Original Article

Assessment of Structural Changes in Translucency and Opacity of Tooth Enamel against a Direct Demineralization Process: An *In Vitro* Study

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ABSTRACT

Objective: The objective of this study was to assess *in vitro* the structural changes in translucency and opacity of tooth enamel following a direct demineralization process. **Materials and Methods:** This experimental *in vitro* study evaluated 45 thirds (cervical, middle, and occlusal) of the tooth enamel surface of premolar teeth extracted from young adults divided into three groups of 15 specimens each: Group 1 (solution based on calcium, phosphorus, and fluorine), Group 2 (orthophosphoric acid 37%), and control group (distilled water). All underwent optical macroscopic examination with ×3 magnification to determine the initial translucency according to the variation of the medium in their intercrystalline spaces, and Thylstrup and Fejerskov Index was used. The experimental groups were then subjected to an artificial caries process during which the specimens were placed in an inorganic and organic solution of calcium, phosphorus, and fluorine at 37°C for 90 days with the acidic solution at pH 5 and the neutral solution at pH 7. The control specimens were placed in distilled water. Finally, all the specimens were assessed by polarization microscopy. **Results:** In relation to the occlusal third, the highest proportion in Groups 1 and 2 was in Grades 2 and 3 (80%). A significant association was only observed between the experimental groups in the degree of translucency in the occlusal third ($P = 0.002$), whereas no association was found in relation to the degree of opacity in the middle and cervical thirds in either study group ($P > 0.05$). **Conclusion:** The resistance of enamel hydroxyapatite crystals increases from occlusal to cervical due to the greater presence of aprismatic enamel in the cervical horizontal third.

KEYWORDS: *Demineralization, dissolution, enamel, opacity, translucency*

INTRODUCTION

Dental caries is an infectious, contagious disease of multifactorial origin that affects the structures of the dental tissue as a result of a demineralization process. It mainly originates from the acids produced by certain acidogenic bacteria. It is the result of a combination of several factors. The same causal insult can produce a wide variety of responses due to differences in host resistance. If the insult or causal factor involves an acid on the host or the part of the tooth that is covered by enamel, the effect or response leads to demineralization or direct dissolution of the enamel. Another factor contributing to deterioration of host resistance is the wear or abrasion of the dental hydroxyapatite or coronal surface.[1-3]

To achieve the adhesion of some biomaterials to dental enamel, variations in the resistance to acid gradation

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of the coronal surface of the tooth must be considered, as well as mineral wear before acid etching. The age of the patient and degree of mineralization of the enamel before acid exposure should also be taken into account. Similarly, aprismatic tooth enamel or Darling coat is slightly more resistant to acid etching but does not require more engraving time. Tooth enamel shows wear with use. For example, aprismatic enamel is found in the cervical part of lower premolars of 97% of adults, being found in 61% and 59% in the mid and occlusal part, respectively, leading to greater susceptibility to dental caries.[4-6]

By varying the existing medium in the intercrystalline spaces, polarization microscopy and macroscopic examination of translucency can detect areas in the tooth enamel that are more or less resistant to direct dissolution. For example, some *in vivo* studies on the resistance of enamel to acids consist of the use of a colorimetric technique. However, other *in vitro* studies have used methods to demineralize tooth enamel using various cyclic pH models plus a demineralizing solution and artificial saliva. With the latter test, the minerals can be extracted from the enamel or the porosity can be increased, thus changing its optical properties, which are detectable with polarization microscopy and macroscopic translucency examination. Nonetheless, translucency may be maintained by the thirds most resistant to direct dissolution.[7-11]

Opacity is defined when a certain structure does not let light through and is generally associated with developmental changes in the tooth. While the Translucency refers to when a body allows light to filter through its structure.[2,10] It is important to verify that the enamel of the cervical third of the permanent tooth has a greater presence of aprismatic enamel, therefore, the hypothesis arises that this third may be more resistant to direct dissolution than in the middle and occlusal third (theoretically with less presence of this contributing factor to deterioration).[5,9,10] Thus, the aim of this study was to evaluate *in vitro* the structural changes of translucency of dental enamel, following a direct demineralization process.

Materials and Methods

Study design and sample size

The study was reviewed and approved by the postgraduate unit of the Faculty of Dentistry of the UNMSM 20170323. This experimental *in vitro* study was carried out in the Multifunctional Laboratory of the Faculty of Dentistry of the Universidad Nacional Mayor de San Marcos (UNMSM) in Lima, Peru, and in the EnviroLab Peru S.A.C. (Environmental Laboratories Peru). The samples included upper and lower premolar pieces of young adults; of which, the dental enamel of each dental piece was analyzed. According to the mean comparison formula with Stata Software, version 15.0 (StataCorp 4905 Lakeway Drive College Station, Texas, USA), with an α of 0.05 and a β of 0.80, it was calculated that a sample size of 45 enamel tooth surfaces of permanent teeth was necessary.

Inclusion criteria

- Permanent adult dental enamel of upper or lower premolars
- Tooth pieces extracted for orthodontic reasons
- Tooth enamel of systemically healthy people

Exclusion criteria

- Tooth pieces with shape alterations
- Tooth pieces with carious lesions
- Tooth pieces with developmental alterations

Ethical statement and allocation

The study had no ethical implications because extracted teeth for orthodontic treatment were used. To establish the groups, a simple random sampling technique was performed of the mesial, distal, buccal, and lingual faces of the upper and lower premolars. The dental specimens (thirds) were formed using a simple random sampling technique to observe any significant changes at that level. The three groups formed were as follows:

Group 1: Experimental group (solution based on calcium, phosphorus, and fluorine), n = 15 tooth crown of the cervical, middle, and occlusal thirds
Group 2: Experimental group (orthophosphoric acid 37%), n = 15 tooth crown of the cervical, middle, and occlusal thirds
Group 3: Control group (distilled water), n = 15 tooth crown of the cervical, middle, and occlusal thirds

Sample preparation

A total of 45 permanent premolars, extracted of young adults between 18 and 25 years of age, were analyzed, which were chosen for being teeth that are suitably indicated to be extracted for orthodontic reasons in the Maxilo-Facial Surgery Service of the UNMSM School of Dentistry. The dental specimens were placed in a 0.1% thymol solution to avoid dehydration. Subsequently, macroscopic examination of translucency (baseline translucency) by thirds of each tooth enamel sample was performed, and enamel samples showing the best degree of translucency (Grade 2 or 3) were selected and recorded. The samples of Groups 1 and 2 were placed in an inorganic/organic solution for 35 min daily during 90 days to mimic what occurs in the oral cavity *in vivo*. The solution was prepared by Laboratorio EnviroLab Peru S.A.C. with test code no. 907185, containing 0.5 mmol/L calcium, 2 mmol/L phosphate, and 3 mmol/L fluoride at an acidic pH (pH = 5) to induce a loss of initial translucency in any region of the outermost surface of the tooth enamel. The samples of Groups 1 and 2 were then stored at 37°C at a pH of 7, with constant circulation every 7 h. The teeth of the control group were placed in distilled water controlling the pH of the solutions and the distilled water every 30 days up to 90 days. Finally at 90 days, the translucency of the dental enamel of the crowns of the experimental groups was observed to assess the final translucency [Figures 1 and 2].

Remineralization of enamel

For evaluation purposes, the roots were removed and only the crowns of the premolars were analyzed. The five permanent teeth or samples were extracted and placed in a 0.1% thymol solution to avoid dehydration. Then macroscopic examination of its translucency was carried out by thirds of each tooth enamel sample (baseline translucency), and the enamel samples showing the best degree of translucency were selected and recorded. The samples of Group 2 were subjected to acid etching *in vitro* for 15 s with 37% orthophosphoric acid, followed by washing with distilled water for 30 s. Then, the macroscopic optical examination of each sample was performed, and the crowns were cut for study under the polarization microscope to assess the final translucency [Figure 3].

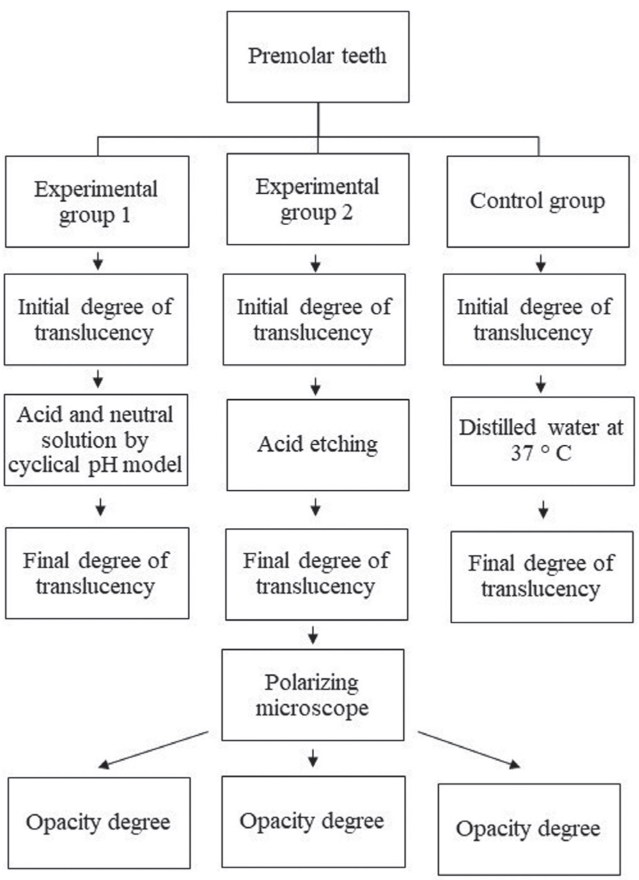

Figure 1: Dissolution of tooth enamel

Opacity evaluation procedures

The polarizing microscope was used for the analysis. To perform the macroscopic evaluation, the following procedures were performed:

Phase 1: Exodontia, cleaning, and conservation of the premolars in thymol
Phase 2: First macroscopic examination of translucency
Phase 3: Direct dissolution, distilled water and acid etching
Phase 4: Second macroscopic examination of translucency [Figure 4]

Degree of translucency and opacity

The following classification was used to assess the degree of opacity of a white spot lesion: Grade 1: intact or healthy area <1%, Grade 2: translucent> 1%, Grade 3: Dark 2%–4%, and Grade 4: Body of the injury> 5%. In addition, the Thylstrup and Fejerskov Index was used to assess translucency, where 0 indicates normal translucency of enamel remains after prolonged air-drying, 1 indicates narrow white lines corresponding to the perikymata, 2 indicates more pronounced lines of opacity that follow the perikymata with occasional confluence of adjacent lines (occlusal surfaces with scattered areas of opacity <2 mm in diameter and pronounced opacity of cuspal ridges), 3 indicates merging and irregular cloudy areas of opacity and

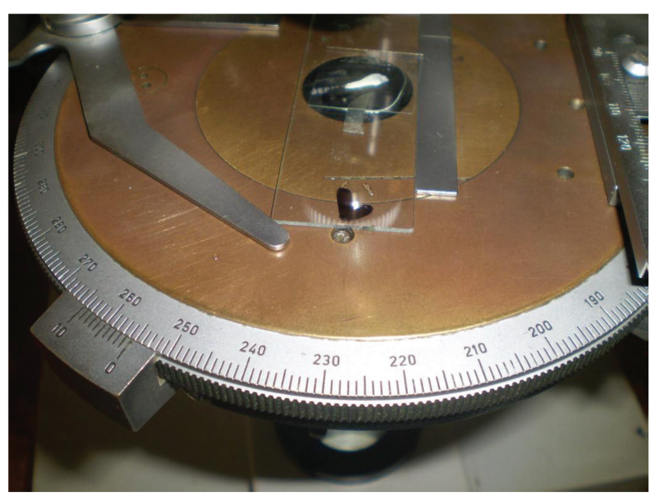

Figure 2: Specimen on the polarizing microscope

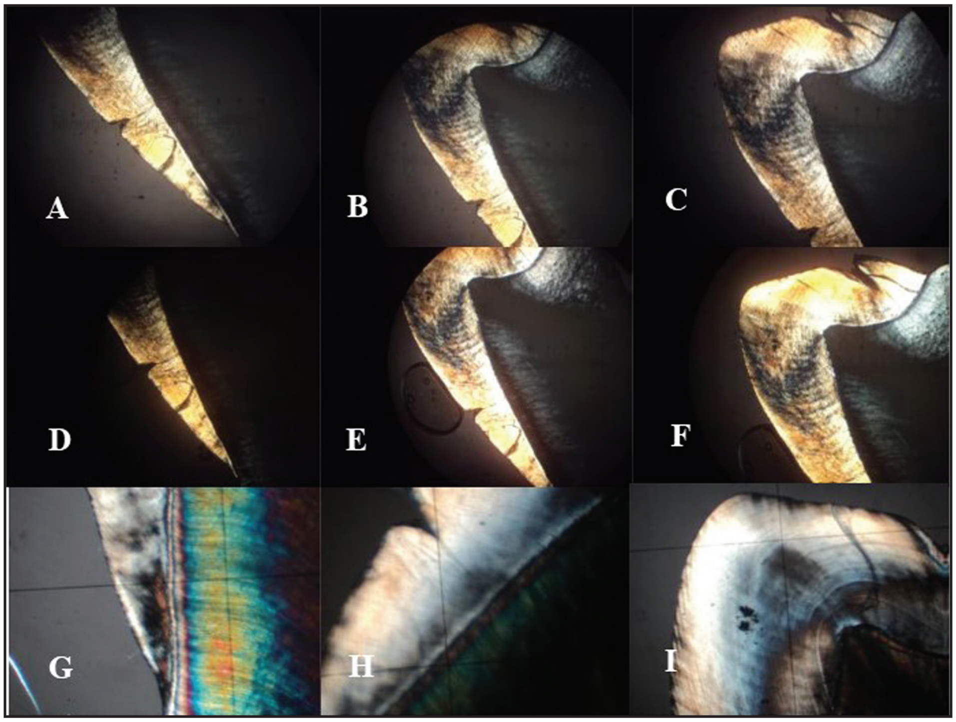

Figure 3: Microscopic analysis of the demineralization–remineralization process. (A–C) Tooth section showing loss of moderate birefringence in the occlusal, middle and cervical thirds. (D–F) Pores filled with distilled water. (G–I) Canadian balm samples with clearance of porous areas

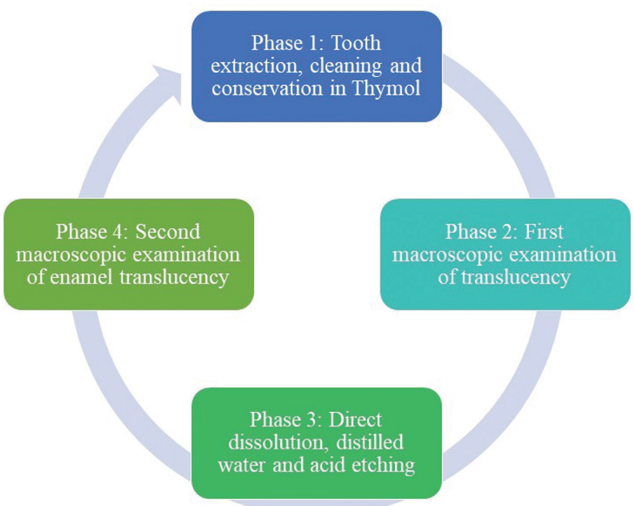

Figure 4: Opacity evaluation procedures

accentuated drawing of perikymata often visible between opacities, and lastly, 4 indicates that the entire surface shows marked opacity or appears chalky white, and parts of the surface exposed to attrition appear less affected.[11]

Statistical analysis

Frequencies and percentages were used for the descriptive analysis of the qualitative variables (degree of opacity and translucency). Subsequently, Pearson's chi-square test was used to make statistical inference. All statistical analyses were performed using Stata software, version 15.0, with P <0.05 being considered as significant.

Results

When determining the degree of translucency in the cervical and middle third of the dental enamel, the highest proportion corresponded to Grade 3 (80%) among the samples exposed to the inorganic–organic solution based on fluoride and phosphate. With regard to the occlusal third, the highest translucency was of Grades 2 and 3 (80%) in the samples in the experimental groups exposed to the inorganic–organic solution based on fluoride and phosphate and to the acid solution. Finally, only the occlusal third showed a significant association between the experimental groups and the degree of translucency, with a $P = 0.002$ [Table 1, Figures 5, 6].

Regarding the degree of opacity, the highest frequencies were found in the cervical third exposed to the inorganic–organic solution based on fluoride and phosphate and to the group of distilled water. In the middle and occlusal third, the highest frequency of opacity was observed in the control group exposed to

Table 1: Associations between degrees of translucency in the cervical, middle, and occlusal thirds in the three study groups

Division	Groups	Degree of translucency								P
		Grade 0	(%)	Grade 1	(%)	Grade 2	(%)	Grade 3	(%)	
Cervical third	Base solution	0	0	0	0	1	20	4	80	
	Acid solution	1	20	2	40	2	40	0	0	0.07
	Distilled water	1	20	2	40	2	40	0	0	
Middle third	Base solution	0	0	0	0	1	20	4	80	
	Acid solution	1	20	2	40	2	40	0	0	0.05
	Distilled water	2	40	2	40	1	20	0	0	
Occlusal third	Base solution	0	0	0	0	1	20	4	80	
	Acid solution	0	0	1	20	4	80	0	0	0.002
	Distilled water	2	40	3	60	0	0	0	0	

*Pearson's χ^2

Significance level P <0.05

All units of measurements were expressed according to the classification of Thylstrup and Fejerskov[11]

Base solution: Inorganic–organic neutral fluoride and phosphate-based solution

Acid solution: Orthophosphoric acid 37%

distilled water. Thus, no significant associations in the degree of opacity were found in any of the groups or in any of the dental thirds ($P > 0.05$) [Table 2, Figure 7].

Discussion

The highest degree of resistance to demineralization shown by dental enamel generally occurs at the level of the cervical third because of its ability to retain translucency or birefringence, even after a direct dissolution test with acids, whereas the middle third or the occlusal third usually loses this property due to the presence of prismatic dental enamel.

In this study, the submission of adult (mature) tooth enamel to a cyclical pH model using an acid solution led to changes in the optical properties or translucency of the tooth with the presence of opaque areas on the surface. These opaque areas were macroscopically located in the middle third or in the occlusal third (tooth enamel structured in prisms), showing that the cervical third (tooth enamel lacking prisms or aprismatics) was more resistant against acidic dissolution. Aprismatic dental enamel protects against acid dissolution of structured dental enamel in maturing prisms, and therefore, is only found externally coating young dental enamel until maturity. Although the prism-structured dental enamel completes its maturation or calcification internally, externally the prismatic dental enamel undergoes wear and tear due to daily use or abrasion, with the prismatic dental enamel disappearing and remaining only in the cervical third in adults.

According to a study by Eisenburger,[12] erosive effects on enamel lead to partial demineralization. He described that softened enamel areas reduce physical stability and features interprismatic porosities and that citric acid causes a loss of substance of 16.0 µm and a softening of the enamel of 2.4 µm. Compared to the original mineral content, the degree of demineralization of the softened enamel was 62% for calcium and 64% for inorganic phosphorus, which may explain the instability of the softened enamel to small chewing forces. On the contrary, Lynch and Ten Cate[13] investigated interactions between enamel and dentin at low pH under conditions simulating dental–dental bonding using enamel blocks demineralized in acid gel systems with a pH of 4.6. They reported that the different demineralization rates in enamel and dentin are produced by differences in their solubility, which may explain the progression of caries.[13]

Another similar study was carried out by He *et al.*[14] who evaluated the potential role of demineralization

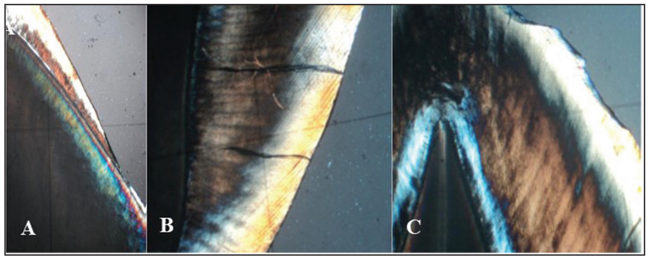

Figure 5: Microscopic evaluation of the opacity of three thirds of the dental enamel. (A) Cervical third. (B) Middle third. (C) Occlusal third

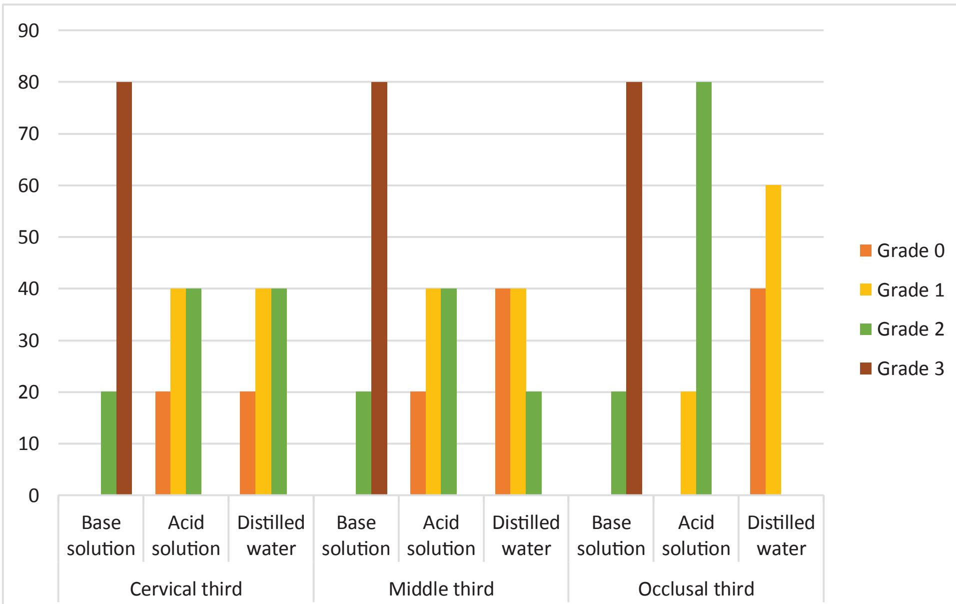

Figure 6: Evaluation of the degree of dental translucency according to the cervical, occlusal, and middle thirds

Table 2: Associations between the degrees of opacity according to the experimental solutions in the different cervical, middle, and occlusal thirds

Division	Groups	Degree of opacity					
		I	II	III	IV	Total	*P*
Cervical third	Base solution	5	0	0	0	5	
	Acid solution	0	2	1	2	5	0.05
	Distilled water	5	0	0	0	5	
Middle third	Base solution	3	0	2	0	5	
	Acid solution	0	0	3	2	5	0.05
	Distilled water	5	0	0	0	5	
Occlusal third	Base solution	2	0	2	1	5	
	Acid solution	0	0	3	2	5	0.05
	Distilled water	5	0	0	0	5	
Total		25	2	11	7	45	

*Pearson's χ^2

Significance level P <0.05

All units of measurements were expressed according to the classification of Thylstrup and Fejerskov[11]

Base solution: Inorganic–organic neutral fluoride and phosphate-based solution

Acid solution: Orthophosphoric acid 37%

of the cervical region of dental structures in the development of non-carious lesions at the cervical level. They concluded that 1- and 2-day demineralization significantly reduced the mechanical properties of the teeth. Demineralized enamel and dentin have low mechanical properties, and they are more prone to

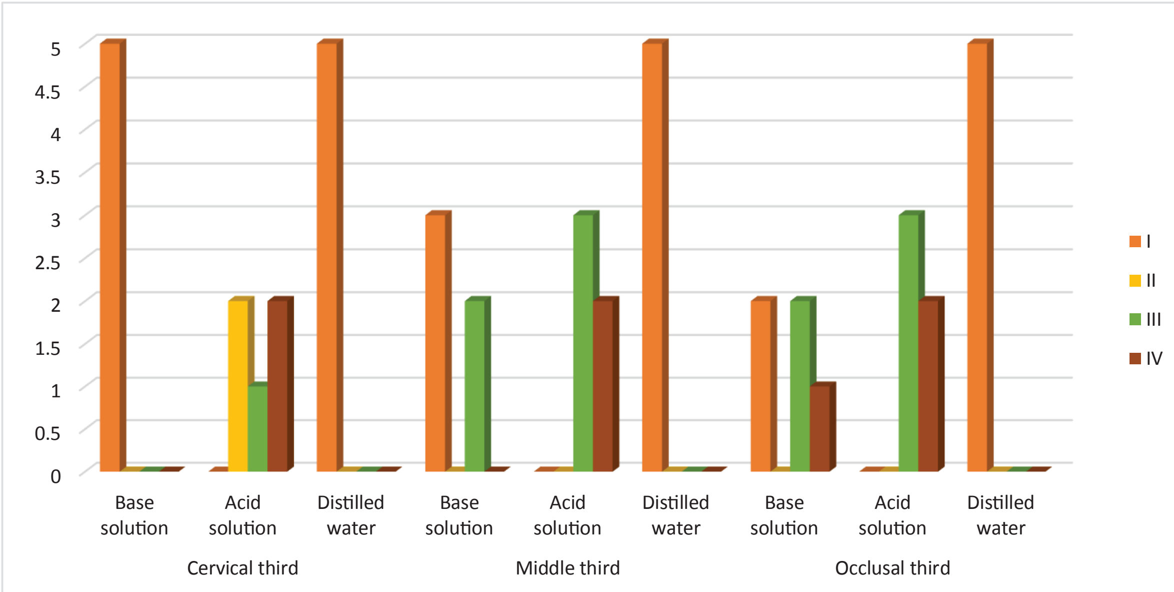

Figure 7: Evaluation of the degree of opacity according to the cervical, middle, and occlusal thirds

wear and abrasion. This agrees with a study by Díaz-Monroy *et al.*[15] who reported that the dissolution rate of enamel decreases with the treatment of the enamel with fluorine or zinc ions, with the dissolution of enamel surfaces being imminent after a period. Another study analyzed the morphological changes produced by acid dissolution of the enamel irradiated with laser, considering that morphological modifications can vary depending on the unwanted effects induced by laser irradiation.[16]

On the contrary, according to Shellis *et al.*,[17] it is important to develop preventive measures for erosion induced in enamel and dentin by hydrochloric acid (HCl). Therefore, sucralfate suspension favors anti-erosive protection to the dental structures induced by HCl. Furthermore, according to Turssi *et al.*,[18] the enamel of human teeth has hydroxyapatite oriented according to the parts of the crown, with a wide range of substrate options for the formation of matrices. Contrary to what is currently understood, reactive amorphous nanoparticles and their transformation efficiently induce a matrix structure.[19]

The main limitation of this study was that by being a purely *in vitro* study, certain covariates, which occur in the oral cavity and can alter the morphology of tooth enamel, were not taken into account. Another important limitation was that opacity and translucency were only evaluated with light microscopy, and it would have been interesting to evaluate these characteristics at a nanometric level using electron microscopy. However, as described above, it is essential to note that aprismatic tooth enamel wears occlusally-cervically over time, and at the same time all the enamel loses a certain degree resistance to direct dissolution. Therefore, in the case of direct dissolution, it is important to statistically verify the superiority of the dental enamel in the cervical third over the dental enamel of the middle and the occlusal third. For the prevention of dental caries and dental aesthetics, it is necessary to differentiate the resistance to direct dissolution of the three thirds of dental enamel, macro- and microscopically evaluating its translucency, porosity, and resistance to acid etching.

The clinical applicability of this study is important because it provides guidelines for the care of tooth enamel, the use of fluoride and acid etching on enamel by the application of two practical tests of resistance to demineralization on dental enamel, the first with a solution in a cyclical pH model and the second with an etching gel. This is relevant because aprismatic dental enamel is the mineral structure with the greatest resistance to demineralization or direct dissolution, and its function is to protect newly erupted young or immature dental enamel until mineralization is completed. However, as prismatic enamel is located on the external surface of the enamel, it undergoes wear and abrasion from use, possibly in the cervical occlusion direction, and all mature dental enamel loses this special protection against dissolution. It is the role of stomatologists to maintain the prismatic tooth enamel as long as possible, especially on newly erupted teeth, by gently cleaning its surface during prophylaxis, so as not to wear the enamel down. Therefore, it is important to preserve, protect, and study aprismatic tooth enamel.

Conclusion

In summary, within the limitations of this *in vitro* experimental study, it was shown that the cervical third of dental enamel presents greater translucency (Grade 3) than the middle and occlusal thirds. However, the differences were not statistically significant except in the region of the occlusal third. Finally, in relation to enamel opacity, there were no significant associations between the degree of opacity and the cervical, middle, and occlusal thirds in any of the experimental groups evaluated.

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Conflicts of interest

None to declare.

Author contributions

Study conception (SR), data collection (ADS, FPV), data acquisition and analysis (ADS, SR), data interpretation (WG, AMD, FMT), manuscript writing (FMT, ADS, FPV).

Ethical policy and institutional review board statement

This project is exempted from ethical approval due to it was an experimental in vitro study. All the procedures have been performed as per the ethical guidelines laid down by Declaration of Helsinki.

Patient declaration of consent

Not applicable.

Data availability statement

The data that support the study results are available from the author (Dr. Frank Mayta-Tovalino, e-mail: fmaytat@unmsm.edu.pe) on request.

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