A Study of the Microstructure of Mineralised Tissues in 50 Human Primary Teeth

Abstract
Background: Mineralised hard tissues in human primary teeth have some peculiarities, in spite of similarities in permanent and primary teeth. Aims and Objectives: To analyse structural and histological features of mineralised tissues in human primary teeth and their correlation with single-rooted and multi-rooted teeth. Materials and Methods: Study comprises of 50 human primary non-carious teeth which were sectioned by ground technique into two samples from each tooth. Single rooted teeth were sectioned in facio-lingual direction and multi-rooted teeth in mesio-distal direction. Sectioned primary teeth were examined under light and polarised microscope to analyse mineralised microstructure in human primary teeth. Results: Tufts, spindles, lamellae were constantly present in dental samples. Low prevalence of Hunter Schreger bands was observed. Non-scalloped dentino-enamel junction and edge to edge cemento-enamel junction are commonly present in primary teeth. Interglobular dentin was not present in all primary teeth. Dead tracts were mainly observed in single-rooted teeth below areas of dental attrition. Areas of cellular and acellular cementum were observed in two dental types. Conclusion: Human primary teeth have some microstructural variation as compared to established structure of permanent teeth and are better observed in polarised microscopy.

Keywords: Cementum; Dentin; Enamel; Microstructures; Mineralised Tissues; Primary Teeth.

Introduction
In spite of much literature on the histological aspects of mineralised hard dental tissues, few studies have been carried out to assess the distribution of these structures in primary teeth. Tooth enamel is the most mineralised tissue in the human body and enamel rods are its main structural entity. Enamel matrix is deposited in layers which is demarcated by Striae of Retzius.1 When ameloblasts are subjected to internal or external noxious stimuli, rhythmic enamel matrix formation gets altered leading to its deposition in layers called striae of Retzius. One such accentuated Retzius line corresponds to the event of birth and is known as neonatal line.2

The outermost layer of enamel is usually devoid of prism structure in primary teeth.3 Enamel tufts, spindles, lamellae and Hunter-Schreger bands are the other structures present in enamel. Hunter-Schreger bands are not the prominent feature in primary teeth when seen at light microscopic level. The prisms are gently curved in comparison with those of permanent enamel in which Hunter-Schreger banding was always pronounced in inner two-third to three-quarter of the tissue.4 Dentinal tubules are most prominent features of dentin and they are less dense and narrower at the dentino-enamel junction and become more dense and wider near the pulp.5,6

Dead tracts present in dentin are observed as dark zones in undemineralized tooth sections when viewed under light microscope due to presence of air caused by retraction or degeneration of odontoblasts in dentinal tubules by external stimuli.1 Secondary dentin is formed as a result of normal physiologic stimuli while tertiary or reparative dentin is formed due to pathologic process such as caries, attrition and restorative procedures.5

In the roots of teeth, a granular layer is present adjacent to cemento-enamel junction called as Tome’s granular layer which is the result of either small hypomineralized areas of dentin or small entrapped spaces that form around tubules.7 When observed under polarised light, alternate translucent and opaque bands are observed in cementum due to rhythmic deposition of matrix in the form of layers.8 The cementum may be classified as cellular and acellular, in terms of presence or absence of cells inside its matrix, respectively. Incremental lines are not frequently seen or may even be absent, in the cellular cementum of primary teeth.1

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Original Article

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The understanding of these histological aspects of teeth is of great importance to improve materials and technique of clinical dentistry. Therefore, present study analyses the structural and histological features of mineralised tissues in human primary teeth and their correlation with single-rooted and multi-rooted teeth.

Materials and Methods
Fifty human non-carious primary teeth were extracted at various stages of root resorption and used in the present study (24 Single-rooted and 26 multi-rooted). Single rooted teeth were longitudinally sectioned in facio-lingual direction, while multi-rooted teeth were sectioned in mesio-distal direction using carborundum abrasive stone (course and fine). Each tooth was divided into two samples. The two samples were manually worked in order to reduce thickness to 60-70 µm. After the desired thickness was obtained, samples were washed in distilled water, dried and later histological slides were mounted with Dystrene Polystrene Xylene (DPX) topped with cover slips for the light microscope analysis.

Analysis: Samples were inspected under 2.5X, 5X, 10X and 40X magnifications using a binocular light and polarized microscope. All surfaces of the samples were analyzed and presence of histological structures was recorded. Quantitative and descriptive analysis of morphology of mineralised tissues was done and statistically analysed using Z-test. Statistical significant difference was considered if p value <0.05.

Results
Samples were analysed for different aspects of each tooth and observations were noted accordingly. The root structures were not analysed at greater extent as some samples were with root resorption. Distribution of microstructures in the enamel, dentin and cementum of primary teeth were statistically analyzed. Results and statistical significance were noted as shown in Table 1 (Figure 1 & 2). Dentino-enamel junction presented with straight / non-scalloped form in 95.83% of the single rooted and all of multi-rooted teeth (Figure 1b). Enamel tufts (Figure 3a), and spindles were observed in occlusal surfaces of all multi-rooted teeth. Neonatal line was not observed in all primary teeth (Figure 3b) and was presenting most frequently internal to the striae of Retzius. Hunter-Schreger bands (Figure 3c) were observed in 61.53% multi-rooted teeth than 33.33% single rooted teeth which showed significant difference.
Incremental lines of Retzius were constantly observed in 80% of dental samples. Enamel lamellae (Figure 4a) were also observed in occlusal surface of all multi-rooted teeth. Incremental lines of dentin were also found in three samples. In root dentin, both dental types presented the Tome's granular layer (Figure 4b) in almost all samples analysed. Dead tracts (Figure 4c) were most often present in single rooted teeth (75%) especially in the incisal region of these teeth \( (p \text{ value } < 0.05, \ Z \text{ test}) \). 'Y' shaped tubules (Figure 4a) were found to be present more often in 70.83% single rooted teeth than 30.76% multi-rooted teeth \( (p \text{ value } < 0.05, \ Z \text{ test}) \). Areas of tertiary dentin (Figure 3b) were observed in incisal and occlusal regions of pulp chamber especially in the teeth with dental wear. Interglobular dentin (Figure 4a) was not present in all primary teeth and its frequency was more in multi-rooted teeth (50%) than in single rooted teeth (29.16%).

In the cervical region, few cementocytic lacunae were observed in cellular cementum (Figure 5a & b). Acellular cementum (Figure 5c) was present in almost all teeth while in middle and apical third, cellular cementum was found to be present. Most common type of cemento-enamel junction observed was edge to edge followed by overlapping and then gap junction (Figure 2c). Thickness of cellular cementum was increasing from cervical to apical region.

**Discussion**

In spite of the similar features in permanent and primary teeth, some peculiar characteristics were observed in the present study as regards to the mineralised hard tissues in primary teeth. As enamel and dentin have inherent crystalline nature, their histological features are better visualized under polarized microscopy than transmitted...
light microscopy because some structures such as crystals and collagen fibres have property to split a polarised light into two, called birefringence.\textsuperscript{12}

<table>
<thead>
<tr>
<th>Teeth Structure</th>
<th>Single rooted (N = 24)</th>
<th>Multi-rooted (N = 26)</th>
<th>p-value (z test)</th>
<th>Interpretation</th>
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Table 1 Distribution of microstructures in primary teeth
* Significant difference if \( p \) value < 0.05, N = Total number of teeth analyzed, \( n \) = Number of teeth with findings, \% = Percentage distribution of structures, NS = Not significant, S = Significant, Mixed = Overlapping Cemento-Enamel junction at one side and edge to edge at other side.

Hypo mineralised structures of enamel such as lamellae, tufts and spindles showed high prevalence in primary molars especially in their occlusal surfaces which can be linked to caries susceptible areas.\textsuperscript{1} In the present study spindles and tufts were also commonly observed mainly in multi-rooted teeth in comparison to single rooted teeth and most commonly observed in occlusal region of the teeth.

The presence of lamellae on occlusal surfaces and on incisal edges may be a defect in formation of the tissue due to the action of masticatory forces over erupted tooth. These lamellae would manifest as cracks on the tissue surfaces. The intercrossed rod pattern of gnarled enamel provides more resistance against masticatory impacts. However, cracks on the occlusal surface were observed despite the presence of gnarled enamel on the same side.\textsuperscript{9} We observed a statistically significant difference in presence of enamel lamellae in multi-rooted teeth and single rooted teeth, showing a greater prevalence in the occlusal region of multi-rooted teeth.

Hunter-Schreger bands are an optical phenomenon produced by changes in direction of the enamel rods. These bands are most commonly observed in the inner two-thirds of the enamel. These bands appear as dark and light alternating zones that can be reversed by altering the direction of incident illumination. They are not seen
clearly in longitudinal ground sections when viewed under reflected light. Therefore, the teeth were analyzed in both light and polarised microscope. Our study showed that there were low prevalence of Hunter-Schreger bands in primary teeth and are more frequently present in multi-rooted teeth than in single rooted teeth and were better appreciated in polarised microscope. These Hunter Schreger bands may be result of ameloblasts displacement by pressures exerted on them during amelogenesis and has developed as a consequence of an evolutionary process. The possible reason for preponderance of these bands in multi-rooted teeth is their evolutionary or functional adaptation to the considerable loading forces during articulation and mastication.

The ‘S’ shaped dentinal tubules are the characteristic feature of permanent teeth. However, in primary molars, straight course of dentinal tubules were found at greater prevalence. This structural appearance in primary molars may contribute to faster progression of caries. Thickness of the coronary dentin is smaller while pulpal chamber is bigger in primary teeth when compared to permanent teeth. We also observed straight course of dentinal tubules in primary molars predominantly which may be due to less crowding of the odontoblasts. The possible reason for this crowding could be the lower difference between the surface area of dentin near dentino-enamel junction and that near the pulp. Y' shaped tubules also showed significant difference in multi-rooted teeth and single rooted teeth. They were mostly found in single rooted teeth. Dead tracts are empty areas in non-functioning dentinal tubules. These are result of odontoblasts being injured sufficiently to inhibit a defence response. When ground sections were examined under light microscope, they appear dark in transmitted light and white in reflected light. Dead tracts usually circumscribed by areas of reparative dentin or sclerotic dentin or both. High prevalence of reparative dentin was observed under attrition than caries and restoration which may be due to slow and progressive loss of dentinal tissue. Reparative dentin was frequently present in relation to dead tracts and attrition simultaneously.

In deciduous teeth, the types of CEJ were different from that of permanent teeth in that enamel and cementum meeting edge to edge the commonest type, followed by cementum overlapping enamel and occasionally scalloped which is in accordance with the other studies and literature. Dreyfuss and Frank (1964) also stated that cellular cementum is usually lamellar. Cellular cementum is frequently formed on the surface of acellular cementum, but it may comprise the entire thickness of apical cementum. Both acellular and cellular cementum are separated by incremental lines into layers which indicate periodic formation. In cellular cementum, cementocyte lacunae were found to be less in number as compared to that in permanent teeth which is in accordance with other studies. A more detailed investigation is required to assess the cementum and dentin of root portion of the teeth as they couldn't...
be studied to complete extent due to root resorption in few of the samples.

This study provides us information regarding various microstructures which are present in human primary teeth with greater sample size which may help in further systematic review and also in the field of forensic of forensic odontology. In our study, hypomineralized microstructures in multi-rooted teeth predominantly occur in occlusal region. As caries spreads progressively through these microstructures, a thorough knowledge of the same shall enable us not only to restore carious teeth with innovative materials, but also prevent progression of secondary caries. Also, the characteristics and properties of enamel, dentin and cementum are the key determinants of nearly all restorative, preventive and disease processes of the teeth. Hence, improved understanding of their structure and nature will have important consequences for today’s dental procedures.

**Conclusion**

It can be concluded that human primary teeth have some microstructural variations as compared to permanent teeth and mineralized structure of hard dental tissue are better observed in polarized microscopy in comparison to light microscopy. Enamel spindles and Hunter-Schreger bands were significantly more in multi-rooted teeth as compared to the single rooted, while Y-shaped tubules and dead tracts were significantly greater in single rooted teeth than multi-rooted teeth. Dentinal tubules showed predominantly straight course in primary teeth as compared to S-tubules in permanent teeth. Edge to edge CEJ was more common in primary teeth which are in contrary to the overlapping CEJ in permanent teeth. Thus, primary teeth have some peculiar characteristics which should be investigated further to facilitate better clinical implications.

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