Relationship between external and internal structures in deciduous teeth: a multidisciplinary study

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Abstract

Aim The different dental structures of deciduous teeth have always been regarded with great interest in endodontics and restorative dentistry. Nevertheless, there are still few investigations on the morphology of deciduous teeth compared to the permanent ones, due to the difficulty in obtaining sound deciduous teeth suitable for measurement. The aim of this research is to evaluate and compare the internal and external structures of deciduous teeth from completely preserved hominid findings, using X-ray and micro-CT examination.

Study Design Twenty-two archaeological tooth finds were collected and analysed. Samples were divided into two subgroups according to gender and arch region.

Methods Samples were photographed using a digital camera and were then analysed by X-ray and micro-CT. Different parameters of external and internal structures were calculated to obtain a normalisation coefficient, called the P-factor.

Statistics The statistical analysis was performed using one-way analysis of variance (ANOVA) and Holm-Sidak multiple comparison test, setting the significance at $p < 0.05$.

Results The P-factors obtained show a similar result (0.25 ± 0.05), and no significant differences were found between all calculated parameters ($p > 0.05$).

Conclusion There is a constant correlation between external and internal structure, regardless of gender, type, shape or arch sector.

KEYWORDS Deciduous teeth, Root Canal Morphology, X-ray, Micro-CT, 3D reconstruction

Introduction

The different dental structures have always represented a great source of interest for clinicians and researchers to better understand all the characteristics of both permanent and deciduous teeth. Differences in terms of microstructural and chemical features between permanent and deciduous teeth play a crucial role in particular situations, mainly in preventive, restorative and endodontic dentistry [Wang et al., 2006; Zamudio-Ortega et al., 2014] In the literature, morphology investigations of deciduous dentition are lacking, although nowadays there is an increasing interest for the evaluation of similarities and discrepancies with permanent ones. Indeed, it has been documented that deciduous teeth have a different enamel and dentin microstructure, presenting enamel with major organic content and fewer mineral substances, a lower density of prismatic crystal structures and smaller prisms [Gentile et al., 2015b], while dentin displays some differences in the mineral content [Anwar Alebrahim et al., 2014]. Moreover, several studies report that the enamel of the deciduous teeth is thinner compared to the enamel of permanent ones, which means that the pulp chamber is closer to the tooth surface [Cheong et al., 2019; Amano et al., 2006]. Therefore, during the removal of a deep carious lesion there is a high risk of exposing the pulp. It is known that the deciduous teeth as compared to the permanent one presents a bigger pulp chamber, due to the fact that the pulp takes up a larger volume of the crown and that dentin secretion and pulp repair activity decrease with aging [Cheong et al., 2019; Borges et al., 2007]. To date the internal volume of root canals has not been well documented. In this light, the knowledge of deciduous tooth morphology is essential for clinicians, in particular for root canal treatment and dental traumatology. However, the relationship between the external and internal structure of deciduous teeth in the scientific literature is still debated [Desoutter et al., 2019]. Unfortunately, obtaining sound deciduous teeth suitable for measurement is difficult due to severe attrition and physiological root resorption prior to the eruption of permanent teeth. Usually, plaster casts of deciduous dentitions from paediatric patients are examined, which focused on crown form and size measured using a caliper [Jung et al., 2012]. However, roots cannot be analysed with a dental cast. Several studies have evaluated intact deciduous teeth derived from hominid fossils [Bermúdez de Castro et al., 2017; Pan et al., 2021; Ortiz et al., 2020]. These findings are needed to obtain odontometric data, which are useful in many fields, including anthropology, genetics, forensic science, and dentistry. The presence in the hominid findings record of deciduous teeth is limited in comparison with the larger collections of permanent samples recovered worldwide [Bermúdez de Castro et al., 2017]. Several studies suggest that deciduous nonmetric dental traits are useful to evaluate the biological correlations of human populations [Tillier et al., 2013; Peretto et al., 2015; Tocheri 2002]. Furthermore, the scientific community underlines that the deciduous dentition maintains its morphology and structure more than the permanent dentition "and is therefore relatively more useful for distinguishing specimens at various taxonomic levels" [Bailey et al., 2016]. Several methods have been used to analyse the different structures of deciduous teeth, such as dental radiographs, morphometric analysis, cone beam computed tomography (CBCT), magnetic resonance imaging, spiral computed tomography [Aps 2014], and micro-computed tomography (micro-CT) [Cheong et al., 2019]. Micro-CT is a non-destructive technique that provides a high-resolution, three-dimensional (3D) imaging of the deciduous teeth to better understand the shape, location and size of the pulp chambers and root canals [El Hachem et al., 2019]. A multidisciplinary analysis is the best approach to investigate the morphology and chemical structure of deciduous tooth. The results obtained provide valuable information on deciduous teeth morphology that can help clinicians select better treatment choices. The aim of this research is to evaluate the structure and the morphology of deciduous teeth from fully preserved hominid findings, with more focus on the relationship between the external and internal dental structures using a multidisciplinary approach.

Materials and methods

2.1 Specimen preparation

For this study the archaeological findings stored at the University Museum of the Gabriele d'Annunzio University of Chieti-Pescara (Piazza Trento and Trieste, Chieti, Italy) were analysed. The sample consisted of 22 deciduous teeth belonging to different skeletal individuals of the population of Herculaneum (ID: E) and of Val Fondillo (ID: Opi-T), respectively in Naples and in Opi (Aquila, Italy), as displayed in Table 1. The sample was divided into two subgroups according to gender and arch region (anterior and posterior), respectively Subgroup A and Subgroup B. Subsequently, samples were photographed using a digital camera (Nikon D7200 Reflex, Nikon Digital) in each projection.

2.2 X-ray examination

The samples were examined with intraoral radiographs using the cordless digital imaging scanner PSPIX² (AS702001 Phosphor System, Acteon Group, de Gotzen S.r.l., Rome, Italy). The operating conditions for the radiological analysis were as follows: voltage = 70 kV, current = 8 mA and exposure time 0.25 s. The radiographic images were then processed using OsiriX (Pixmeo, Switzerland), an open source DICOM (Digital Imaging and Communications in Medicine) viewer for two-dimensional (2D) rendering, and for each tooth the following parameters were calculated, expressed in μm^2:

- the total area of the tooth in the buccal-occlusal direction (aExt_vo),
- the total area of the tooth in the mesio-distal direction (aExt_md),
- area of the internal structure in the buccal-occlusal direction (aInt_vo),
- area of the internal structure in the mesio-distal direction (aInt_md).

Afterward, aExt_vo with aInt_vo and the aExt_md with aInt_md were compared and from these ratios, a normalisation coefficient, called the P-factor, was calculated. Consequently, P_vo and P_md were obtained respectively. The same parameters were calculated for Subgroup A and Subgroup B.

2.3 Micro-CT imaging, reconstruction, and analysis

After xX-ray evaluation, each sample was individually scanned by means of micro-CT Bruker SkyScan 1174 (SkyScan-Bruker, Antwerp, Belgium), installed at the Laboratories of the Research Center and Microscopy Service of Nanostructures (CISMiN), at the Polytechnic University of Marche, Ancona, Italy. The operating conditions for the micro-CT analysis were: voltage = 50 kV, current = 800 μA, aluminum filter of 1 mm, pixel size of 11.5 μm, rotation of 180◦ with 0.4° steps and exposure time per projection of 10 s. Tooth projections were converted to cross-sectional slices by NRecon software (Version 1.6.10.2, Bruker Billerica, Massachusetts, USA) with the following correction settings: ring artefacts (8.0); smoothing (6.0); beam hardening (70%) and proper misalignment compensation. The total number of slices was approximately 950 for all the samples, obtaining axial information on approximately 10 mm tooth thickness with an average scan time of 5 h per tooth.

Subject	Age (y. o.)	Gender	Arch Region	Tooth
E129	3.5 - 4	M	P	74
E129	3.5 - 4	M	A	81
E129	3.5 - 4	M	A	83
E118	8-9	M	A	53
E118	8-9	M	A	73
E118	8-9	M	P	85
E96	2-2.5	M	A	52
E96	2-2.5	M	A	72
E007	5-6	F	A	62
E007	5-6	F	P	75
E007	5-6	F	P	24
E008	7-8	M	A	73
E074	7-8	M	A	53
E082	3-4	F	A	72
E088	1-1.5	F	A	61
E088	1-1.5	F	A	63
E094	4-5	M	P	64
E094	4-5	M	P	65
Opi-T143	3-4	F	A	82
Opi-T143	3-4	F	P	54
Opi-T143	3-4	F	A	71
Opi-T143	3-4	F	A	51

Table Legends: (M) Male, (F) Female, (A) Anterior region, (P) Posterior region.

TABLE 1 Characteristics of samples collected

The two-dimensional information was processed using specific algorithms and ultimately a three-dimensional (3D) specimen reconstruction was generated. The 3D analysis software DragonFly (ORS, Montreal, Quebec) was used to perform image analysis and assessment of tooth structures.

In particular, the parameters quantified expressed in μm3 were:

- the total volume of the tooth (vT)
- the volume of the internal structure of tooth (vInt).

Similarly to the surface area values, the ratio of the two volumetric parameters obtained (vT and vInt) was calculated, obtaining the volume P-factor (Pv). Finally, Pv of Subgroup A and Subgroup B were also calculated, respectively.

2.4 Statistical Analysis

The statistical analysis was performed using Prism9 (GraphPad software, San Diego, CA, USA). The following morphometric parameters, obtained by xX-ray and micro-CT measurements, were tested: P_vo, P_dm and Pv, expressed as mean value ± standard deviation. The test used was one-way analysis of variance (ANOVA) and Holm-Sidak multiple comparison test on P_vo, P_dm and Pv groups. The group size was set to $n = 22$ for all experimental groups and the significance was $p < 0.05$.

Results

The qualitative results of digital photographs, radiological and micro-CT analysis are reported in Figure 1 and Figure 2.

The quantitative results of x-ray examination are reported in Table 2. The results, expressed as mean value ± standard deviation, showed a P_vo of 0.25±0.04 while P_md of 0.25±0.05.

The micro-CT quantitative results of all samples display a Pv of 0.25±0.05 as reported in Table 3.

The results of Subgroup A and Subgroup B are showed in Table 4 and Table 5 respectively. Subgroup A displays a P_vo of 0.25±0.04, a P_md of 0.24±0.05 and a Pv of 0.25±0.05; while Subgroup B presents a P_vo of 0.25±0.04, a P_md of 0.24±0.05 and a Pv of

	aExt_vo in μm^2	aInt_vo in μm^2	P_vo	aExt_md in μm^2	aInt_md in μm^2	P_md
Mean	1.08E+05	2.74E+04	0.25	1.09E+05	2.65E+04	0.25
SD	2.76E+04	7.46E+03	0.04	4.00E+04	9.53E+03	0.05

TABLE 2 X-ray examination results

	vInt in μm^3	vT in μm^3	Pv
Mean	4.88E+05	1.95E+06	0.25
SD	3.94E+05	1.26E+06	0.05

TABLE 3 Mean and standard deviation of micro-CT evaluation results of all samples

0.25±0.05.

The results of the statistical analysis of the two P-factors of the area and that of the volume of all samples, are shown in Figure 3. The results highlight that there are no significant differences between the parameters of P_vo, P_dm and Pv ($p>0.05$).

The same results were obtained for Subgroup A and Subgroup B, showing no differences between gender and arch region, as displayed in Figure 4 and Figure 5, respectively.

Discussion

Knowledge of the teeth structures and their correlations contributes to the success of treatment, especially in endodontics and restorative dentistry. Recently, several studies aimed at investigating the morphology of the different anatomical structures of deciduous teeth [Nava et al., 2022; Ticona-Flores e Diéguez-Pérez 2022], although in the literature there are few that analyse and investigate the morphology of deciduous teeth compared to the permanent teeth ones [Haberthür, Hlushchuk, e Wolf 2021; Buhamer et al., 2021]. This scarcity stems from the difficulty of finding healthy and intact deciduous teeth for in vitro evaluations. Indeed, several studies display the CBCT analysis of in vivo research or results derived by teeth belonging to hominid remains [Jung et al., 2012; Bermúdez de Castro et al., 2017; Pan et al., 2021; Azim et al., 2014].

Therefore, this study aimed to describe the 3D tooth morphology of hominids deciduous molars and incisors and to identify and consolidate positional factors that could potentially influence tooth preparation during the restorative treatment. It is well known that the deciduous maxillary anterior teeth have relatively longer roots compared to the crown length of their permanent counterparts, although the root form varied according to the teeth [Jung et al., 2012]. Furthermore, the dental crown of posterior primary teeth demonstrates a great anatomical variability, especially concerning the volume ratio of the pulp chamber and dental crown [Diéguez-Pérez e Ticona-Flores 2022]. Traditionally, the mandibular second molar is the tooth with the largest coronal dimension, since it has the largest number of cusps and pulp horns [Silva et al., 2014]. The buccolingual dimension of the crown is shorter than the mesiodistal dimension in all deciduous maxillary anterior teeth [Amano et al., 2006]. Furthermore, several studies evaluated and measured proportions and dimensions of deciduous teeth [Amano et al., 2006; Jung et al., 2012; Diéguez-Pérez e Ticona-Flores 2022; Silva et al., 2014; Gentile et al., 2015a], although there is a lack of research that indagate the comparison of external and internal structures. The present is the first study that evaluates the correlation between the total tooth area and the internal volume, ratio that we called P-factor, which was constant for all samples. Indeed, the values obtained for the area display a P_vo and P_dm values (both of 0.25±0.05), which represent the same result that was also obtained by the volumetric analysis by micro-CT (Pv = 0.25±0.05). It is interesting to note that there is a correlation between the area and the volume, obtaining as average the same P-factor value. Furthermore, the statistical analysis shows that there is no difference between the P-factors obtained ($p>0.05$). Therefore, it is assumed that a correlation exists between the external and internal structure, represented by the space of the dental pulp. In this light, it can be considered that the volume of the pulp space tends to be a quarter of the total volume of the tooth and despite root resorption, it remains constant with respect to the external structure of the tooth.

Contrary to some studies that indicate a great anatomical variability, especially in the maxillary first molar, and higher volumes in the maxillary second molar [Diéguez-Pérez e Ticona-Flores 2022], this work presents a constant relationship between the external and internal structures, regardless of the gender, type, shape and root resorption of the tooth. This finding can improve the clinical knowledge of the anatomy of the crown-root pulp structure, representing the key to successful root canal treatment. Indeed, due to the complex root canal system of the maxillary molars,

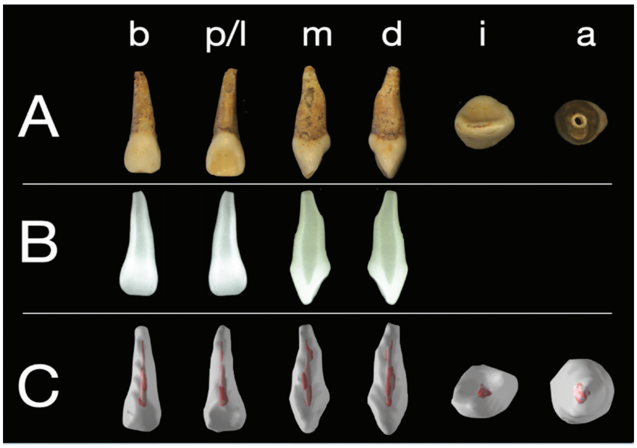

FIG. 1 Representative image of Opi-T143 individual. (A) digital photographs, (B) X-ray and (C) micro-CT analysis were displayed for each projection: buccal (b), palatal or lingual (p/l), mesial (m), distal (d), incisal (i) and apical (a) view. In micro-CT 3D reconstruction (C), the internal structure was colored in red

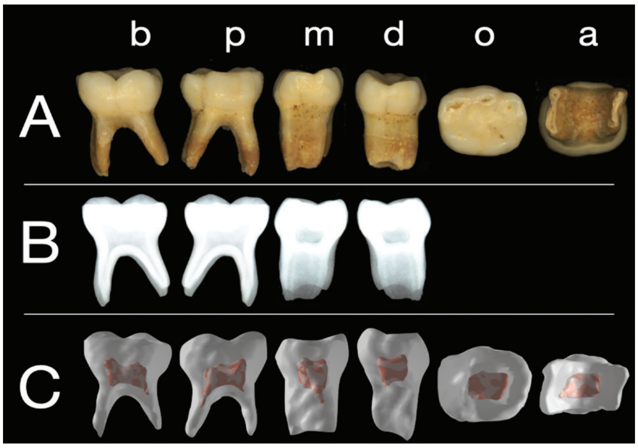

FIG. 2 Representative image of E118 individual. (A) digital photographs, (B) x-ray and (C) micro-CT analysis were displayed for each projection: buccal (b), palatal or lingual (p/l), mesial (m), distal (d), occlusal (o) and apical (a) view. In micro-CT 3D reconstruction (C), the internal structure was colored in red

		aExt_vo in μm^2	aInt_vo in μm^2	P_vo	aExt_md in μm^2	aInt_md in μm^2	P_md	vInt in μm^3	vT in μm^3	Pv
Male	Mean	1.07E+05	2.74E+04	0.26	9.71E+04	2.44E+04	0.26	4.42E+05	1.86E+06	0.25
	SD	2.66E+04	7.99E+03	0.05	3.33E+04	7.24E+03	0.06	2.28E+05	9.03E+05	0.05
Female	Mean	1.10E+05	2.74E+04	0.25	1.24E+05	2.90E+04	0.23	5.42E+05	2.05E+06	0.25
	SD	3.15E+04	7.61E+03	0.03	4.59E+04	1.21E+04	0.03	5.42E+05	1.64E+06	0.04

TABLE 4 X-ray and micro-CT results of Subgroups A

		aExt_vo in μm^2	aInt_vo in μm^2	P_vo	aExt_md in μm^2	aInt_md in μm^2	P_md	vInt in μm^3	vT in μm^3	Pv
Anterior	Mean	9.83E+04	2.41E+04	0.25	1.05E+05	2.45E+04	0.24	3.63E+05	1.50E+06	0.25
	SD	2.74E+04	6.52E+03	0.04	3.54E+04	7.45E+03	0.05	9.83E+04	4.67E+05	0.05
Posterior	Mean	1.30E+05	3.44E+04	0.27	1.19E+05	3.08E+04	0.27	7.54E+05	2.91E+06	0.24
	SD	1.60E+04	4.56E+03	0.04	5.26E+04	1.31E+04	0.06	6.32E+05	1.86E+06	0.04

TABLE 5. X-ray and micro-CT results of Subgroups B

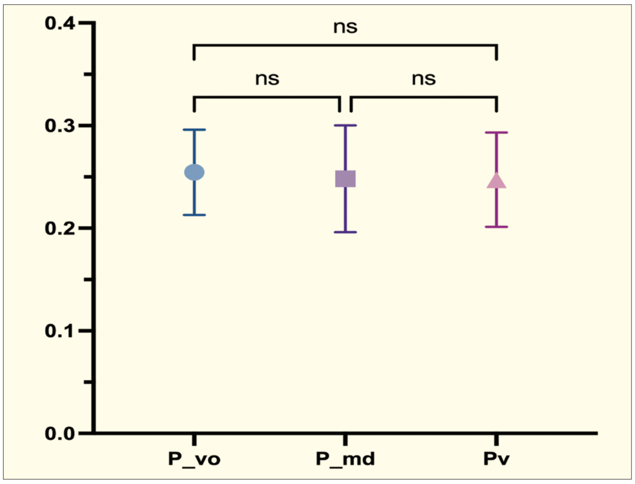

FIG. 3 All samples statistical analysis results. One-way ANOVA with Holm-Sidak multiple comparison test; p> 0.05 is not significant (ns) between all parameters evaluated. Results are reported as average value and standard deviation

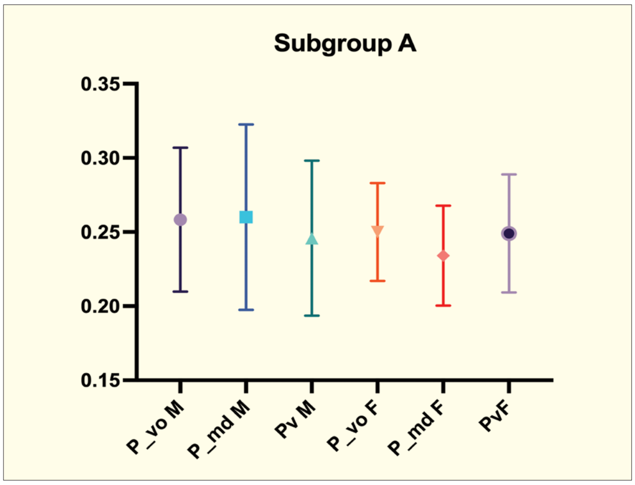

FIG. 4 Subgroup A statistical analysis results. One-way ANOVA with Holm-Sidak multiple comparison test; p> 0.05 is not significant between all parameters evaluated. Results are reported as average value and standard deviation

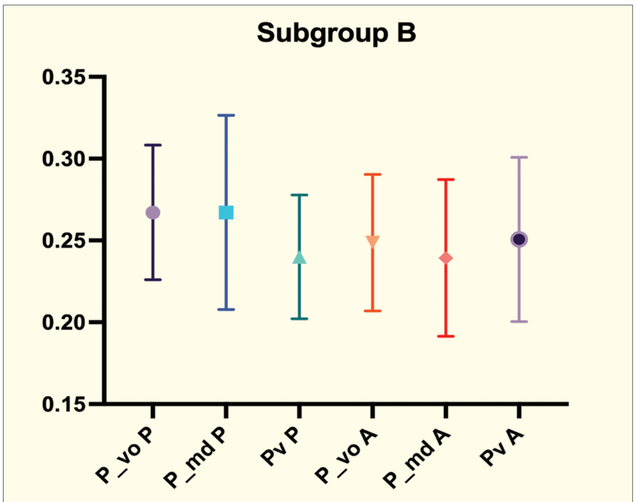

FIG. 5 Subgroup B statistical analysis results. One-way ANOVA with Holm-Sidak multiple comparison test; p> 0.05 is not significant between all parameters evaluated. Results are reported as average value and standard deviation

several mistakes can occur during the preparation of the access cavity [Angerame et al., 2020]. Generally, the clinical approach to perform a diagnosis or planning a dental treatment usually is based on a 2-dimensional radiographic image, which may not always be reliable. The conventional in vitro studies on the internal anatomy are destructive methods which can lead to alterations of the sample. Therefore, to obtain a more realistic information about the internal and external morphology of tooth anatomy is needed a more accurate investigation. Nowadays, micro-CT has proven to be a proper and effective non-invasive and non-destructive analysis tool that allows clinicians to accurately evaluate internal aspects, regardless of the shape or size of the sample. Thanks to the 3D imaging capabilities, micro-CT data can be reconstructed to obtain qualitatively and quantitatively valuable information, and hence, represents the preferred non-destructive method for determining the precise volume and position of the external and internal morphology of the teeth, providing objective data. The 3D information and measurements of the crown dimensions, pulp chamber, root canal and apex anatomy will improve the endodontic success [Azim et al., 2014]. In addition, restorative materials, voids and tooth structures can be distinguished with high precision and spatial resolution [Putignano et al., 2021]. For these reasons, micro-

CT is a useful tool for dental investigations. Furthermore, it can be useful in forensic dentistry, to assess the chronological age of a child [Lugliè et al., 2012]. In this light, further studies are needed to correlate the volume of the pulp with the biological and chronological age of the child. As of today, for in vivo studies, CBCT is the most accurate analysis [Ozcan et al., 2016], given that micro-CT is used for in vitro evaluation. This is a limitation of this study, since samples belonging to hominid residues are evaluated, although several intact samples tested derived from populations of the Italian peninsula of which there are not many studies in the literature. For this reason, further studies are needed to evaluate and confirm this relationship between the area and volume of the external and internal structure of deciduous teeth, and hence, providing relevant data for didactic and clinical application.

Conclusion

The relationship between external and internal tooth structures, discovered for the first time and called P-factor, can help the clinicians to better understand the structure of the dental pulp of deciduous teeth, which accounts for a quarter of the total tooth volume, despite radical resorption. Indeed, the P-factor can be useful for deciding the most suitable instrumentation and technique in restorative or endodontic treatment, since it is constant in both single- and multi-rooted teeth.

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