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Volume measurement of crowns in mandibular primary central incisors by micro-computed tomography

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Abstract
Objective. To determine sex differences in the tissue proportions of crowns of mandibular primary central incisors in Chinese children and to quantify the volume of crown components in three dimensions using micro-computed tomography (micro-CT). ***Materials and methods.*** The specimens used in this study were 41 mandibular first deciduous incisor teeth with intact crowns (21 males and 20 females) obtained from patients between 5–6 years of age. Each specimen was scanned using micro-CT at a resolution of 0.05 mm and 3D-rendered images were created. The volume of each component of the crown was measured and examined for differences in different sex and ages. ***Results.*** The pulp chamber volume decreased with age and the volume ratio of the pulp chamber to the whole crown was significantly smaller in 6-year-olds than in 5-year-olds ($p < 0.05$). ***Conclusions.*** Males had significantly larger tooth crown volumes and dentin volumes than females did ($p < 0.001$), while the volume of enamel showed no sexual dimorphism.

Key Words: *deciduous incisor, micro-computed tomography, sexual dimorphism, three-dimensional observation, volumetry*

Introduction

Tooth crowns are a composite of dentin, pulp and enamel covering, all of which are ectodermal tissues. Studies of tooth crown dimensions, such as maximum mesiodistal and buccolingual diameters, have frequently been the subject of analysis by dentists, geneticists and physical anthropologists [1–5]. Quantitative investigations of hard tissues of crowns have focused on the measurement of enamel thicknesses and surface areas [6–8]. Traditionally, mesiodistal or buccolingual measurements have been used to express crown sizes of teeth. However, in this study, crown volume was used as a reference parameter. Martin [9] argued that the most ideal measurement of the amount of enamel on a tooth would be the volume of the tissue. Unfortunately, normative values for crown volumes of individual teeth or for the primary or permanent dentition are limited for incisors and there is no information regarding the extent to which discrimination of tissue according to sex can be improved by examining volume of teeth.

Conventional destructive approaches such as physical sections [10,11] and two-dimensional lateral flat-plane radiographs [12,13] cannot allow a full and three-dimensional distribution of the volume of dental tissues to be examined. Fanibunda [14] provided a method to produce an accurate dental replica of a human pulp cavity, from which the volume of the pulp cavity can be measured; however, this method is also destructive. Due to the limitations of previous methods, little data are available regarding the different volumes of dental structures in incisors.

In recent years, the development of micro-CT has enabled a non-destructive, detailed and quantitative evaluation of tooth components from a three-dimensional perspective [15,16]. Odontometric studies of dentition have played an important role in investigations of biological variations in human tissues, but most of these studies were based on traditional measures of tooth crown diameters. In the present study, we extend the previous studies conducted on crown dimensions with two-to-three dimensions. The aim of this study was to gather normative crown volume data of primary

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Table I. Age and sex distribution of the sample.

Age (years)	Male	Female	Total
5	7	9	16
6	14	11	25
Total	21	20	41

mandibular incisors, which have not been studied previously, and to explore sexual dimorphism in each part of the crown volume in a sample of Chinese children, a population that has not been studied previously in this regard.

Materials and methods

Sample preparation

Forty-one mandibular first deciduous incisors with intact and caries-free crowns were selected. Each specimen had minimal tooth attrition and root resorption of less than 2/3 of the original length of the root. All of the teeth had been extracted for clinical reasons and were selected from the Research Institute of Pediatric Dentistry in Tongji University. Immediately after extraction, all teeth were cleaned under tap water and stored in a 0.9% sodium chloride solution until use. Only teeth from individuals whose gender and age were documented at the time of extraction were used for analysis. Based on the patient's age at extraction, the specimens were classified into two age groups: 5- and 6-year-olds. The age and sex distributions of the samples are shown in Table I.

Micro-CT scanning

Each specimen was scanned at a resolution of 50 μm using the micro-CT system (HMX-225 Actis 4, TESCO, Tokyo, Japan). This system consists of an imaging device and a computer. The imaging device consists of a microfocus X-ray generator, a two-dimensional detector array and a specimen stage that can be rotated 360° by a computer-controlled motor. Specimens were placed with the long axes of the teeth lined up along the rotational axis of the scanner. The tube voltage was set at 120 kV, the tube current was set at 140 μA and the focal spot size of the X-ray generator was set at 5 μm. The source-to-object distance (SOD) was 60 mm, the source-to-image distance (SID) was 600 mm and the magnification was at ×10. The detector was equipped with an image intensifier tube coupled to a CCD camera with a scanning line of 1024 × 1024 dots/16 bits per memory. Based on the raw data obtained from the camera, two-dimensional slice images were produced by the back projection method. Each image had a resolution of 1024 × 1024 pixels and a voxel size of 35 × 35 × 50 μm. For each sample, ~180–280 microtomographic slices with thicknesses of 50 μm were acquired (Figure 1), covering the entire incisal to apex length of the teeth. The average scanning time for each specimen was ~30 min.

Production of 3-D micro-CT models

First, with each image slice obtained by micro-CT, contrast adjustment and elimination of noise were performed using photo-retouching software (PHOTOSHOPCS3, Adobe, Inc, California, USA). Next, three-dimensional images were reconstructed from the slices imaged by the volume rendering method using a three-dimensional reconstruction software TRI/3D BON (Ratoc, Tokyo, Japan). In this way, 3D renderings of the exterior and interior regions of the teeth were obtained (Figure 2). As all the slices were consecutive and had the same thickness, the image obtained from the slices was a true three-dimensional image volume. Furthermore, by making the enamel and dentin translucent and the pulp chamber stained, the morphology and positional relationship between the enamel, dentin and the pulp chamber were clearly observed. By rotating the 3D images, we were able to observe the teeth in various directions.

Measurements

The cemento-enamel junction (CEJ) of the mandibular incisor is sinuous, which makes defining a single cervical plane (i.e. above which is crown and below which is root) difficult. In order to define a plane, we

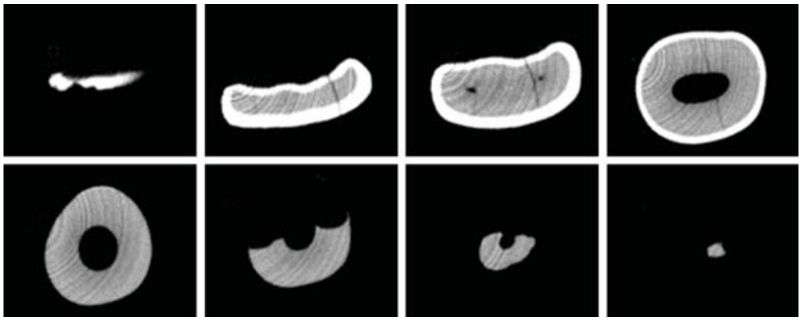

Figure 1. Micro-CT scanning. Eight of the 280 slices of the micro-CT data for a mandibular deciduous central incisor. Enamel, dentine and pulp space could be identified. The position of the pulp horn and the absorption of the root could also be identified.

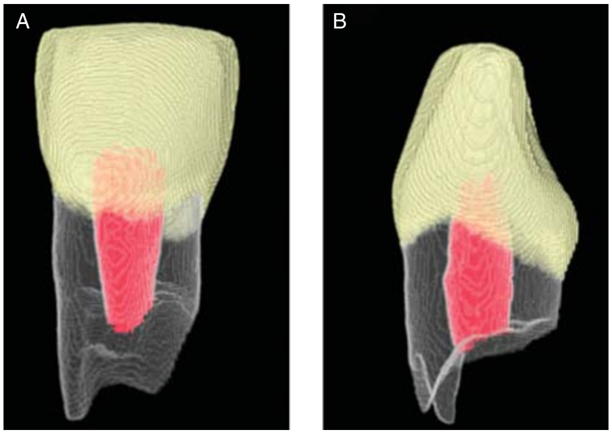

Figure 2. Three-dimensional reconstruction of a tooth using micro-CT.

located the first plane of the cross-section through the incisal edge. Next, the most apical plane that contained the most apical extension of the enamel in the lingual surface was taken as the cervical margin plane above which was defined as the crown in our study.

The crown volume measurements were performed using the TRI 3D BON software (Ratoc) by a single observer on the 3D reconstruction imaging to prevent the introduction of inter-observer error. The crown volume data were recorded for each tooth as follows: the total crown volume (mm^3), enamel volume (mm^3), dentin volume (mm^3) and pulp chamber volume (mm^3). Then the volume ratio of the pulp chamber to the total crown was calculated.

Descriptive statistics for crown tissue volumes (i.e. mean, standard deviation, coefficient of variance and percentage of sexual dimorphism) were calculated. The percentage of the coefficient of variance of the tissue volumes was calculated by dividing the standard deviation by the mean measurement, multiplied by 100. The percentage of sexual dimorphism was calculated following Garn et al. [17] (i.e. [(mean in male − mean in female)/mean in female] × 100). The results between the different genders and ages were performed with the Student's t-test. The significance level was set at $p < 0.05$. Statistical analyses were performed using SPSS11.0 for Windows.

Table II. The basic descriptive data of crown volumes in the primary mandibular central incisors.

Measurements	N	Mean (mm^3)	SD	CV
Total crown volume	41	35.413	5.893	16.641
Enamel volume	41	8.087	1.631	20.168
Dentin volume	41	25.386	4.398	17.324
Pulp chamber volume	41	1.941	0.630	32.457

n, number of subjects; SD, standard deviation; CV, coefficient of variance.

Table III. The crown volume measurements in different ages (mean ± standard deviation, mm^3).

Measurements	5 years	6 years	t	p
Total crown volume	33.985 ± 6.822	36.328 ± 5.154	1.251	0.219
Enamel volume	7.531 ± 1.476	8.443 ± 1.654	1.793	0.081
Dentin volume	24.400 ± 5.400	26.017 ± 3.599	1.153	0.256
Pulp chamber volume	2.054 ± 0.709	1.868 ± 0.577	0.917	0.365
Pulp chamber volume ratio (%)	6.035 ± 1.568	5.106 ± 1.323	2.040	0.048*

*$p < 0.05$.

Results

Values of the components of the crown volume are shown in Table II. Coefficients of variation showed that the total tooth crown volume measurements were the most stable in size, followed by dentin volume, enamel volume and the pulp volume, which showed the greatest variation of all. Table III shows the results of the Student's t-test for differences in crown volume components between the different ages. The pulp chamber volume was non-significantly smaller in individuals who were 6 years of age. The volume ratio of the pulp chamber in relation the whole crown was significantly smaller in 6-year-olds compared to 5-year-olds ($p < 0.05$). Table IV depicts the descriptive statistics and p-values for the measured crown components in the different sexes. Males showed significantly greater total crown volumes, dentine volumes and pulp volumes. No differences in the enamel volume were found between the males and females.

Discussion

The study of dental morphology is important for dentists to understand dental forms and internal structures in three dimensions. Different methods have been used to investigate tooth morphology and these methods include serial sectioning of extracted teeth [18,19], scanning electron microscopic studies [20], casts of the pulp cavity with metal or resin [21], transparent tooth models [22] and conventional dental radiographs [13]. All of these methods have unavoidable pitfalls because they do not allow one to study the external and internal anatomy of teeth three-dimensionally at the same time. In the early 1980s, the first micro-CT scanners were developed that reached micrometer resolutions. Micro-CT had been successfully used for experimental explorations in the field of dentistry. Micro-CT, based on several two-dimensional dental radiographs from a series of

Table IV. The crown volume measurements in different genders (mean ± standard deviation, mm^3).

	Males			Females			Sex difference		
	n	Mean	SD	*n*	Mean	SD	%	*t*	*p*
TCV	21	38.358	4.835	20	32.321	5.370	18.678	3.787	0.001**
EV	21	8.503	1.735	20	7.650	1.428	11.150	1.713	0.095
DV	21	27.707	3.521	20	22.948	3.933	20.738	4.086	0.000**
PV	21	2.148	0.658	20	1.723	0.531	24.667	2.273	0.029*

TCV, total crown volume; EV, enamel volume; DV, dentin volume; PV, pulp chamber volume.
**$p < 0.01$, *$p < 0.05$.

slice scans, allows three-dimensional reconstruction of teeth and detailed exploration of teeth and volumetry of root canals. As an alternative and refined approach, we made three-dimensional observations of the morphology of the surface and interior of the mandibular deciduous central incisors using micro-CT, which is non-destructive and allows for observations and analyses of the morphological characteristics of teeth in detail from many directions. By adjusting the colors in different tissue, the topographic relationships of the pulp cavity and the hard tissues of the teeth can be clearly visualized.

Philippas [23] carried out a radiological investigation of pulp chambers, which was the first study to provide quantitative data on their size. However, only one permanent tooth was used in the study. The pulp morphology and volume of maxillary second deciduous molar crowns have been studied using micro-CT by Amano et al. [24]. They studied the pulp morphology of the tooth crown and measured the volume ratio of the pulp chamber in relation to the whole crown in patients of different ages. However, genders of the specimens in the sample were not indicated. In this study, we obtained three-dimensional images of the mandibular primary incisors and measured the volumes of the different tissues of crowns in individuals of both genders and of different ages (Table IV).

Pulp chamber volumes, in mandibular first molars from patients aged between 20–60 years, have been described by Ketterl [25]. The volumes decreased from 76 to 56 mm^3 (27%) in the 40-year period. In the present study, pulp chamber volumes of teeth in 6-year-olds were smaller than in 5-year-olds, decreasing from 2.054 mm^3 to 1.868 mm^3 (9.06%) in 1 year. The volume ratio of the pulp chamber in the 6 year olds was significantly smaller. It is apparent that the volume of the pulp chamber is strongly related to age in deciduous teeth. The reduction in the size of the pulp chamber was mainly caused by the continued deposition of secondary dentin with increasing age. The hard tissue volume was significantly increased for 6-year-olds, although enamel wear would be higher in those of 5 years of age. Philippas et al. [26,27] studied secondary dentin in different teeth and showed that age, rather than external irritation, is the primary factor in the production of irregular secondary dentin.

Studies on sexual dimorphism in human teeth showed that males possess larger tooth crowns than females for both permanent and deciduous dentitions. However, all of these studies relied on linear measurements of tissue thickness derived from dental casts [28,29], radiographs [12] or sectioning [11]. In these studies, enamel and dentin thickness measurements were confined to the mesiodistal or buccolingual directions. Linear measurements of tissue thickness may be less ideal representations of total tissue volumes [9]. Studies on the volumes of dental pulp chambers have used a rubber material drawn into teeth under a vacuum [14]. Three-dimensional volume distributions of enamel and dentin in primary teeth between males and females have never been explored.

In our study, the volumes of the hard tissue and pulp chamber of the crown showed significant sexual dimorphism. The males had significantly larger crown and dentin volumes than did females. It was demonstrated that sexual dimorphism with regard to mesiodistal tooth size was primarily due to differences in dentin thickness rather than enamel thickness [6]. Studies on tooth crown sizes and structures of individuals with chromosomal aneuploidies and their normal male and female relatives have demonstrated that sex chromosomes are associated with dental growth and development, while human X and Y chromosomes showed differential direct effects on the dental hard tissues. The Y chromosome enhances the mitotic activity of developing tooth germs, resulting in greater dentin thickness and hence larger crowns in males than in females. The X chromosome exerts its influence in enamel deposition and has little or no influence on the growth of dentin [28–31].

Hietala and Larmas [32] showed that estrogen deficiency induced by ovariectomized rats showed enhanced dentin formation. By using immunological methods, they also demonstrated that estrogen receptors are present in odontoblasts and endothelial cells of blood vessels in human teeth [33]. Quigley [34] demonstrated that human males and females undergo 'mini-puberty' during the first 6 months of life, in which sex steroid hormone concentrations reach pubertal levels. In human male infants, serum testosterone peaks in concentration at ~2 months of age, while estrodial levels in females are often elevated above the pre-pubertal range, especially during the first 6 months of life [34].

The crowns form inside mandibular deciduous incisors at ~16 weeks *in utero* and reach their final sizes and shapes ~2 months after birth. During this time, the sex hormone concentrations reach peak levels. The results of our study showed that the crown and dentin volumes and sizes were significantly sexually dimorphic and we suggest that both sex chromosomes and the sex hormone had effects on primary mandibular central incisors.

Conclusion

Micro-computed tomography is a non-invasive technique that enables one to visualize morphological characteristics of teeth in a three-dimensional manner and while simultaneously allowing one to accurately measure the volume of a tooth for evaluating sexual dimorphism of children. The primary mandibular central incisors showed significant sexual dimorphism in their crown volumes and dentin volumes, while volumes of enamel showed no sexual dimorphism.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Morrees CFA, Thomsen S, Jensen E, Yen PKJ. Mesiodistal crown diameters of the deciduous and permanent teeth in individuals. J Dent Res 1957;36:39–47.
[2] Yuen KK, Tang EL, So LL. Relations between the mesiodistal crown diameters of the primary and permanent teeth of Hong Kong Chinese. Arch Oral Biol 1996;41:1–7.
[3] Liversidge HM, Molleson TI. Deciduous tooth size and morphogenetic fields in children from Christ Church, Spitalfields. Arch Oral Biol 1999;44:7–13.
[4] Hughes T, Dempsey P, Richards L, Townsend G. Genetic analysis of deciduous tooth size in Australian twins. Arch Oral Biol 2000;45:997–1004.
[5] Harris EF, Lease LR. Mesiodistal tooth crown dimensions of the primary dentition: a worldwide survey. Am J Phys Anthropol 2005;128:593–607.
[6] Stroud JL, Buschang PH, Goaz PW. Sexual dimorphism in mesiodistal dentin and enamel thickness. Dentomaxillofac Radiol 1994;23:169–71.
[7] Kono RT, Suwa G, Tanijiri T. A three-dimensional analysis of enamel distribution patterns in human permanent first molars. Arch Oral Biol 2002;47:867–75.
[8] Schwartz GT. Enamel thickness and the helicoidal wear plane in modern human mandibular molars. Arch Oral Biol 2000; 45:401–9.
[9] Martin L. Significance of enamel thickness in hominoid evolution. Nature 1985;314:260–3.
[10] Green D. Morphology of the pulp cavity of the permanent teeth. Oral Surg Oral Med Oral Pathol 1955;8:743–59.
[11] Smith TM, Olejnicak AJ, Reid DJ, Ferrell RJ, Hublin JJ. Modern human molar enamel thickness and enamel-dentine junction shape. Arch Oral Biol 2006;51:974–95.
[12] Harris EF, Hicks JD. A radiographic assessment of enamel thickness in human maxillary incisors. Arch Oral Biol 1998; 43:825–31.
[13] Chandler NP, Pitt Ford TR, Monteith BD. Coronal pulp size in molars: a study of bitewing radiographs. Int Endod J 2003; 36:757–63.
[14] Fanibunda KB. A method of measuring the volume of human dental pulp cavities. Int Endod J 1986;19:194–7.
[15] Olejniczak AJ, Tafforeau P, Feeney RN, Martin LB. Three-dimensional primate molar enamel thickness. J Hum Evol 2008;54:187–95.
[16] Suwa G, Kono RT. A micro-CT based study of linear enamel thickness in the mesial cusp section of human molars: reevaluation of methodology and assessment of within-tooth, serial, and individual variation. Anthropol Sci 2005;113:273–89.
[17] Garn SM, Lewis AB, Swindler DR, Kerewsky RS. Genetic control of sexual dimorphism in tooth size. J Dent Res 1967; 46:963–72.
[18] Mikrogeorgis G, Lyroudia KL, Nikopoulos N, Pitas I, Molyvdas I, Lambrianidis TH. 3D computer-aided reconstruction of six teeth with morphological abnormalities. Int Endod J 1999;32:88–93.
[19] Lyroudia K, Pantelidou O, Mikrogeorgis G, Nikopoulos N, Pitas I. Three-dimensional reconstruction: a new method for the evaluation of apical microleakage. J Endod 2000;26:36–8.
[20] Wrbas KT, Kielbassa AM, Hellwig E. Microscopic studies of accessory canals in primary molar furcations. ASDC J Dent Child 1997;64:118–22.
[21] Melton DC, Krell KV, Fuller MW. Anatomical and histological features of C-shaped canals in mandibular second molars. J Endod 1991;17:384–8.
[22] Shivapathasundharam B, Berti AE. Transparent tooth model system. An aid in the study of root canal anatomy. Indian J Dent Res 2000;11:89–94.
[23] Philippas GG. Influence of occlusal wear and age on formation of dentin and size of pulp chamber. J Dent Res 1961;40: 1186–98.
[24] Amano M, Agematsu H, Abe S, Usami A, Matsunaga S, Suto K, et al. Three-dimensional analysis of pulp chambers in maxillary second deciduous molars. J Dent 2006;34:503–8.
[25] Ketterl W. Age-induced changes in the teeth and their attachment apparatus. Int Dent J 1983;33:262–71.
[26] Philippas GG, Applebaum E. Age factor in secondary dentin formation. J Dent Res 1966;45:778–89.
[27] Philippas GG, Applebaum E. Age change in the permanent upper canine teeth. J Dent Res 1968;47:411–17.
[28] Alvesalo L, Osborne RH, Kari M. The 47, XYY male, Y chromosome, and tooth size. Am J Hum Genet 1975;27: 53–61.
[29] Alvesalo L, Kari M. Sizes of deciduous teeth in 47, XYY males. Am J Hum Genet 1977;29:486–9.
[30] Alvesalo L, Portin P. 47, XXY males: sex chromosomes and tooth size. Am J Hum Genet 1980;32:955–9.

[31] Alvesalo L, Tammisalo E. Enamel thickness of 45, X females' permanent teeth. Am J Hum Genet 1981;33: 464–9.
[32] Hietala EL, Larmas M. The effect of ovariectomy on dentin formation and caries in adult rats. Acta Odontol Scand 1992; 50:337–43.
[33] Hietala EL, Larmas M, Salo T. Localization of estrogen-receptor-related antigen in human odontoblasts. J Dent Res 1998;77:1384–7.
[34] Quigley CA. Editorial: the postnatal gonadotropin and sex steroid surge-insights from the androgen insensitivity syndrome. J Clin Endocrinol Metab 2002;87:24–8.