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Short Communication

Forensic significance of dental cementum: pilot study on new micro-CT energy parameters for age estimation in human teeth

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Aim: To determine new energy parameters in micro-CT to allow appropriate segmentation of dental cementum from the surrounding structures. **Methods:** For this pilot study, 11 single-rooted premolar teeth of young subjects aged 12 to 21 were selected. The teeth were scanned using high-energy microtomography (SkyScan 1173, Bruker) with varying acquisition energies (60, 70, and 80 kV). Each tooth was scanned three times. The images were reconstructed and analyzed by calculating the volume (mm^3) of dental cementum in the cervical, middle, and apical root thirds among the different scanned stacks. The mean cementum volume in the root thirds was obtained, and correlated with the patient's age, using Pearson's correlation. **Results:** The mean age of the patients in the sample was 16,73 years. For the 60kV energy, the cementum volumes were 1.01 mm^3, 1.00 mm^3, and 0.58 mm^3, for the cervical, middle, and apical thirds, respectively. For 70kV, it was 1.08 mm^3, 1.20 mm^3, and 0.71 mm^3 for the respective thirds. And for 80kV, 0.55 mm^3, 0.91 mm^3, and 0.67 mm^3 for the respective thirds. **Conclusion:** An acquisition energy of 70kV resulted in the best segmentation and reproducibility of the results. Micro-CT can be a useful tool to non-destructively evaluate the dental cementum.

Keywords: Forensic dentistry. X-ray microtomography. Dental cementum.

Introduction

Dental cementum is a vital tissue with a continuous apposition throughout life. However, it is distributed unevenly in layers on the root surface, which are larger in the apical areas and smaller in the areas near the cementoenamel junction[1]. This physiological characteristic makes it an excellent source of information for age estimation in cases of human identification[1].

The adjacent position and mineral content of cementum-like dentin make it difficult to differentiate from conventional radiographs[2]. And the micro-computed tomography (micro-CT), a microscopic version of cone-beam computed tomography (CBCT), which also uses X-radiation to obtain images[3], can contribute to dental tissue analysis.

Currently, the main applications for micro-CT involve its use in qualitative hard tissue analysis, such as three-dimensionally visualizing specific characteristics of bone trabeculae and their quality[4], or quantitative, such as measuring the area, volume, porosity, density, and concentration of minerals[5,6]. Other than bone tissues, micro-CT has also been used for quantitative analysis of dental tissues, such as enamel, dentin, and root canal, often associated with age estimation methods[1,7].

Only one study has been conducted to analyze dental cementum using micro-CT, albeit with dental samples from rodents[2]. Thus, this pilot study aimed to establish acquisition parameters for images obtained by micro-CT that allow the qualitative and quantitative analysis of human dental cementum and its volumetric quantification correlating with age.

Materials and Methods

After approval by the Research Ethics Committee (CAAE 92868518.0.0000.5257), patients with scheduled extraction of premolars due to various clinical indications were approached as potential participants of the study. Upon thorough review and comprehension of the informed consent document, patients who willingly consented to contribute their extracted teeth became integral participants in this study.

Eligibility criteria and sample selection

We included single-rooted premolar teeth of individuals aged between 12 and 21, with intact root portions. Tooth elements that had been damaged during the extraction procedure or presented any alteration in the root portion were excluded. After selection, the teeth (n=11) were stored individually, in containers containing formaldehyde 10%, identified by codes and patient age.

Micro-CT scanning and reconstruction procedures

All specimens were scanned using a high-energy microtomographic device (SkyScan, 1173; Bruker-micro-CT, Kontich, Belgium), with each tooth individually fixed with utility wax on its base. Each specimen was scanned at three different energy inputs (60, 70, and 80 kV) to generate reconstructions with different contrasts. The other parameters were fixed and consisted of a current of 100 µA; aluminum filter of 1.0 mm in thickness; rotation step of 0.5°; average rotation of 5 and 360; exposure time of 1100 ms; pixel size of 8.98 µm per image in a matrix of 2240 by 2240 pixels each image[8-11].

After the acquisition, all images were reconstructed using InstaRecon™ (v.1.7.3.0) software (Skyscan, Kontich, Belgium). Some reconstruction parameters were adjusted to ensure the best image quality, such as smoothing, misalignment compensation, beam hardening, and ring artifacts. Image processing was performed using CTAn™ (v.1.18.4.0) software (Skyscan, Kontich, Belgium).

Cementum evaluation

Using the CTAn™ (v.1.18.4.0) software (Skyscan, Kontich, Belgium), the length of the roots of each tooth was measured and divided into three-thirds of equal lengths: cervical, middle, and apical thirds. From this, regions of interest (ROIs) were established manually throughout the length of the tooth where the dental cementum is normally located (Figure 1).

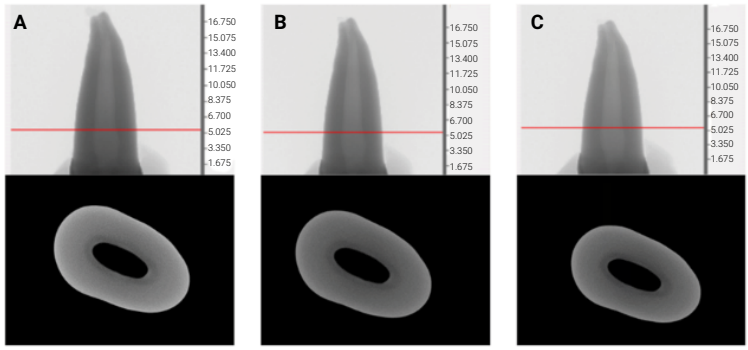

Figure 1. Axial and coronal reconstructions of a scanned tooth with three different energies using image correction factors. The acquisition parameters were: 8.98 µm pixel size, 100 ms integration time, 0.50 thick pitch, and 1.0 mm thick aluminum filter. (a). 60 kV; (b). 70 kV; (c). 80kV.

A gray value histogram was obtained from each ROI, allowing segmentation of the cementum (Figures 2 and 3). Furthermore, a global threshold technique was employed to obtain the total volume of the cementum for each third. These steps were performed in each of the respective thirds three times for each tooth, all by a single experienced and trained examiner, for all acquisition parameters evaluated (60kV, 70kV, and 80kV).

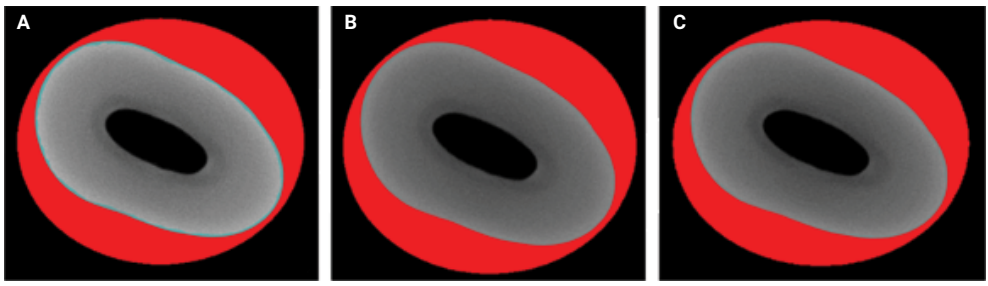

Figure 2. Axial reconstructions of the same tooth show the region of interest (ROI). (a). 60 kV; (b). 70 kV; (c). 80kV.

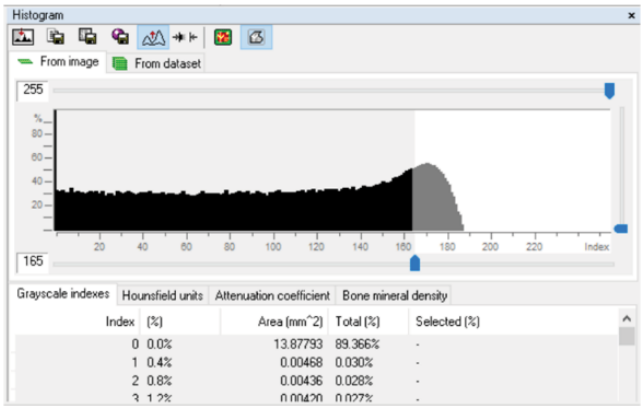

Figure 3. Illustrative representation of the Histogram. Is a software tool capable of allowing the selection of the gray levels of the image. The representation shows the selection of all gray levels between 165 and 255.

Statistical analysis

The variables were represented by the mean and standard deviation for all three regions of the tooth and discriminated against for the different energy levels. The mean of the values measured in the three regions was calculated and correlated with the patient's age using Pearson's Correlation test. The software IBM-SPSS v.25 was used for the analyses, and Excel™ was used for the graphs. The adopted significance level was 5%.

Results

The volume of dental cementum (mm³), obtained in each root third of the same tooth, and their respective means, for the three energies tested, are shown in Table 1.

Table 1. Values of dental cementum volume (mm³) and average between the thirds obtained in each tooth, in the three energies tested, about age.

Energy	Tooth	Age	Cervical	Middle	Apical	Mean	Pearson's Correlation
60 kV	1	14	4.14	1.99	1.22	2.45	r = -0.227 (p=0.504)
	2	21	1.09	1.51	1.36	1.32	
	3	17	0.93	0.63	0.37	0.64	
	4	16	0.93	0.89	0.38	0.73	
	5	14	1.97	2.55	1.64	2.05	
	6	16	0.09	0.16	0.25	0.17	
	7	17	0.87	0.41	0.33	0.54	
	8	21	0.83	1.4	0.23	0.82	
	9	16	0.15	0.86	0.29	0.43	
	10	16	0.01	0.35	0.09	0.15	
	11	16	0.13	0.3	0.23	0.22	

Continue

Continuation

70 kV	1	14	2.75	1.22	1.08	1.68	r = -0.539 (p=0.088)
	2	21	0.34	0.85	0.97	0.72	
	3	17	0.9	0.57	0.28	0.58	
	4	16	1.52	1.07	0.41	1.00	
	5	14	2.4	2.77	1.69	2.29	
	6	16	2.4	2.77	1.69	2.29	
	7	17	0.69	0.54	0.3	0.51	
	8	21	0.34	0.71	0.15	0.40	
	9	16	0.1	0.92	0.27	0.43	
	10	16	0.27	1.32	0.45	0.68	
	11	16	0.19	0.5	0.5	0.40	
80 kV	1	14	1.71	0.79	0.62	1.04	r = -0.045 (p=0.899)
	2	21	1.11	1.59	1.4	1.37	
	3	17	0.59	0.53	0.22	0.45	
	4	16	0.54	0.69	0.2	0.48	
	5	14	0.57	1.75	1.65	1.32	
	6	16	0.57	1.75	1.65	1.32	
	7	17	0.23	0.46	0.32	0.34	
	8	21	0.41	0.82	0.15	0.46	
	9	16	0.02	0.51	0.27	0.27	
	10	16	0	0.45	0.2	0.22	
	11	16	0.25	0.68	0.68	0.54	

The mean age of the participants was 16.73, with a standard deviation (SD) of 2.3 years and a variance of 5.42. For the 60-kV energy, the means (SD) and variance obtained among all cementum volumes were 1.01 (1.19) mm^3 and 1.42 variance, 1.00 (0.77) mm^3 and 0.59, and 0.58 (0.54) mm^3 and 0.30 for the cervical, middle, and apical thirds, respectively. For the 70kV energy, they were 1.08 (1.01) mm^3 and 1.02 variance, 1.20 (0.82) mm^3 and 0.67 and 0.71 (0.56) mm^3 and 0.32 for the respective thirds. At 80kV, the values were 0.55 (0.50) mm^3 and 0.25 variance, 0.91 (0.52) mm^3 and 0.27 and 0.67 (0.60) mm^3 and 0.37 for the respective thirds.

The gray levels used to visualize the dental cementum in each root third were also established to measure the volume, employing the histogram (Table 2). These data were subsequently correlated with age as shown in Figure 4. The mean (SD) and variance of the gray level values for the 60 kV energy were 147.6 (11.7) and 136.85 variances, for the 70 kV energy was 118.6 (10.0) and 100.1 variances, and for the 80 kV, energy was 105.5 (28.0) and 784.5 variances.

Table 2. Gray levels were adopted to visualize the dental cementum in each root third to measure the volume.

Energy	Tooth	Age	Cervical	Middle	Apical	Mean	Pearson's Correlation
60 kV	1	14	165	165	165	165	r = 0.033 (p=0.924)
	2	21	150	150	150	150	
	3	17	147	147	147	147	
	4	16	135	135	135	135	
	5	14	147	147	147	147	
	6	16	145	145	145	145	
	7	17	162	162	162	162	
	8	21	151	151	151	151	
	9	16	152	152	152	152	
	10	16	122	122	122	122	
	11	16	148	148	148	148	
70 kV	1	14	132	132	132	132	r = 0.055 (p=0.871)
	2	21	122	122	122	122	
	3	17	114	114	114	114	
	4	16	100	100	100	100	
	5	14	116	116	116	116	
	6	16	126	126	126	126	
	7	17	134	134	134	134	
	8	21	120	120	120	120	
	9	16	118	118	118	118	
	10	16	107	107	107	107	
	11	16	116	116	116	116	
80 kV	1	14	138	138	138	138	r = -0.289 (P=0.388)
	2	21	90	90	90	90	
	3	17	93	93	93	93	
	4	16	82	82	82	82	
	5	14	96	96	96	96	
	6	16	176	176	176	176	
	7	17	115	115	115	115	
	8	21	93	93	93	93	
	9	16	95	95	95	95	
	10	16	90	90	90	90	
	11	16	92	92	92	92	

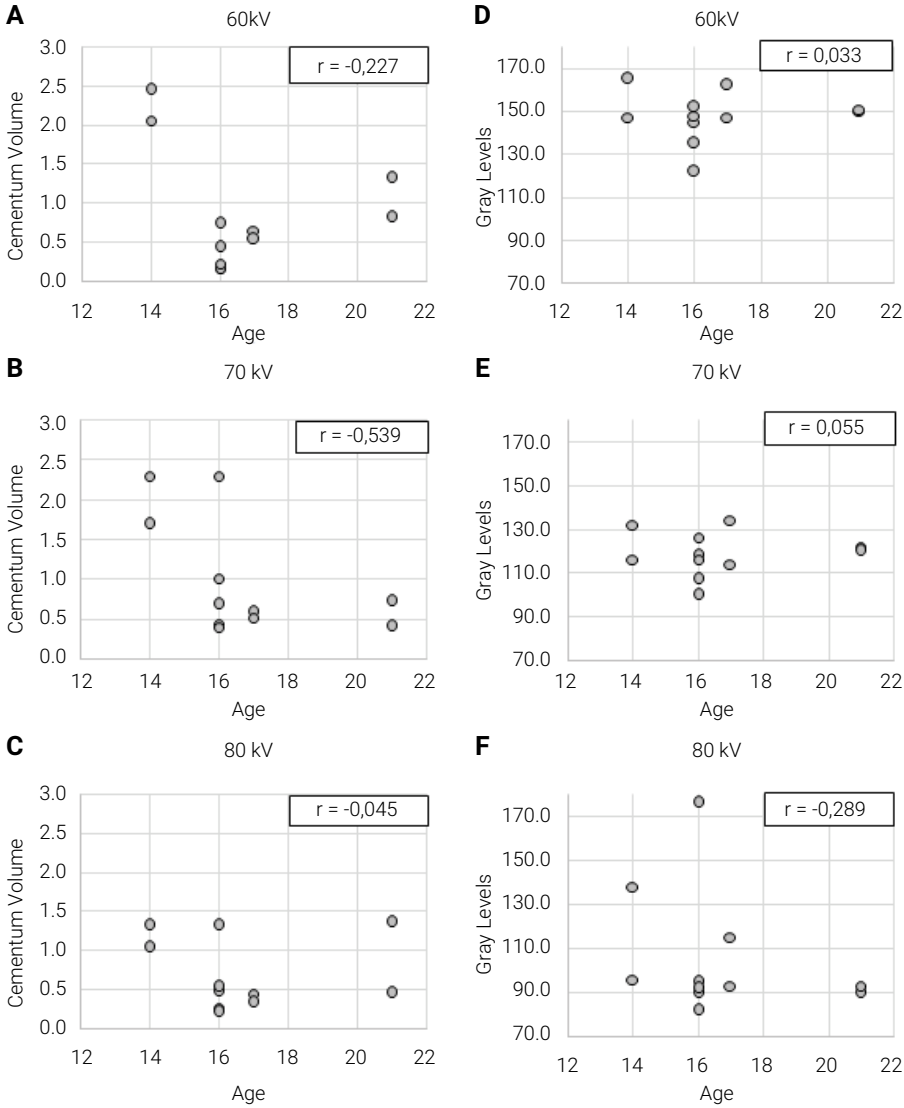

Figure 4. Graphs of correlations between age and cementum volume in mm^3 (A. B. and C) and between age and gray levels (D, E, and F). for each of the three energies tested (60, 70, and 80 kV).

None of the obtained dental cementum volumes means, or gray levels were correlated with age ($p>0.05$) (Figure 4). Nevertheless, the highest correlation (in absolute value) between dental cementum volume and age was found when using 70 kV energy ($r = - 0.539$; $p=0.088$).

Discussion

Many studies have been conducted to analyze and measure the volume and thickness of cementum, but most of them used destructive methods, such as histologic analysis[1,12,13]. In contrast, micro-CT allows the scanning of samples in a non-destructive

manner, maintaining the physical integrity of the analyzed structure[3,8], which could be advantageous, especially in keeping counter-evidence in forensic cases.

As this was a pilot study, and due to the scarcity of previous similar studies, the sample size used was 11 teeth. This number was supported in preliminary studies based on microtomographic analysis of other biological tissues or microscopic analysis of the dental cementum itself[1,8,11].

Earlier studies have outlined dental tissue analysis parameters encompassing energy levels (70-100kV), amperage (100-114μA), isotropic resolution (5-20μm), and integration time (300-900ms). Image reconstruction employed NRecon software (V1.6.10.4; Bruker, Belgium), quantitative analysis utilized CTAn software (V.1.14.4, Bruker, Belgium)[9-11], with some studies also incorporating filters[2]. On the other hand, none of them presented values adapted for the evaluation of the dental cementum over the entire root length.

According to the results of this study, the mean volume of cementum in all root thirds was measured and correlated with the patients' age, showing better results when the images were obtained with 70kV ($p=0.088$). Despite this, the values were not statistically significant ($p<0.05$), suggesting that a study with a larger number of samples may provide better results. In summary, existing studies differ regarding the selection of micro-CT acquisition parameters for dental tissue analysis. Specifically regarding the energy, where the values found vary from 60 to 100kV[3,9,11], there is, in fact, no literal consensus on the subject.

Based on the analysis of the different gray levels used in the histogram to delimit the region of the dental cementum, it is believed that the large variation in these numbers was due to the different thicknesses of cementum among the root thirds of each sample, making this value unique for each one. Most studies conducted to analyze dental tissues such as enamel and dentin do not have the habit of disclosing the values used in the histogram[14,15]. However, in order to make the research method reproducible, we have chosen to disclose the gray levels that were manually determined by the operator.

Due to the density similarity between dentin and cementum, it is challenging to segment and differentiate them. But, based on the results of the present study, and in comparison with data from a previous study[1], it is possible to suggest that the combination of an exposure time greater than 1000ms and a voxel less than 5μm along with adequate power and filter should be used in future studies.

An important consideration to acknowledge in this study pertains to the exclusivity of the tooth type selected for analysis, i.e., premolars, which are commonly subjected to orthodontic extraction. While this choice was made for specific research reasons, it is crucial to recognize its potential impact on the generalizability of findings to a broader population of tooth types.

In light of this limitation, future investigations could substantially enhance the depth and breadth of knowledge in this field. It is recommended that forthcoming studies encompass a broader spectrum of tooth types to represent dental tissues comprehensively. Moreover, expanding the scope to include larger sample sizes and more

diverse age groups will undoubtedly facilitate a more nuanced understanding of the correlation between cementum thickness and age. This approach has the potential to yield invaluable insights into the intricate dynamics of dental tissue aging. By broadening the study's focus and incorporating a wider range of tooth specimens and patient demographics, future research endeavors can effectively address the limitations mentioned and foster a more comprehensive comprehension of the subject matter.

Therefore, we conclude that the micro-CT allows a non-destructive analysis of the dental cementum, possibly becoming a tool for human age estimation, and that the acquisition parameters to ensure a better quality of the quantitative analysis are: 8.98 µm pixel size, 2240 x 2240 pixels matrix per image, 100 µA, 1100 ms integration time, 0.5º rotation step, 1.0 mm thick aluminum filter, and 70kV energy. Additionally, for a subjective visual analysis of the cementum, the 60kV energy guaranteed greater sharpness in the teeth analyzed.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author Contribution

Pedro Américo Felizardo dos Santos: study concepts and study design, data acquisition, data analysis and interpretation, statistical analysis and manuscript editing, manuscript preparation. **Vanessa Moreira Andrade:** statistical analysis and manuscript editing, manuscript preparation, manuscript review. **Luan Ferreira Bastos:** data acquisition. **Luciana Pereira da Silva:** data acquisition, data analysis and interpretation. **Ricardo Tadeu Lopes:** data acquisition. **Aline de Almeida Neves:** study concepts and study design, manuscript review. **Andreia Cristina Breda de Souza:** study concepts and study design, manuscript review. **Maria Augusta Visconti:** study concepts and study design, manuscript review. All authors actively participated in the manuscript and approved the final version of the manuscript.

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