sciendo
10.2478/AMB-2025-00059

ORIGINAL ARTICLE

ASSESSMENT OF ENAMEL MICROROUGHNESS IN PRIMARY AND PERMANENT TEETH WITH INITIAL CARIOUS LESIONS BEFORE AND AFTER ACID APPLICATION

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Abstract. Aim: *To evaluate the microroughness of primary and permanent teeth in the initial carious lesion (white spot) area before and after orthophosphoric and hydrochloric acid application.* ***Material and Methods:*** *The in vitro study included physiologically exfoliated primary molars and permanent third molars extracted for orthodontic reasons. The teeth were divided into eight groups according to the presence of initial caries lesions, type of dentition, the acid used, and the time of its application. The surface microroughness in the area of the carious lesion, both before and after etching, was assessed using a profilometer, and the results were compared. Carious lesions were etched either with 37% orthophosphoric acid for 30 seconds or with 15% hydrochloric acid for 120 seconds.* ***Results:*** *Higher microroughness values were found in the area of the initial carious lesion compared to the area with intact enamel for both dentitions. The microroughness of the enamel surface in the carious lesion area increased after applying orthophosphoric and hydrochloric acids in both primary and permanent teeth. The highest microroughness was recorded after conditioning with 15% hydrochloric acid. Microroughness data from carious primary and permanent teeth showed increased microporosity after etching with 37% orthophosphoric acid.* ***Conclusion:*** *Etching (with hydrochloric and orthophosphoric acid) of initial carious lesions of primary and permanent teeth significantly increases their microroughness.*

Key words: *microroughness, profilometer, hydrochloric acid, orthophosphoric acid, primary teeth, permanent teeth, white spot lesions*

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Received: *31 December 2024;* ***Accepted:*** *20 January 2025*

INTRODUCTION

The enamel is the hardest substance in the human body [1]. It serves as a protective barrier, shielding teeth from decay or sensitivity. Enamel consists of tightly packed hydroxyapatite crystals, making it resilient against wear and tear from chewing and acidic substances [2]. It forms a barrier that protects the tooth from physical, thermal, and chemical influences that could threaten the vitality of the dental pulp [3]. The acidic environment on the tooth, resulting from bacterial metabolism or the erosive potential of various products and materials, can damage the enamel, gradually dissolving hydroxyapatite nanocrystals and losing tooth structure [4, 5]. The first visible clinical manifestation of these chemical processes is the development of an incipient carious lesion.

There are various established techniques for non-operative treatment of initial carious lesions, as well as those for improving aesthetics, since they are often accompanied by a change in the color of the tooth structures (from chalky white to brown), which is a problem, especially in anterior teeth [6, 7]. Some authors recommend microabrasion with hydrochloric acid-based products, while others suggest erosion of the carious surface area with 15% hydrochloric acid and subsequent infiltration with a low-viscosity resin material [7-9]. Etching the enamel surface is a standard approach in daily clinical practice, ensuring the retention of aesthetic fillings. It leads to the chemical dissolution of the enamel prisms, increasing the microroughness of the enamel surface and creating micropores into which the adhesive system can enter.

Roughness is defined as irregularities or small protrusions and depressions, which can be assessed using electronic devices [10]. The roughness of hard tooth structures greatly influences the retention of oral microorganisms and the formation of dental biofilm, one of the main etiological factors for the development of caries [1]. The critical roughness threshold is defined as 0.2 µm, above which bacteria are more likely to adhere to the tooth surface [11]. Increasing surface roughness leads to faster colonization of microorganisms and enhanced maturation of the dental biofilm [11]. The inherent morphological and physiological differences in the enamel of primary and permanent teeth are expected to be accompanied by differences in enamel surface microroughness [12].

This study aimed to evaluate the microroughness of the enamel surface of primary and permanent teeth (with initial white carious lesions) before and after orthophosphoric and hydrochloric acid application. We are not aware of any such study being conducted to date.

MATERIALS AND METHODS

The objects of the in vitro study were physiologically exfoliated primary teeth and permanent third molars extracted for orthodontic reasons. The teeth included in the study were clinically examined with a microscope (Semorr DOM 3000E) under 8x magnification, selecting 64 teeth (32 primary and 32 permanent teeth) with initial carious lesions (white spot) with code 02 (ICDAS II) and 64 intact teeth without carious lesions on their smooth surfaces (32 primary and 32 permanent). Carious lesions were located on smooth surfaces and had a size of at least 2 mm in their most expansive area, which fell within the measuring range of the profilometer (a device used for measuring microroughness). Teeth with restorations, fractures, cracks, erosion, or abrasion were excluded from the study.

Prior to the tooth extraction, consent forms were signed by the patients or their parents for the use of the extracted teeth in the study. The research ethics committee approved the use of human teeth.

PREPARATION OF THE SAMPLES FOR SURFACE MICROROUGHNESS MEASUREMENTS

After extraction, the teeth were cleaned of soft tissue debris, and the roots of permanent molars and those present in primary teeth were removed. Then, the teeth were stored in 0.1% thymol solution at 4°C until the beginning of the experiment. The crowns were embedded halfway in silicone, exposing the smooth (buccal or proximal) carious or intact tooth surfaces, and ensuring the specimens remained stable during the experiment (Figure 1C). The teeth thus prepared were mounted in a specially made 3D-printed positioner (custom-designed) (Figure 1A and B).

MICROROUGHNESS MEASUREMENT

The surface roughness of all samples was measured using a Surface roughness tester (model – SRT-6210S, Shenzhen Graigar Technology Co, Shenzhen, China). It has a five µm radius needle tip with a cutoff value of 0.08 mm (λc), a transverse length of 0.25 mm, a measurement speed of 0.25 mm/sec, and a Gaussian Filter (Figure 1C).

The initial distribution of the samples is presented in Table 1.

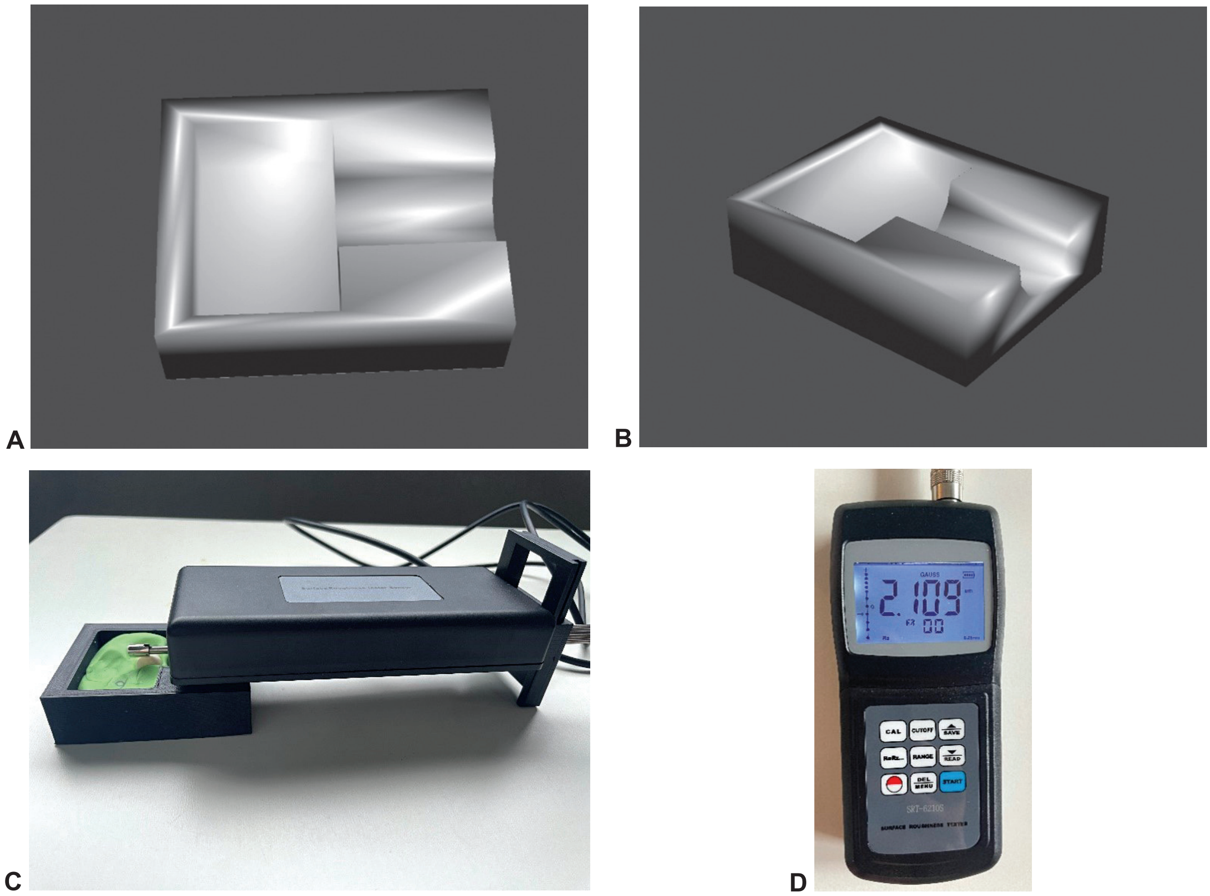

Fig. 1. Experimental setting: **A, B** – custom-designed printed positioner, **C** – tooth positioning unit, **D** – Surface roughness tester

Table 1. Distribution of samples by type of dentition and health status of the studied enamel surface

Dentition	Condition of the examined enamel surface	
	With initial carious lesion (N)	Sound (N)
Primary	Group 1 N=32	Group 3 N=32
Permanent	Group 2 N=32	Group 4 N=32

In the carious lesion groups (groups 1 and 2), microroughness measurements were made in the central area of each carious lesion. The diamond tip of the measuring device was placed in the central part of the carious lesion, and the device was turned on (Figure 1C). During operation, the diamond tip of the stylus moved horizontally along the tooth surface over the carious lesion back and forth with a linear displacement of 1.2 mm. For each sample, the average value of the Ra parameter in micrometers (arithmetic mean of the height of peaks and the depth of valleys from a mean line along the measuring length) was measured, and its value was displayed on the device screen (Figure 1D). For each tooth, five measurements were taken to obtain the average Ra value.

The microroughness of intact smooth enamel surfaces (groups 3 and 4) was measured and recorded using the same methodology in the same areas corresponding to the localization of the initial carious lesions in the specimens with such lesions (groups 1 and 2). The results for the average microhardness value in all groups (groups 1, 2, 3, and 4) were recorded and subjected to comparative analysis.

For teeth from groups 1 and 2, the change in microroughness in the area of the existing carious lesion after etching was assessed. For this purpose, after the initial measurement of microroughness in the area of the carious lesion, the group of primary and permanent carious teeth was randomly divided into two new subgroups, each according to the type of etchant used and its exposure time (Table 2). The samples were then etched (for 30 or 120 seconds) and rinsed with a water-air spray for 30 or 120 seconds. Their microroughness in the area of the etched initial carious lesion was measured again (according to the methodology described above). Although perfect repositioning accuracy is impossible at the micron level, the sample was roughly in the same position for every measurement. The results obtained were subjected to comparative analysis.

Table 2. Distribution of samples into groups according to the type of dentition, the etchant and the duration of exposure

Group	Type dentition	Number of teeth	Acid	Duration
Group 5	Primary	16	37% orthophosphoric acid	30 s
Group 6	Permanent	16	37% orthophosphoric acid	30 s
Group 7	Primary	16	15% hydrochloric acid	120 s
Group 8	Permanent	16	15% hydrochloric acid	120 s

Statistical analysis

SPSS v. 16.0 (SPSS Inc., Chicago, IL, USA) was used to analyze the data. Through a Kolmogorov–Smirnov test, the data distribution was evaluated, and comparisons of the microroughness values between groups were performed using a pairwise Mann–Whitney U test. The significance level was set at $P<0.05$.

RESULTS

Table 3 presents the results of the difference in microroughness of sound and carious enamel of primary and permanent teeth.

The data in Table 3 show low and close values in the microroughness of intact enamel of primary and permanent teeth ($p=0.568$). In contrast, enamel with signs of initial carious lesions (white spots) shows microroughness values of about 2.5 μm. Higher microroughness values were found in the area of the initial carious lesion compared to the area with intact enamel for both dentitions.

Table 4 presents the results of the microroughness changes after etching white carious lesions of primary and permanent teeth.

Table 4 shows that microroughness increased after applying orthophosphoric and hydrochloric acids in both primary and permanent teeth. A statistically significant difference in microroughness was found within the respective dentition groups between baseline and post-etching values, as well as between group 1 and groups 5 and 7, and between group 2 and groups 6 and 8. The difference in microroughness is statistically significant depending on the applied acid, between groups 5 and 7 and between groups 6 and 8. The highest microroughness was recorded after conditioning with 15% hydrochloric acid, with no statistically significant differences found between the teeth from both dentitions (groups 7 and 8). Microroughness data from carious primary and permanent teeth after etching with 37% orthophosphoric acid showed almost the same results for microporosity.

Table 3. Microroughness of sound and carious enamel in primary and permanent teeth

Group	Enamel surface	Microroughness (μm) Mean ± SD (μm)	Mann-Whitney U Test
Group 1 (primary teeth)	initial carious lesion	2.432 ± 1.725	$p_{1,2}=0.709$
Group 2 (permanent teeth)	initial carious lesion	2.483 ± 0.641	$p_{1,3}=0.011$
Group 3 (primary teeth)	sound	1.930 ± 0.440	$p_{2,4}=0.018$
Group 4 (permanent teeth)	sound	1.980 ± 0.569	$p_{3,4}=0.568$

Table 4. Microroughness of the enamel carious lesion of primary and permanent teeth – initial and after orthophosphoric and hydrochloric acid application.

Tooth	Baseline	37% orthophosphoric acid	15% hydrochloric acid	Mann-Whitney Test
Group	**Group 1**	**Group 5**	**Group 7**	
Primary	2.4329 +/- 0.8531	3.1629 +/- 0,7136	4.1067 +/- 0.5014	$p_{1,5}=0.007$ $p_{1,7}<0.001$ $p_{5,7}=0.001$
Group	**Group 2**	**Group 6**	**Group 8**	
Permanent	2.4834+/- 0.6417	3.2325 +/- 0.6682	4.2599 +/- 0.7826	$p_{2,6}=0.004$ $p_{2,8}<0.001$ $p_{6,8}=0.006$
Mann-Whitney Test	$t_{1,2}=361.500$, p=0.709	$t_{5,6}=92.500$, p=0.871	$t_{7,8}=71.500$, p=0.255	

DISCUSSION

The present study compared the change in surface microroughness of enamel in both dentitions after applying 37% orthophosphoric and 15% hydrochloric acid using a profilometer to measure microroughness. Our results showed differences in microroughness data among the tested groups. The two applied etchants on the initial enamel lesions (white spots) resulted in higher values of surface roughness parameters than before their application (Table 4). This suggests an increase in the surface area available for adhesive bonding with infiltrants or other materials for treating the lesions.

The microroughness of intact enamel in primary and permanent teeth and that over initial carious lesions classified with code 02 (ICDAS II) was measured and compared. Significant differences were not found between the two groups – the microroughness of sound enamel was approximately 2 µm, while that of white carious lesions was about 2.5 µm. This increased microporosity is due to both the subsurface demineralization typical of the initial carious lesion and the additional etching with the two acids, leading to microporosities in the surface layer [13, 14].

Other studies have described results similar to ours [15, 16]. The lack of a significant difference in microroughness between sound and carious enamel with an initial carious lesion can be explained by the dissolution/precipitation mechanism that acts on the enamel surface, ensuring a continuous supply of mineral ions from the subsurface zone of the enamel [17].

Etching with orthophosphoric acid is a conventional technique that creates microporosities by partially demineralizing the enamel surface (dissolving the inorganic structure) [18, 19]. This process enhances the penetration of monomers and ensures effective resin infiltration into the enamel [19]. The type and concentration of etching agents are crucial for material retention afterward. The microroughness after etching with 37% orthophosphoric acid increased by about one unit compared to the initial values in the study (Table 4).

Enamel micro-abrasion using hydrochloric acid efficiently and effectively achieves aesthetic results [20]. This technique is considered safe, conservative, and atraumatic in removing superficial enamel stains and defects on tooth surfaces [21]. The method is widely used to treat fluorosis, hypomineralization, and other stains and has been promoted in several studies [21]. Hydrochloric acid 15% is used in resin infiltrating systems to remove the surface layer of the decalcified zone due to its penetration depth of 58 ± 37 µm [22]. This etching opens access to the lesion body, allowing the resin to penetrate deeper. Our data showed a more than 1.5 increase in enamel microroughness after application of 15% hydrochloric acid compared to baseline in both dentitions (Table 4).

Opalustre is another product on the market that uses hydrochloric acid's erosive action in combination with silicon carbide's abrasive action [23]. These components work synergistically to remove the rough and porous enamel surface, and studies have reported a smoothing and leveling of the enamel surface after treatment with this product [23].

The orthophosphoric acid etched groups in the present study had lower values of microroughness created than the hydrochloric acid groups (Table 4). However, decades of application and its proven effectiveness in the protocol of aesthetic adhesive restoration, especially in the enamel margins, are undisputed for both dentitions. Mineral loss caused by acid action contributes to surface erosion [15].

CONCLUSION

Etching (with hydrochloric and orthophosphoric acid) of initial carious lesions in primary and permanent teeth significantly increases their microroughness, which would lead to an increase in the contact area and increase the adhesion of various materials used to treat initial carious lesions. More pronounced microroughness is achieved after exposure to hydrochloric acid. Despite the differences in enamel between primary and permanent teeth, applying approved etching agents did not result in significant differences in the microroughness created. This indicates the lack of risk after application of hydrochloric acid, especially in primary dentition, whose enamel has a lower degree of mineralization.

Funding: *The article was developed with the support of a scientific project "Grant-2023", Contract No. 184/03.08.2023.*

Conflict of interest: *The authors declare no conflicts of interest related to this work.*

Ethical approval: *All patients gave written informed consent for using their teeth for research purposes, and all teeth were irreversibly anonymized immediately after extraction. Approval from the Ethics Committee of the Medical University of Sofia was also obtained.*

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