Enamel Surface Morphology: An Ultrastructural Comparative Study of Anterior and Posterior Permanent Teeth

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Abstract

Introduction: Enamel is one of the most important structures of the tooth, both functionally and aesthetically. Because of the highly mineralized nature of enamel, its structure is difficult to study under routine light microscopy. Since scanning electron microscopy (SEM) offers the possibility of studying structures under very high magnification without altering the gross specimen, it is one of the best methods to study the enamel surface. **Aims and Objectives:** To study and compare the surface morphology and morphometry of enamel on various surfaces of permanent dentition. **Materials and Methods:** A total of 20 permanent teeth were analyzed under the SEM. In both anterior and posterior teeth, four surfaces – mesial, distal, labial, and lingual – in three thirds – cervical, middle, and incisal – were studied. In addition, the occlusal surface was also studied for the posterior teeth. The different prism morphology and prism dimensions were recorded. **Results:** Based on our observations, we could definitely identify striae of retzius, debris, and cracks under ×50 magnification. Three morphological patterns of prism arrangement were identified: Type 1 - shallow prisms, Type 2 - well-defined prisms, Type 3 - microporosities, on analyzing the mesial, distal, labial, lingual, and occlusal surfaces of the permanent teeth, at ×3000 magnification. The prisms were measured under ×6000 magnification and the results showed larger prisms in posterior than in anterior teeth. **Conclusion:** The study aided us in categorizing the enamel prism structure based on morphology and morphometry in anterior and posterior teeth of the permanent dentition.

Keywords: Dental enamel, enamel prism, scanning electron microscope, morphology, morphometry

Introduction

Enamel is the hardest calcified matrix of the body and is the most important structure of the tooth.[1-4] Because of the highly mineralized nature of enamel, the conventional demineralized sections reveal only an empty space in areas previously occupied by mature enamel, thus making the study of enamel difficult routinely. One of the best ways to study and understand this structure and difference is by scanning electron microscopy (SEM) as it offers a three-dimensional picture of the enamel and thus helps in its better understanding. Enamel rods are the structural and fundamental units of enamel, and thus, detailed study about it is needed.[2] In literature, although there is overall information about the ultrastructure of the enamel, not much is known about the variations and differences in its structure on different surfaces of permanent teeth. Hence, the present study is undertaken to use it as an approach to determine and compare the morphological patterns of the enamel in anterior and posterior teeth of permanent dentition, with the aim of understanding the clinical implications.

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Materials and Methods

A total of 20 permanent extracted teeth were studied. Out of which, 8 anterior teeth extracted due to compromised periodontal status and 12 posterior teeth extracted for therapeutic purpose were collected. Four surfaces (mesial, distal, labial, and lingual) in three thirds (cervical, middle, and incisal) were studied for 16 tooth samples (8 each of anterior and posterior), and in addition, the occlusal surface was also studied for four posterior teeth [Figure 1]. These teeth were cleaned of blood using saline solution. The soft tissue and calculus were removed manually using hand scalers and stored in 10% formalin solution.

Each tooth was sectioned to study the enamel structure on different surfaces. Using a low-speed diamond disc

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(40,000–60,000 rpm), under copious irrigation, the teeth were sectioned labiolingually to study the mesial and distal surfaces and also sectioned mesiodistally to study the labial and lingual surfaces. For posterior teeth, a cross-section through the middle third of the crown was done to study the occlusal surface.

The teeth were then coded (based on the position of the tooth in the arch, i.e., anterior or posterior, the serial number, and the surface studied, i.e., mesial/distal/labial/lingual/occlusal) and transferred to a sterile container to be taken to the SEM center for analysis. Smear layer removal was done just before mounting the specimen by agitating the teeth in 2.5% sodium hypochlorite solution for 30 s and then sprayed with the same using 5 ml disposable Dispovan syringe. The prepared sample was then fixed to the aluminum stub using double-sided carbon tape and studied under the Zeiss SEM (EVOLS 15).

Analysis done for whole tooth image was captured at ×50 magnification for all the surfaces, i.e., mesial, distal, labial, lingual, and occlusal. Then, the magnification was increased to ×3000, and the morphology of the enamel prisms was analyzed on all the surfaces. The magnification was further increased to ×6000, and the greatest dimension of enamel prisms was measured. For the analysis under ×3000 and ×6000, all the teeth were divided horizontally into cervical, middle, and incisal thirds on all the surfaces. However, for the proximal surfaces, in addition, a vertical division was also done, dividing the tooth into labial and lingual halves, which were further analyzed. The occlusal surface of only posterior teeth was studied by dividing it in a horizontal direction mesiodistally into facial, middle, and lingual thirds. The results were statistically analyzed using SPSS 20, (IBM, Armonk, NY, United States of America) using descriptive statistics.

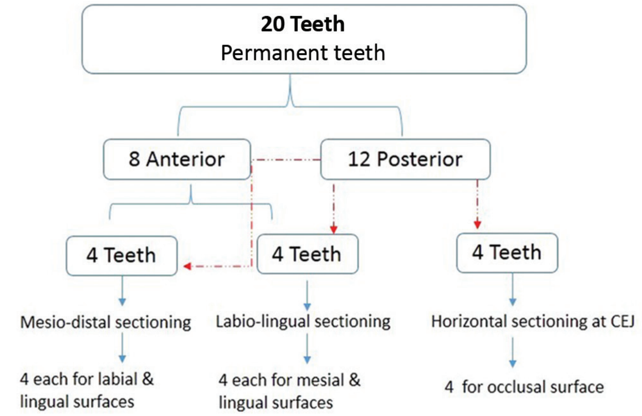

Figure 1: Flowchart showing the study design

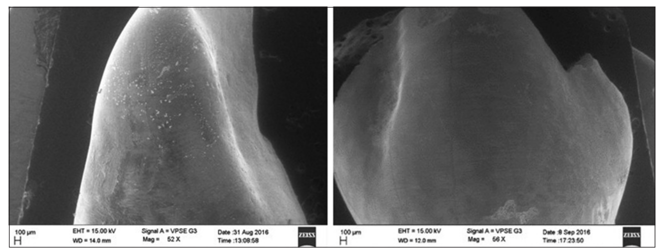

Figure 2: Scanning electron microscopy image of tooth showing the straie of retzius (×50)

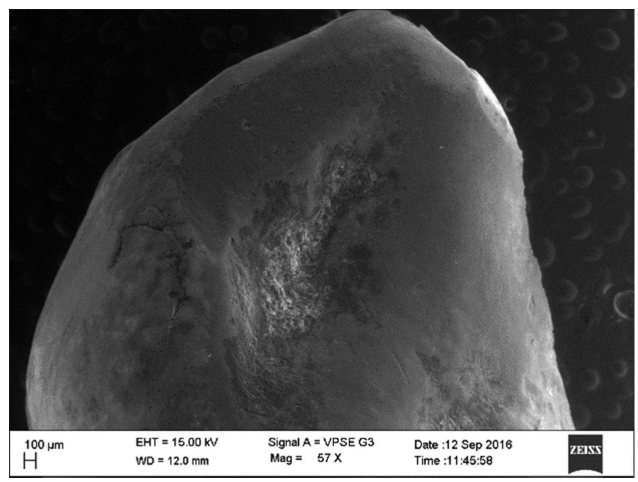

Figure 3: Scanning electron microscopy image of tooth showing debris accumulated on its surface (×50)

Results

The results were recorded with respect to whole tooth under ×50 magnification, pattern of enamel prisms under ×3000 magnification, and morphometry of the enamel prisms under ×6000 magnification. Under ×50 magnification, all the teeth showed straie of retzius [Figure 2], few teeth were masked focally with smear layer and debris [Figures 3 and 4], and few showed cracks on their surface. Three different morphological patterns of enamel prisms – Type 1: Shallow prisms, Type 2: well-defined prisms, Type 3: Prisms with microporosity – were found when viewed at ×3000 magnification [Figure 5].

The most important finding of our study was the presence of Type 2, well-defined prism pattern predominantly in both permanent anterior and posterior teeth.

On the mesial and distal surfaces, Type 2, well-defined prism pattern was seen predominantly in both permanent anterior and posterior teeth, except for incisal third of mesial surface of posteriors which showed predominant Type 1, shallow prism pattern [Table 1].

The incisal third of distal surface showed predominant Type 1, shallow prism pattern in the anterior teeth [Table 2].

The labial surface of anterior teeth showed equal distribution between Type 1 and 2 prism patterns, i.e., shallow and well-defined prisms, respectively, and posterior teeth showed predominant Type 2, well-defined prism pattern [Table 3].

On the contrary, the lingual surface of anterior teeth showed predominant Type 1 shallow prism pattern and posterior teeth showed predominant Type 2 prism pattern [Table 4].

Occlusal surface of posteriors showed predominant Type 2 prism pattern [Table 5].

On an average, the anterior teeth showed slightly smaller prisms (5.0 µm) compared to posterior teeth (5.35 µm) upon morphometry at ×6000 magnification [Table 6].

Discussion

Enamel is a nonvital, noncellular, and the hardest highly mineralized dental tissue, which forms the protective outermost layer of the tooth.[2] The surface enamel is composed of enamel prisms or rods, rod sheaths, and cementing interprismatic substance. The enamel prisms or rods are the basic structural component of enamel. They originate at the dentino-enamel junction and extend through the thickness of the enamel surface. The prisms appear cylindrical in longitudinal sections and take up key-hole or fish-scale pattern in cross-sections.[3,4] Since many features of enamel rods are below the limit of resolution of the light microscope, many questions concerning their morphology can only be answered by electron microscopy. Thus, SEM is one of the best methods to study the enamel surface. In electron micrographs, the surface of the rods is visible because of abrupt changes in crystal orientation from one rod to another.[5] The present study was undertaken to better understand the morphology of the basic building blocks, the enamel prisms in permanent dentition. Further the anterior and posteriors teeth, samples were considered to evaluate any differences between the groups. All the teeth were studied under SEM at three different magnifications. The teeth when viewed under ×50 magnification showed striae of retzius on all the surfaces of all the teeth. Few teeth were masked with smear layer and few with debris in focal areas, which might have been due to improper smear layer removal and dust

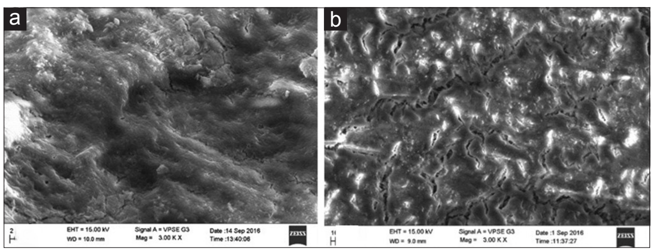

Figure 4: Scanning electron microscopy image of sample with (a) debris, and (b) dust particles on its surface (×3000)

Table 1: Distribution of prism patterns on the mesial surface of permanent teeth in cervical, middle, and incisal thirds

Position	Pattern type	Groups	
		Permanent anterior, frequency (%)	Permanent posterior, frequency (%)
Cervical	Shallow	1 (25.0)	0 (0.0)
	Well-defined	3 (75.0)	4 (100.0)
	Microporosity	0 (0.0)	0 (0.0)
Middle	Shallow	0 (0.0)	1 (25.0)
	Well-defined	4 (100.0)	3 (75.0)
	Microporosity	0 (0.0)	0 (0.0)
Incisal	Shallow	1 (25.0)	3 (75.0)
	Well-defined	3 (75.0)	1 (25.0)
	Microporosity	0 (0.0)	0 (0.0)

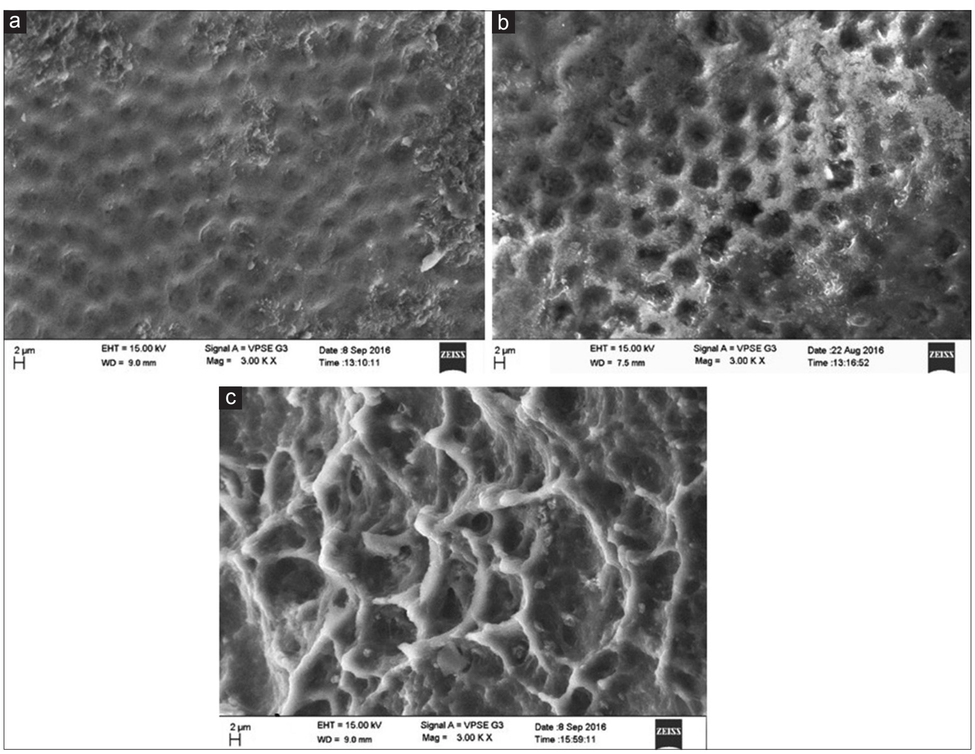

Figure 5: Scanning electron microscopy images of types of enamel prisms (a) shallow prisms, (b) well-defined prisms, and (c) prisms with microporosities

Table 2: Distribution of prism patterns on the distal surface of permanent teeth in cervical, middle, and incisal thirds

Position	Pattern type	Groups	
		Permanent anterior, frequency (%)	Permanent posterior, frequency (%)
Cervical	Shallow	1 (25.0)	1 (25.0)
	Well-defined	3 (75.0)	3 (75.0)
	Microporosity	0 (0.0)	0 (0.0)
Middle	Shallow	1 (25.0)	0 (0.0)
	Well-defined	3 (75.0)	4 (100.0)
	Microporosity	0 (0.0)	0 (0.0)
Incisal	Shallow	3 (75.0)	0 (0.0)
	Well-defined	1 (25.0)	4 (100.0)
	Microporosity	0 (0.0)	0 (0.0)

Table 3: Distribution of prism patterns on the labial surface of permanent teeth in cervical, middle, and incisal thirds

Position	Pattern type	Groups	
		Permanent anterior, frequency (%)	Permanent posterior, frequency (%)
Cervical	Shallow	2 (50.0)	1 (25.0)
	Well-defined	2 (50.0)	3 (75.0)
	Microporosity	0 (0.0)	0 (0.0)
Middle	Shallow	2 (50.0)	0 (0.0)
	Well-defined	2 (50.0)	4 (100.0)
	Microporosity	0 (0.0)	0 (0.0)
Incisal	Shallow	2 (50.0)	1 (25.0)
	Well-defined	2 (50.0)	3 (75.0)
	Microporosity	0 (0.0)	0 (0.0)

deposition respectively. Few of the anterior teeth revealed cracks and fracture lines on their surface.

According to a number of literature sources, there are various forms of prisms and a variety of options regarding their orientation to each other. A prism head can appear above the enamel surface, lie on the same level with slight contouring, or be a cavity, thus forming different patterns or forms.[6]

According to a study done by Zamudio-Ortega *et al*., on morphology, chemical composition, structure, and crystalline phases of deciduous enamel, they observed three patterns when examined by SEM. Majority of the prisms were smooth with some grooves, and few showed abundant microporosities. Another secondary pattern seen only in the incisal third was the exposed prisms.[7]

In our study, we found three different morphological patterns (Type 1–3) of enamel prisms on analyzing the mesial, distal, labial, lingual, and occlusal surfaces of permanent anterior and posterior teeth when viewed at ×3000 magnification. Type 1 - shallow prisms: They are smoothly rounded prisms arranged uniformly on the surface with

Table 4: Distribution of prism patterns on the lingual surface of permanent teeth in cervical, middle, and incisal thirds

Position	Pattern type	Groups	
		Permanent anterior, frequency (%)	Permanent posterior, frequency (%)
Cervical	Shallow	2 (50.0)	2 (50.0)
	Well-defined	2 (50.0)	1 (25.0)
	Microporosity	0 (0.0)	1 (25.0)
Middle	Shallow	3 (75.0)	1 (25.0)
	Well-defined	1 (25.0)	2 (50.0)
	Microporosity	0 (0.0)	1 (25.0)
Incisal	Shallow	3 (75.0)	2 (50.0)
	Well-defined	1 (25.0)	1 (25.0)
	Microporosity	0 (0.0)	1 (25.0)

Table 5: Distribution of prism patterns on the occlusal surface of permanent teeth in labial, middle and lingual thirds

Position	Pattern type	Permanent posterior, frequency (%)
Labial	Shallow	2 (50.0)
	Well-defined	2 (50.0)
	Microporosity	0 (0.0)
Middle	Shallow	0 (0.0)
	Well-defined	4 (100.0)
	Microporosity	4 (100.0)
Lingual	Shallow	0 (0.0)
	Well-defined	4 (100.0)
	Microporosity	4 (100.0)

delineated prism outlines. Their surface is in the level with the tooth surface. Type 2 - well-defined prisms: The prisms are uniformly arranged having open concave centers with well-defined peripheral prism outlines and their surfaces also in level of the tooth surface. Type 3 - microporosities: Ill-defined irregular, large prisms, having a wide central depression and irregular peripheral borders.

In our study, on an average, the permanent teeth showed well-defined prism pattern, followed by shallow prism pattern. However, we could not establish any clinical correlation with the prism pattern in the permanent teeth. Electron microscopic studies have recorded the average diameter of the enamel prisms or rods to be about 4–7 μm.[7,8] However, the literature describing in detail regarding the enamel prism dimension on different teeth (anterior and posterior) are few. A study conducted by De Menezes Oliveira *et al*. has shown the mean rod diameter to be 3.22–3.47 μm for deciduous teeth and 3.84 μm to 4.34 μm for the permanent teeth.[9] However, higher diameters were recorded for permanent teeth in another study, wherein the prism diameter was 10 μm at the outer enamel surface.[5,10] However, lower measurements of 2.9 μm (±1.2 μm) were recorded for primary teeth.[2,11] An extensive literature search did not reveal any study regarding

Table 6: Morphometry of enamel prisms of permanent teeth in the cervical, middle, and incisal thirds on all the surfaces at ×6000 magnification

Surface_position	Groups	Mean	SD
Mesial_cervical	Permanent anterior	5.5750	1.08436
	Permanent posterior	4.7000	1.01325
Mesial_middle	Permanent anterior	4.2750	1.49081
	Permanent posterior	5.2000	0.93808
Mesial_incisal	Permanent anterior	5.3000	0.92014
	Permanent posterior	4.1500	0.80623
Distal_cervical	Permanent anterior	4.7750	0.47170
	Permanent posterior	5.6250	0.78899
Distal_middle	Permanent anterior	4.6750	0.94296
	Permanent posterior	5.1000	0.83666
Distal_incisal	Permanent anterior	5.3250	1.04682
	Permanent posterior	5.6000	0.25820
Labial_cervical	Permanent anterior	4.9250	1.44539
	Permanent posterior	6.1750	1.04682
Labial_middle	Permanent anterior	5.4000	1.81842
	Permanent posterior	5.5500	0.34157
Labial_incisal	Permanent anterior	5.7000	1.13725
	Permanent posterior	5.0750	0.53151
Lingual_cervical	Permanent anterior	5.2500	1.57797
	Permanent posterior	4.8750	2.04185
Lingual_middle	Permanent anterior	4.8500	1.23962
	Permanent posterior	5.9000	0.45461
Lingual_incisal	Permanent anterior	4.4000	0.84459
	Permanent posterior	5.2250	1.65404
Occlusal_cervical	Permanent posterior	5.7000	0.47610
Occlusal_middle	Permanent posterior	5.4750	0.45735
Occlusal_incisal	Permanent posterior	5.9250	0.40311

SD: Standard deviation

the comparison of measurements of enamel prisms between anterior and posterior teeth or between the different thirds of the tooth in a horizontal direction. In our study, the enamel prisms or rod diameter was measured and compared between all the surfaces and in all the thirds of the anterior and posterior teeth at ×6000 magnification. The range of prism dimension that was measured varied from 4.8 to 7.7 µm. Further, a mean of the dimensions between the anterior and posterior permanent teeth was established and compared, which revealed that the mean dimensions were 5.0 and 5.3 µm for permanent anterior and permanent posterior, respectively, in the greatest dimensions. Thus, we could conclude that the permanent posteriors showed slightly larger prism dimension than permanent anterior teeth.

The present study was undertaken to understand the structure and morphology of the enamel surface in its indigenous form, without any surface alterations. Therefore, there was no use of any physical or chemical methods such as acid etching in the tooth preparation before observation under SEM. The study aided us in categorizing the enamel prism structure based on morphology and morphometry in anterior and posterior teeth of permanent dentition.

Conclusion

An extensive study on the morphology and morphometry of the enamel prisms in permanent teeth was done. We could definitely identify and categorize three different prism patterns. Further, the morphometry of the prisms revealed a wide range of prisms in the greatest dimension. However, we could not establish a significant difference in the prism dimensions when the three thirds are compared.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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