Microstructure of Mineralized Tissues in Human Primary Teeth

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*The **aim** of this study was to analyze the structural characteristics of the mineralized dental tissues - enamel, dentin and cementum - in primary teeth and to correlate the histological aspects observed in function of the dental type – single-rooted or multi-rooted. **Method**: Eighteen human primary noncarious teeth were sectioned in facial-lingual (single-rooted) and mesio-distal direction (multi-rooted). One to three samples from each tooth were obtained. The samples were prepared by the ground technique and analyzed under light microscopy at different magnifications. A quantitative and descriptive analysis of the morphology of the mineralized tissues was performed. **Results**: Spindles, tufts and lamellae were consistently observed mainly in the occlusal surface of the primary molars. The scalloped pattern of the dentinoenamel junction was not always present. The same was seen for zones of interglobular dentin. Dead tracts in dentin and tertiary dentin were observed mainly in single-rooted teeth below areas of dental attrition. Areas of cellular and acellular cementum were observed in the two dental types. **Conclusions**: Primary teeth have some structural peculiarities and these should be investigated concerning the clinical repercussion.*
Keywords: *dental enamel; dentin; dental cementum; tooth, deciduous, primary.*
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INTRODUCTION

The current literature on the histological aspects of permanent teeth offers information for the development of research on this class of teeth. Yet, concerning primary teeth the main obstacle encountered is the lack of scientific information on their histological characterization. Some studies about the microstructure of primary teeth have been reported.[1-15] Nevertheless, authors have reached consensus over the need for more comprehensive investigations on the topic.

The tooth enamel is the most mineralized tissue in the human body. The enamel is a compact mass of crystals, and rods are its main structural entity.[16,17] In the process of enamel formation, the striae of Retzius demarcate the deposited layers of the tissue.[17] In primary teeth a markedly visible stria of Retzius is called neonatal line. These are dark bands and are the result of the metabolic stress that affects tooth development at birth.[18,19] The enamel inside this line is formed before birth (prenatal), while the outer enamel is deposited after birth (postnatal). Variations in thickness and composition of these two regions have been described in the literature.[12,13]

The region where the enamel joins the dentin is called dentinoenamel junction, and the pattern commonly displayed is scalloped.[15] Apart from the structures described above, other enamel structures are the bands of Hunter and Schreger, tufts, spindles, lamellae, and areas with gnarled enamel.[16,17]

Dentin is less mineralized than the enamel and it supports this tissue.[20,21] The dentin of primary teeth is slightly less hard than the permanent teeth. It is believed that hardness depends on degree of mineralization and, based on this fact, permanent dentin is more mineralized.[22,23]

The main structural characteristic of dentin is defined by its tubular pattern and an abundant anastomosis system is also observed.[6,20,24] External stimuli may lead to retraction or death of odontoblasts, which in turn generates empty dentinal tubules. The occurrence of these dead tract areas on dentin is observed by microscopy as dark zones in undemineralized tooth slices, due to the presence of air within the tubular lumen.[20,21]

Tertiary dentin is a tissue formed around the pulp as response to external stimuli like attrition, caries or restoration procedures. Histologically, tertiary dentin may present dentinal tubules in connection with the adjacent dentin, irregular and scattered tubules, or even the absence of then.[20,21,25]

When the radicular dentin is observed by the tooth

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ground technique in a light microscope, granules are observed in what has been called the granular layer of Tomes. It is believed that this layer is the result of the coalescence and the curved terminal portion of tubules.[20,21]

Less mineralized areas are found in the dentin of primary and permanent teeth, which are described as interglobular dentin.[5,26] These are the results of the inappropriate fusion of the mineralization globules, called calcospherites.[20,21]

The cementum is a mineralized connective tissue which covers the root surface of the tooth, which main role is the insertion of fibers of the periodontal ligament to the tooth root.[27] The cementum may be classified as cellular and acellular, in terms of the presence or absence of cells inside its matrix, respectively.[27] The acellular cementum in primary teeth is most visible in the cervical root third, while the cellular cementum is predominant in the apical root third. Incremental lines are not frequently seen, or may even be absent, in the cellular cementum of primary teeth.[1]

The knowledge of these histological aspects of teeth is greatly important in function of the improvement in materials and techniques of clinical dentistry. Therefore, the present study analyzes the main structural characteristics of mineralized dental tissues (enamel, dentin and cementum) in human primary teeth, and establish the correlation of histological aspects observed to the type of primary tooth (single-rooted and multi-rooted).

METHODS AND MATERIALS

Tooth sectioning

Eighteen human non carious primary teeth were used in the present study (nine single-rooted and nine multi-rooted). The selected teeth presented fully formed roots or up to two thirds of root resorption. They were obtained as required by therapeutic prescription. The teeth used were kept in distilled water at 4°C upon the beginning of the experiment.

All procedures were carried out with the adequate understanding and written consent of the subjects. The present study was approved by the Committee of Ethics in Research on Human and Animals of the Lutheran University of Brazil- ULBRA.

Preparation of tooth samples

The teeth were mounted on a metallic plate using cyanoacrylate glue (Super Bonder Gel, Loctite, Brazil). Single-rooted teeth were longitudinally sectioned in facial-lingual direction, while multi-rooted teeth were sectioned in mesio-distal direction using a double-faced diamond disk (KG Sorensen, Brazil) under refrigeration. Two sections were performed as close as possible to the middle of each teeth, which afforded to obtain between 1 and 3 samples per tooth.

The tooth samples were manually worked in order to reduce thickness to 100 µm, using sandpaper grit 400, 600, and 1000 (3M, Brazil), in this order. After the desired thicknesses were obtained, samples were washed in distilled water in ultrasound for 10 minutes. Next, samples were left to dry for 24 hours and latter mounted on histological slides topped with cover slips for the light microscopy analysis.

Light microscopy analysis

Samples were inspected by an examiner under 40, 100, 200, and 400 x magnifications using a binocular light microscope (Olympus Optical, model U-MDOB3, Japan). The examiner analyzed the same samples at two different times within a one-week interval. All sample surfaces were analyzed and the presence of histological structures recorded. This enabled to conduct a quantitative and descriptive analysis of the morphology of mineralized tissues.

RESULTS

The distribution of structures in mineralized tissues varied as function of the tooth surface inspected. Samples that presented some imperfection during the preparation process were not considered in the results. The 18 teeth used in the present study provided 34 samples in total, 17 for each dental type (single-rooted and multi-rooted). The samples were used to analyze different aspects of the same tooth and the results were presented by tooth.

The distribution of teeth into two groups (single-rooted and multi-rooted) was based on the different embryological development stages at which each tooth type is formed. The histological structures were described in terms of absolute and relative frequencies on the tables.

Table 1 shows the results of the distribution of structures in the enamel of primary teeth analyzed. The striae of Retzius were constantly observed in dental samples, as well as the presence of structures like spindles, tufts and lamellae (Figures 1, 2 and 3).

Enamel inspection revealed the presence of one pronounced stria of Retzius (neonatal line) in this tissue. Striae of Retzius were more frequent in the enamel external to the neonatal line (Figure 1). Lamellae, spindles and tufts were observed in all occlusal surfaces of multi-rooted teeth.

The presence of a straight dentino-enamel junction along the whole extension of the tooth prevailed in single-rooted teeth (67%), while in multi-rooted teeth the combination of the two patterns, straight and scalloped, was more often observed (77%). The distribution of the kind of junctions did not show statistically significant difference in single-rooted teeth ($P>0.05$; chi-square test). Statistical significance was

Table 1. Distribution of the enamel structures in primary teeth.

Structures	Teeth (n)					
	Single-rooted			Multi-rooted		
	N*	n	%	N*	n	%
Striae of Retzius	9	8	89	9	9	100
Smooth dentinoenamel junction	9	6	67	9	2	22
Scalloped dentinoenamel junction	9	1	11	9	0	0
Smooth/scalloped dentinoenamel junction	9	2	22	9	7	77
Tufts	9	8	89	9	9	100
Spindles	9	9	100	9	9	100
Lamellae	9	9	100	9	9	100
Gnarled enamel	9	3	33	9	7	77
Bands of Hunter and Schreger	9	0	0	9	0	0

* Teeth number analyzed

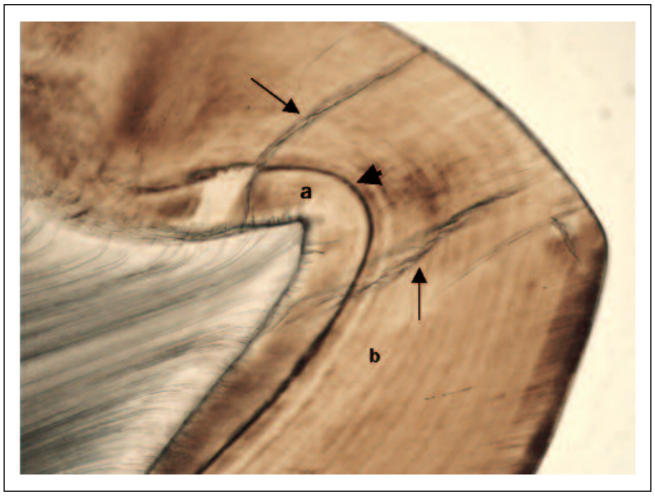

Figure 1. Neonatal line (short arrow) separating the prenatal enamel (a) from the postnatal enamel (b). Striae of Retzius are more evident in the postnatal enamel. Dentinoenamel junction with spindles. Lamellae follow a long extension of the enamel in the region (long arrows). (100x; tooth ground technique-TGT; light microscopy-LM).

observed for patterns in multi-rooted teeth ($P<0.05$; chi-square test).

The rods in the enamel of primary teeth were more linearly arranged, with few areas of gnarled enamel in single-rooted teeth (33%). This structure was observed in the occlusal surface of 78% of the primary molar teeth analyzed (Figure 3). No variation in the arrangement of rods, characterized by the bands of Hunter and Schreger, was observed in the teeth analyzed.

Table 2 shows the distribution of structures in the dentin of primary teeth analyzed. Primary curvature of dentinal tubules was present in 50% of the single-rooted teeth, though this characteristic was not observed in the incisal region of these teeth. Tubular branches were observed in both the coronary and the radicular dentin, along the peripheral region of the dentin as well as in the tubular arrangement (Figures 2 and 5).

A higher occurrence of dead tracts in dentin was seen in

Table 2. Distribution of the dentin structures in primary teeth.

Structures	Teeth (n)					
	Single-rooted			Multi-rooted		
	N*	n	%	N*	n	%
Primary curvature of tubules	8	4	50	9	6	66
Dead tracts of dentin	9	7	77	9	2	22
Interglobular dentin	9	0	0	9	2	22
Incremental lines	8	4	50	9	3	33
Granular layer of Tomes	8	7	87	8	7	87

* Teeth number analyzed

single-rooted teeth, especially in the incisal region of these teeth (77% of the samples). Areas of tertiary dentin were observed in incisal and occlusal regions of pulp chambers, especially in the pulp horns. In single-rooted teeth, normally these regions of tertiary dentin were related to the wear of adjacent enamel and/or dentin. They were characterized by a less tubular pattern as compared to the remaining dentin tissue (Figure 4).

Interglobular dentin was not observed in single-rooted primary teeth, while in molars the frequency of the event was low (22%), observed only in the occlusal region.

The presence of incremental lines in dentin was 33% and 50% in multi-rooted and single-rooted teeth, respectively. As regards root dentin, both dental types presented the granular layer of Tomes in almost all samples analyzed (87%) (Figure 5).

About the distribution of cellular and acellular cementum areas, the latter was observed more often in more cervical areas towards the root (Table 3). This distribution of cementum types did not present statistically significant difference either in single-rooted ($P>0.05$; chi-square test) or in multi-rooted teeth ($P>0.05$, chi-square test).

In the cervical region, the cementum presented the acellular pattern (Figure 5). A few cementocyte lacunae were observed in the cellular cementum, in the middle and apical thirds of roots (Figure 6). The frequency of incremental lines in the cementum was low (Table 3).

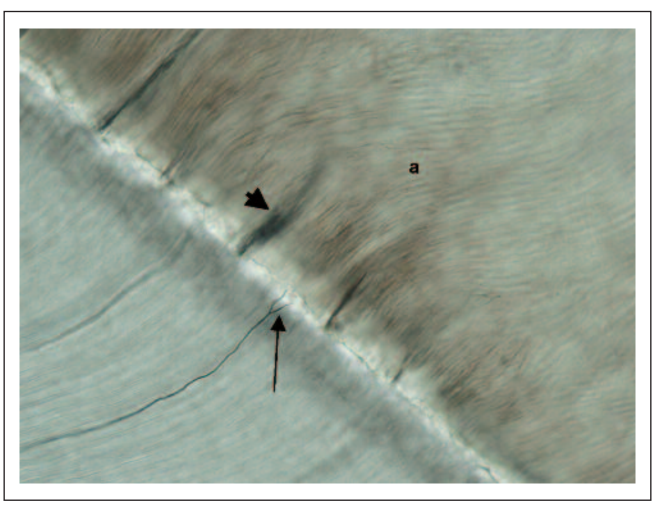

Figure 2. Dentinoenamel junction with tufts in the enamel (short arrow). Undulated pattern of rod arrangement (a). Dentinal tubule with terminal ramification (long arrow). (400 x; TGT; LM)

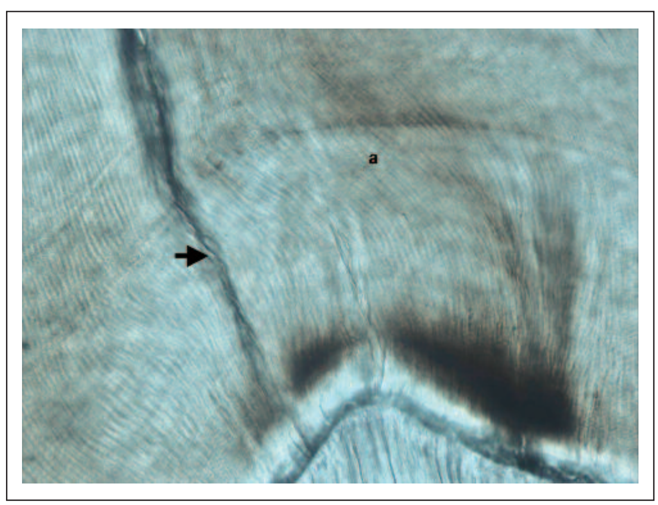

Figure 3. Cusp presenting the gnarled enamel characterized by the undulated rod arrangement (a). One lamella that reaches the dentinoenamel junction is also observed in the region (arrow). (400 x; TGT; LM).

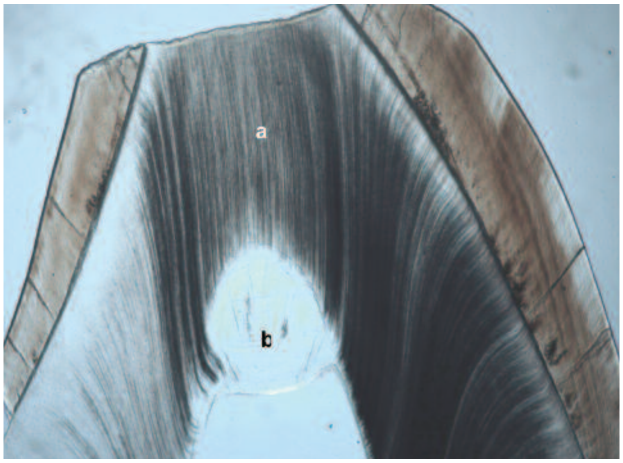

Figure 4. Wear of the cusp with dead tracts in dentin (a) and tertiary dentin (t). (40x; TGT; LM)

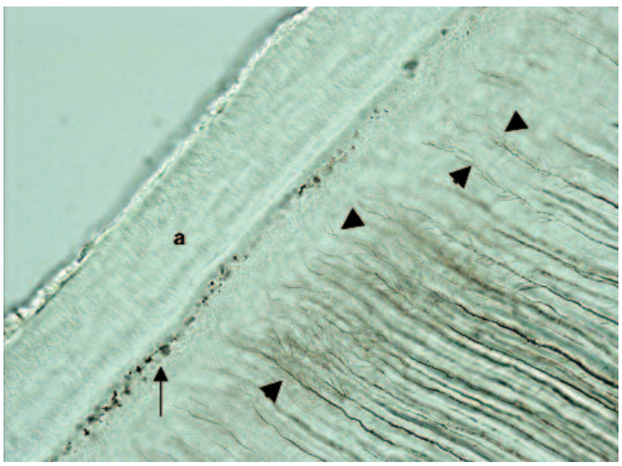

Figure 5. Granular layer of Tomes in the periphery of the root dentin (long arrow) adjacent to the acellular cementum (a). Tubule branches are present in the tubules of the adjacent dentin (short arrows). (400 x; TGT; LM)

Table 3. Distribution of the cementum structures in primary teeth.

Structures	Teeth (n)					
	Single-rooted			Multi-rooted		
	N*	n	%	N*	n	%
Cellular cementum	9	6	66	8	4	50
Acellular cementum	9	8	89	8	7	87
Incremental lines	9	1	11	8	2	25

* Teeth number analyzed

DISCUSSION

In spite of the comprehensive literature on the histological aspects of mineralized hard dental tissues, few studies have assessed the distribution of these structures in primary teeth. Because of that, the present study aimed to contribute on the investigation of the histological aspects related to the microstructure of mineralized tissues of primary teeth.

Considering the enamel, it is important to point out the high prevalence of areas of increased organic nature, as lamellae, spindles and tufts, especially in the occlusal surface of primary molars. The presence of these structures in the occlusal region may be linked to the higher complexity in macromorphology of the enamel in this area. It is important to emphasize the likely contribution of these hypomineralized structures on the occlusal surface to the higher carious development velocity when reaching these regions. Nevertheless, for Eisenmann although lamellae and tuft displayed higher protein contents as compared to the remaining enamel structures, they do not seem to represent sites of greater vulnerability to dental caries.[16]

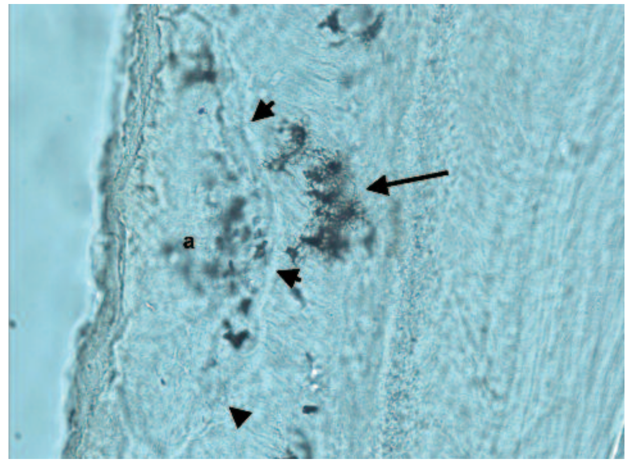

Figure 6. Cementocyte lacunae with canaliculi in the cellular cementum in the apical root region (long arrow). Reversion line (short arrows) indicates a previous resorption area repaired by the deposition of new cementum (a). (400x; TGT; LM).

The presence of lamellae on the occlusal surfaces and on the incisal edge may be a defect in the formation of the tissue, and also to the action of masticatory forces over an erupted tooth. In this case, these lamellae would represent cracks on the tissue surface.[17] The present study demonstrated the high prevalence of this structure on occlusal surfaces of primary molars, which are subject to more intense forces during mastication, as compared to the other surfaces analyzed on these teeth. The cracks on the occlusal surface were observed even when gnarled enamel were seen on the same side, which intercrossed rod pattern is a factor that give more resistance against masticatory impacts.[17] Therefore, these higher fragility may be explained based on the lesser thickness and degree of mineralization of the enamel on primary teeth.[4,28]

It is possible to question the absence of the bands of Hunter and Schreger on the teeth analyzed. These structures are an optical phenomenon characterized by the presence of light and dark bands on the structure of the tissue, caused by the change in the arrangement of rod groups. It is possible that this histological finding is related to the shorter development times presented by primary teeth, as compared to permanent teeth.[4,28] This shorter development period may determine the development of a more homogeneous rod arrangement, differently from that was observed in bands.

The enamel of the teeth analyzed presented one stria of Retzius markedly observable in this tissue, which suggests that it is in fact the neonatal line. Higher striae frequencies were observed outside this neonatal line, which gave the prenatal enamel a more homogenous development pattern and also presented less structural defects than the postnatal enamel. These characteristics have also been reported in other studies [18,19].

Although the literature describes the dentinoenamel junction as an usually scalloped contact surface,[15,20] a smooth aspect was also observed in the primary teeth analyzed. This smooth surface has been related to lateral dental surfaces and the scalloped pattern has been linked to regions that suffer greater masticatory forces, as seen below the cusp and the incisal edges.[29] It is important to underline the observation that this smooth pattern was present in over half the number of single-rooted teeth, in all sides analyzed. Further studies are needed to understand this smooth pattern of the contact surface between enamel and dentin, as observed in primary teeth.

As regards the dentin tissue, the teeth analyzed presented tubules arranged under different directions. Branches of tubules were also observed. These characteristics are common not only in primary but also in permanent teeth.[20,21,30,31] Straight tubules were seen in the occlusal surfaces and incisal edges, whereas curvatures were more commonly observed on the other dental surfaces. This tubule distribution pattern is commonly described in the literature and represents the pathway that odontoblasts take when building up on the dentin tissue.[20]

Dead tracts in dentin were often observed in the incisal region of single-rooted teeth. The wearing process of this region may have favored this dentin response, as well as the formation of tertiary dentin on the region under it. Klinge also observed the development of tertiary dentin with smaller number of tubules in areas of the pulp horns under the region dentin wears away.[32] A well defined limit between physiological dentin and tertiary dentin was observed in primary teeth analyzed in this research. Klinge also reports the presence of this well clear limit between the dentinal tissues.[25]

Moreover, concerning the dentin of primary teeth, low prevalence of interglobular spaces was observed. The literature describes the presence of these spaces in the central zone of the coronary dentin in primary incisives.[5,11,26] Since these areas are described as hypomineralized,[20,21] their low occurrence may indicate a more homogeneous pattern of mineralization of the dentin matrix in primary teeth. Nevertheless, in spite of this likely uniformity in tissue mineralization, the literature describes a lower degree of mineralization in primary teeth as compared to permanent teeth.[22,23]

A lower frequency of incremental lines, the presence of acellular cementum on the cervical region and of cellular cementum on the middle and apical root region are in agreement with the pattern described in primary teeth by Furseth.[1] However, the cellular cementum seemed to have a small number of cementocyte lacunae, in comparison to what is normally observed in permanent teeth. A more detailed investigation is required in order to compare the development of this tissue in primary and permanent teeth.

In spite of the similarities in permanent and primary teeth, some peculiarities were observed in the present study as regards the mineralized hard tissues in primary teeth. These characteristics and the clinical repercussions should be better investigated. For example, future studies are warranted to assess whether the histological patterns observed could affect the interaction and adhesion of restorative materials in these substrates, e.g. due to the high number of hypomineralized areas in the enamel. Such knowledge could in turn benefit the dental industry in the sense of developing and improving materials specifically designed for this type of tissue. Therefore, original studies will offer greater support to the pediatric dentist in the conduction of its clinical practice.

CONCLUSIONS

The present study concluded that primary teeth present some histological peculiarities:

a) the presence of areas with greater protein content - tufts, spindles and lamellae - was consistently observed in the enamel;
b) the dentinoenamel junction presented a non-scalloped pattern in more than half of the single-rooted teeth analyzed;
c) hypomineralized areas in dentin were seldom observed;
d) tertiary dentin was detected in regions of cusps and incisal edges in association to dental wear;
e) cellular cementum was observed to have few cementocyte lacunae.

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