

Evaluation of superficial microhardness in dental enamel with different eruptive ages

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Abstract: This study evaluated the superficial microhardness of enamel in teeth at different posteruptive ages (before eruption in the oral cavity, 2-3 years after eruption, 4-10 years after eruption and more than 10 years after eruption). The study sample was composed of 134 specimens of human enamel. One fragment of each tooth was obtained from the flat-test central portion of the crown to produce specimens with 3 x 3 mm. The enamel blocks were minimally flattened out and polished in order to obtain a flat surface parallel to the base, which is fundamental for microhardness testing. Microhardness was measured with a microhardness tester and a Knoop diamond indenter, under a static load of 25 g applied for 5 seconds. Comparison between the superficial microhardness obtained for the different groups was performed by analysis of Student's *t* test. The results demonstrated that superficial microhardness values have a tendency to increase over the years, with statistically significant difference only between unerupted enamel and that with more than 10 years after eruption. According to the present conditions and methodology, it was concluded that there were differences between the superficial microhardness of specimens at different eruptive ages, revealing an increasing mineralization. However, this difference was significant only between unerupted specimens and those with more than 10 years after eruption.

Descriptors: Dental enamel; Hardness; Tooth eruption; Tooth, unerupted.

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Introduction

Amongst the problems of oral health, dental caries still constitutes one of the biggest challenges of dentistry.¹ Its understanding as a multifactorial process led researchers to pursue a broader comprehension of it and a search for several methods for its prevention.

Dental caries is a dynamic process that initiates as a located enamel lesion, provoked by alternating periods of demineralization and remineralization. It is caused by intimate contact of the enamel with dental biofilm which is influenced by the oral environment.^{2,3} As an effect of the complexity of this environment, several factors such as the surface of the enamel, the saliva and the presence of dental biofilm, among others, modulate the severity and the development of caries lesions in an individual.

When a tooth erupts in the oral cavity, the enamel has not yet undergone post-eruptive maturation, and presents special characteristics that render it more susceptible to demineralization.^{4,7} At this point, the enamel is more porous,^{5,8-10} with larger carbonated apatite concentration^{11,12} and a greater percentage of impurities (sodium, magnesium, etc) in its composition.¹³ As a consequence, enamel crystals become more soluble in the oral environment. Thus, at eruption, the mineral structures of the enamel are vulnerable to variations of the oral environment.¹⁴ The saliva, since it presents calcium, phosphorus and fluorine ions – which are the main mineral components of the crystalline structure of the tooth – naturally protects the enamel, favoring the maturation and rendering it less soluble.

Previous studies have shown the different characteristics of the enamel that has not yet undergone post-eruptive maturation from those of enamel that has undergone this type of dynamic in the oral cavity.^{5,8-12,15} However, the nature of this maturation is not well understood. Furthermore, studies have demonstrated that immature enamel is more susceptible to demineralization than mature enamel,^{6,7,12,13,16} probably due to post-eruptive maturation.

Taking those reasons into account, the purpose of this study was to evaluate the superficial microhardness of human enamel with different eruptive ages.

Material and Methods

This study was approved by the Research and Ethics Committee, School of Dentistry of Bauru, University of São Paulo (Proc. n. 144/2005).

Collection and grouping of the teeth

Permanent caries-free teeth extracted from patients from a fluoridated area (0.70 mgF/L) with widely different ages were used. They were collected in the emergency center, School of Dentistry of Bauru, University of São Paulo (FOB-USP), and were divided into 4 groups: Group I - unerupted third molars with incomplete root formation; Group II - premolars extracted for orthodontics reasons with 2-3 years after eruption and with incomplete root formation; Group III - premolars extracted for orthodontics reasons with 4-10 years after eruption and with complete root formation; Group IV - teeth extracted for periodontal or surgical reasons with more than 10 years after eruption. The teeth were stored in a stoppered bottle with a 0.1% thymol neutral solution.

Teeth with caries, areas of hypomineralization or attrition affecting the enamel and those damaged during extraction were excluded from the study. The teeth finally selected for the study are shown in Table 1.

Specimen preparation

In order to produce specimens with 3 x 3 mm, one fragment of each tooth was obtained from the flattest central portion of the buccal aspect of the crown, which was sectioned with a precision sectioning machine (Isomet Low Speed Saw; Buehler, Lake Bluff, IL, USA) and two double-faced diamond discs (Diamond wafering blade, series 15 HC diamond, arbor size ½ inch, Isomet 1000; Buehler,

Table 1 - The 134 teeth used in this study grouped according to their eruptive age.

Age group	Number of teeth	Teeth	Eruptive age (years)	Root formation
I	47	Third molar	Unerupted	Not completed
II	32	Premolar	2 to 3	Not completed
III	29	Premolar	4 to 10	Completed
IV	26	Different kind	More than 10	Completed

Lake Bluff, IL, USA), separated by a 3-mm spacer adapted to it.

The dentinal aspects of the blocks were initially flattened with silicon carbide paper grit 320 (Buehler, Lake Bluff, IL, USA). Then, the enamel blocks were minimally flattened out (600 and 1,200 grades of Al₂O₃ paper; Buehler, Lake Bluff, IL, USA) and polished with felt paper wet by 1 µm diamond spray (Buehler), resulting in the removal of about 100 µm in depth of the enamel which was controlled with a micrometer.

Microhardness tests

To investigate the mineral content of the specimens, microhardness tests were performed. The microhardness of the specimens was measured with a microhardness tester (HMV- 2000; Shimadzu Corporation, Tokyo, Japan) and a Knoop diamond indenter, under a static load of 25 g applied for 5 seconds. Five indents were made on each fragment with a distance of 100 µm.

Statistical analysis

Comparison of the mean values of the post-eruptive age groups was performed by applying Student's *t* test. The significance level was set at 5%.

Results

The values of superficial microhardness found in human enamel with different eruptive ages, unerupted, 2-3 years of eruption, 4-10 years of eruption and with more than 10 years of eruption, are shown in Table 2.

The values of superficial microhardness presented an increasing trend over the years. Significant differ-

ence was found only between unerupted enamel and that with more than 10 years of eruption ($p < 0.05$).

Discussion

In academic literature, there is no pattern to be considered for age groups in studies regarding post-eruptive maturation. This lack of standardization in studies^{4-10,12,13,15-18} generates difficulties for the comparison of results and its trustworthiness.

Therefore, in this study, the collected human teeth presented different post-eruptive ages, and were divided in four groups: Group I - unerupted; Group II - 2-3 years in the oral environment which did not present completely formed roots; Group III - 4-10 years in the oral environment with completely formed roots; and Group IV - more than 10 years in the oral environment. We opted for these age intervals due to the fact that each one of them represents different characteristics, as follows.

The unerupted tooth has never been exposed to the oral environment, its enamel is completely mineralized, and it has not yet undergone post-eruptive maturation.

The 2-3 years interval group includes teeth in the period of eruption or with complete eruption, which already had been exposed to the oral environment. This is a critical period for the development of caries lesions. Since teeth remain long periods in infra-occlusion and children are unable to perform their teeth brushing properly, there is a greater risk of accumulation of dental biofilm. However, teeth in eruption are exposed to dental biofilm during several months before functional occlusion occurs. According to Ekstrand *et al.*¹⁹ (2003), the time required for the first and second permanent molars to erupt ranges, respectively, from 5 to 32 and 9 to 45 months.

The 4-10 years interval group presents teeth that already are in occlusal contact and have been exposed to the oral environment for a longer period of time.³

The last group, which contains tooth specimens with more than 10 years of eruption, represents those teeth that already are in the oral environment for a much longer period of time.

In the present study, the values of superficial microhardness of the different eruptive age groups

Table 2 - Mean microhardness values (KHN) and standard deviation for the human enamel of the studied groups.

Eruptive age	Unerupted	2-3 years	4-10 years	More than 10 years
N	47	32	29	26
Mean*	375.79 ^a	384.72 ^{ab}	383.55 ^{ab}	390.69 ^b
Standard Deviation	21.62	14.92	16.42	11.90

*Means followed by different letters are significantly different ($p < 0.05$).

presented an increasing trend with the increase in age. Significant difference was found only between unerupted enamel specimens and those with more than 10 years of eruption. As known, a direct relation exists between the values of hardness and the percentage of mineral volume.^{20,21} This finding can be related to the exposure of the enamel to the oral-salivary environment¹⁴ and to normal dynamic processes of demineralization and remineralization during and after the eruptive process.³

The results of the present study are in accordance with the findings of Crabb⁵ (1976), who observed, through reflected light, polarized light and scanning electron microscopy, a defined outer white zone with a honeycomb aspect. He concluded that unerupted or just-after-eruption teeth show a greater susceptibility to demineralization than erupted teeth, as a function of the superficial layer characteristics of the enamel. Brudevold *et al.*¹⁵ (1982), albeit following a different methodology, found similar results. They demonstrated that post-eruptive maturation of the enamel involves a reduction in permeability. Furthermore, Fejerskov *et al.*⁸ (1984), through scanning electron microscopy, observed that the surface of the enamel at the time of eruption presented many varieties of irregularities in its surface, as well as great inter-crystalline spaces, which render the tooth more susceptible to aggression. Still in 1984, Thylstrup *et al.*²² compared unerupted with just-after-eruption teeth. They found small differences between them. The most important was the size of the inter-crystalline spaces, which were smaller in erupted teeth that were exposed to the oral environment.

Driessens *et al.*¹³ (1985) also have a study which agrees with the present results. They suggested that

post-eruptive maturation occurs in minerals of the superficial layer of the enamel, thus producing a greater resistance to caries lesions. Schulte *et al.*⁹ (1999) and Ten Bosch *et al.*¹⁰ (2000) observed an increase in electric resistance with the increase of the post-eruptive ages of the teeth. This characteristic is directly related to a reduction in the porosity of the enamel due to post-eruptive maturation.

It can be suggested that this increasing trend of superficial microhardness could be related to a tendency to be less susceptible to caries over the years.^{3,5-10,12,14-16} Unerupted teeth or with incomplete post-eruptive maturation are more liable to suffer demineralization.^{6,7,16} However, the fact that the superficial layer of the enamel was polished and flattened, as well as the fact that different kinds of teeth were used, could have influenced the results of the present study.

A better knowledge of post-eruptive maturation, as well as of the natural processes occurring in the enamel soon after eruption, would be an asset to clinical dentists, pediatric dentists and to public health through the definition of preventive strategies, as it would help to recognize the periods of higher susceptibility for the development of new caries lesions.

Conclusion

The present study revealed differences between the superficial microhardness of specimens with different eruptive ages. The results also indicated an increasing mineralization behavior of enamel. However, significant difference was found only between unerupted enamel specimens and those with more than 10 years of eruption.

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