences, and the other consisted of the internal dentin with a decrease in mineral content toward the center of the tooth (Table 2). Difference between external and internal dentin was found to be about 3.97% for G1 and 8.72% for G2. When comparing the MC values of dentin between G1 and G2, there was a clear tendency towards a higher value for G1 − although this difference was not significant (P=0.052). Throughout the z-axis, a similar pattern for the differences in mineralization degree was observed for dentin in both G1 and G2 − as that described previously for enamel. In other words, a smoother difference was noted in G2 than in G1.

DISCUSSION

In 1991, ten Bosch and Angmar-Månsson[15] in a detailed review of quantitative methods to determine mineral changes recommended the use of radiographic methods to quantify mineral loss in whole teeth. The interest in radiation techniques is due to the ability of X-rays to travel through matter without destroying the specimens.

Currently, transverse microradiography (TMR) is considered as the 'gold standard' for the determination of mineral loss in experimentally induced incipient lesions. The method has been used for the comparison and validation of other newly developed caries diagnostic techniques[16]. A key disadvantage of microradiography lies in its superimposition effect, such that any non-uniformities detected in the direction of the X-ray beam are lost due to this effect. In addition, specimens need to be physically cut into thin sections − which is rather difficult and defi-

nitely destructive. This is especially so for brittle materials or sections which include hard and soft regions − such as caries lesions[17]. It has been reported that micro-CT requires no preparation of cut cross-sections[9,18], and that it enables longitudinal experiments to be conducted in three-dimensional studies − thereby overcoming the disadvantages of microradiography. The aim of this study was to test a commercial micro-CT device for the quantification of mineral content in tissues of a whole tooth or 6 mm-thick samples.

Our findings demonstrated that use of different methodologies led to different quantification values of mineral content. In G2, mineral concentration values of enamel were in agreement with past studies[5,7,13,19−22]; in G1, statistically significant lower values were observed. The authors speculated that a few factors had caused the observed differences in results: a higher exposure time, the positioning of sample in the sample holder − which can either facilitate or make it more difficult for the beam to pass through the sample, and in particular the reduction of sample size by eliminating the dense areas of enamel. Though we could not conclusively pinpoint which given factor influenced the results most, it was thought to be more related to the size of the sample as it highly affects the signal-to-noise ratio[1,23].

For the commercial device used in this study, it was recommended by the manufacturer to use samples up to 17 mm in diameter. However, these recommended instructions are usually more appropriate for bone studies. Recommendations for micro-CT analysis should be adjusted accordingly to the porosity or density of the specimen itself. In this study, the tooth specimen was an extreme compact mineral mass, thus attempt should be made to reduce the specimen size. This was done in line with the general rule of thumb where the energy of X-rays must be higher if the sample were thicker or denser[1].

With regard to the analysis of different areas through the same tissue, neither Group G1 nor G2 yielded a consistent, smooth gradient from the external layer for both enamel and dentin − in disagreement with that which was shown for enamel by Weatherell et al.[24]. Robinson et al.[25] showed that calcium and phosphorus concentrations were relatively high in the middle layers of enamel, which may explain the higher MC in this area for both groups in our experiment. A second possibility could be the incapability of the device to clearly define the gradient through tooth tissues. Nevertheless, lower values of mineralization were found in the innermost part of enamel, near to ADJ, which was consistent with past experiments[5,13,21]. Many factors were associated with this gradient: variation in calcium and phosphorus contents, organic matter, water, and possibly variation in porosity.

When analyzing the MC values of enamel in G1,

abrupt variation from one slice to the next (30 μm in z-axis) were seen throughout the thickness (Fig. 4). These abrupt variations could be attributed to the high coefficient of variation and noise artifacts associated with the G1 settings. Hence, these abrupt changes were not observed in G2 (Fig. 5) − instead, the changes along the z-axis of 6-mm samples were smoother and rarely abrupt.

For dentin, the internal layer also appeared to have a lower degree of mineralization. The decrease in mineral content was already expected due to the following factors: greater amount and diameter of the dentinal tubules, as well as higher portion of water and organic phase. These factors clearly reduced the attenuation of X-rays from the ADJ to the internal layers of dentin. Similar results were already observed with micro-CT by Hayakawa et al.[26]. However, these results disagreed with those of Anderson et al.[7], whereby a lower mineralization degree was indicated at the ADJ (1.42 g/cm^3) and a higher degree in deeper dentin (1.50 g/cm^3) using a non-commercial micro-CT device.

With the first-generation micro-CT system, practicable measurements of mineral content was limited to a small number of 'slices' through lesions formed in cut blocks with a 2×2.5 mm^2 cross-section[5]. This need to physically cut specimens in order to measure mineral concentration ran contrary to one of the greatest advantages of the micro-CT system. In the present study which used a commercial, second-generation micro-CT device, it was once again shown that whole teeth are not recommended for quantification of mineral content. Thus, it is strongly recommended to consider the limited acceleration voltage of the μCT 20 system and to limit sample evaluation to 6-mm thickness − which is already an advantage over other analytic approaches which require the preparation of very thin, micrometric-sized samples. Concerning the accuracy of this device for longitudinal studies to quantify mineral changes, validation studies should be performed in order to provide the important supplementary information about the dynamics of treatment.

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