In vitro Study Regarding the Effect of Different Types of Etching Acids on the Morphology and Chemical Content of the External Surface of Enamel Carious Lesion on Primary Teeth

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The aims of this study were to evaluate the changes in surface morphology and in chemical composition of the artificial carious enamel lesion after etching with different types of etching acids. 25 extracted primary canines and molars were sectioned in mesio-distal direction. The external surface of the enamel was covered with a varnish resistant to acid action excepting a square of 2 mm × 2 mm, situated in the central area of the buccal and lingual faces. Artificial enamel carious lesions were induced. The 50 prepared sections were randomly divided into 5 groups. Control group included 10 enamel samples not subject to acid etching. Each of the rest of 4 study groups included 10 enamel samples etched for 15 , 30, 60 s with 37% phosphoric acid gel respectively, 2 minutes with 15% hydrochloric acid gel. The samples were then analyzed using a scanning electronic microscope coupled with a EDX detector. Phosphoric acid and hydrochloric acid affect the surface morphology of the carious enamel. Etching with 37% phosphoric acid for 15 or 30 s lead to similar morphological changes on artificial enamel carious lesion. A decrease of calcium and phosphorous ions content were recorded after etching with phosphoric acid and hydrochloric acid, as a result of enamel dissolution.

Keywords: phosphoric acid, hydrochloric acid, primary teeth, calcium and phosphorous ions, SEM

Micro-invasive treatment of carious lesions includes sealing and low-viscosity resin infiltration. Very good results were obtained during time when using sealants to prevent the occlusal carious lesions, but also to treat the noncavitated occlusal or proximal caries [1, 2]. Histologically, in noncavitated carious enamel lesions, the pores in the lesion body are diffusion pathways for acids and mineral ions [3]. Blocking these pores by forming a thin, superficial resin layer or by infiltrating the enamel structure prevent the progression of the carious lesion and the appearance of cavitation. Many in vivo or in vitro studies evaluated the penetration of carious lesion by adhesive resins, experimental resins or fissure sealants [4-9]. The action of some materials still remains unclear as regards their penetration into the lesion or precipitation on the lesion surface studies [4, 7-9]. In noncavitated carious lesion, a superficial, hypermineralized enamel layer is formed by precipitation of minerals on the enamel surface. The presence of this layer inhibits or delays the resin penetration into the carious lesion. For a better adhesion of the resin to enamel or for a better infiltration of the low-viscosity resin in the subsurface area of the lesion, some authors proposed that the hypermineralized enamel to be etched with orthophosphoric acid [7]. After etching the enamel surface, both sealants and low-viscosity resin act as diffusion barriers [4, 10, 11]. Some studies showed that infiltration of the adhesive into natural lesions was still superficial even after 2 min of etching with phosphoric acid [12]. As an alternative solution, using 15% hydrocloric acid for 2 min was proposed in the clinical protocol [13]. It was demonstrated that etching with phosphoric acid leaded to a decrease of the enamel organic content, which involves a decrease in enamel permeability, while the increase of the organic matter achieved by hydrochloric acid action still maintains enamel permeability [14].

The aims of this study were to evaluate the changes in surface morphology and in chemical composition of the artificial carious enamel lesion on primary teeth after etching with different types of acid.

Experimental part

In the study were used 25 extracted primary canines and molars having no carious lesions. The teeth were sectioned in mesio-distal direction, using diamond disks (Gebr. Brasseler GmbH&Co, Germany) with low speed, under continuous cooling with water. Two halves, buccal and lingual, were thus obtained. The external surface of the enamel was covered with a varnish resistant to acid action (Resist and shine, L'Oreal, Paris), excepting a square of 2 mm × 2 mm, situated in the central area of the buccal and lingual faces. To induce in vitro enamel carious lesions the samples were submitted for 7 days to this regime: 1 h demineralization, 3 h immersion in artificial saliva, 1 h demineralization, 5 h immersion in artificially saliva, 1 h demineralization, immersion in artificially saliva until next immersion in demineralizing solution. The chemical composition of the demineralization solution was: 1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 150 mM KCl, 0.1 mM sodium acetate, pH=5.0. As artificial saliva AFNOR standard S90-701 saliva was used, having a pH of 8.67 and a chemical composition of: NaCl 0.7g/L; KCl 1.2 g/L; Na_2HPO_4 0.26 g/L; $NaHCO_3$ 1.5g/L; KSCN 0.33 g/L; urea 1.35 g/L. The 50 prepared sections were randomly divided into 5 groups. Control group included 10 enamel samples not subject to

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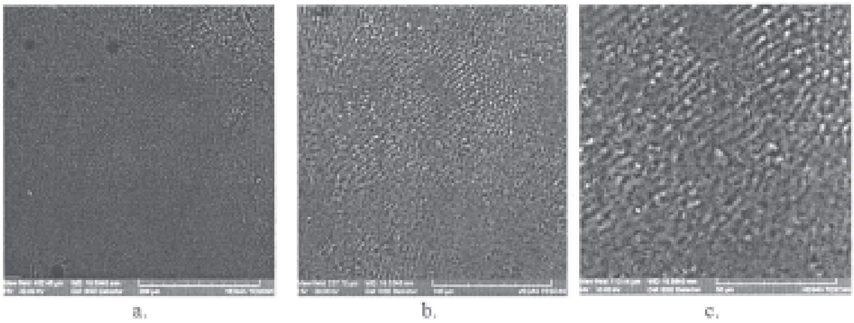

Fig. 1. SEM aspects of enamel carious lesion after etching using 37% phosphoric acid for 15 s: a. 500 X, b. 1000X, c. 2000X

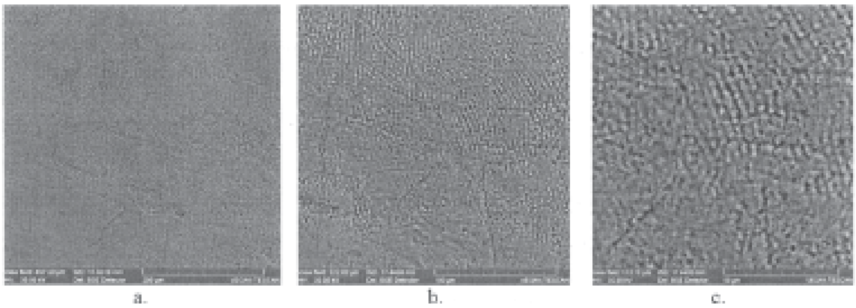

Fig. 2. SEM aspects of enamel carious lesion after etching using 37% phosphoric acid was used for 30 s: a. 500X,b. 1000X, c. 2000X

acid etching. Each of the rest 4 study groups included 10 enamel samples etched for 15, 30, 60 s with 37% phosphoric acid gel (Total Etch, Ivoclar Vivadent) respectively, 2 min with 15% hydrochloric acid gel (Icon-Etch, DMG, Germany). After etching the samples were washed for 30 sec with distilled water. The samples were then analyzed using a scanning electronic microscope (VEGA II LSH, Tescan Czech Republic), coupled with a EDX detector QUANTAX QX2 (Bruker/ Roentec, Germany). The integrally computer-assisted microspcope has a cannon of electrons with tungsten filament, which may reach a resolution from 3nm to 30KV, and a magnification power up to 1.000.000X in the resolution mode, an accelerating voltage between 200 V and 30 kV, scanning rates between 200 ns and 10 ms per pixel. It has an active area of 10 mm^2, so that it may analyze all elements heavier than carbon. Quantax QX2 employs a 3rd generation detector, Xflash, which needs no cooling with liquid nitrogen, being about 10 times more rapid than conventional detectors.

Results and discussions

All the samples etched with 37% phosphoric acid gel and with 15% hydrochloric acid gel showed different degrees of demineralization on the enamel surface. When 37% phosphoric acid was used for 15 s, clear modifications of the external demineralized enamel layer were recorded (fig. 1a). The major pattern of enamel dissolution was represented by interprismatic substance loss (fig. 1b). A random distribution of this pattern and very rare patterns of intraprismatic enamel demineralization are shown at higher magnification (fig. 1c).

37% phosphoric acid exposure for 30 s caused similar demineralization on the enamel surface as 15 s exposure. The major pattern of enamel dissolution was represented by the interprismatic substance loss. A few intraprismatic areas of dissolution or irregular, aprismatic area presented no dissolution were observed (fig. 2a-c).

When 37% phosphoric acid was used for 60 s, accentuated demineralization of the enamel surface was observed, with a generalized pattern of interprismatic dissolution been present (fig. 3a-b). At a higher magnification, the head of the rods or their core remaining intact was the major aspect (fig. 3c).

15% hydrocloric acid action for 2 min resulted in a completed different aspect of enamel surface, with a possible removal of the extern enamel layer and an uniform dissolution of the interprismatic areas (fig. 4a and b).

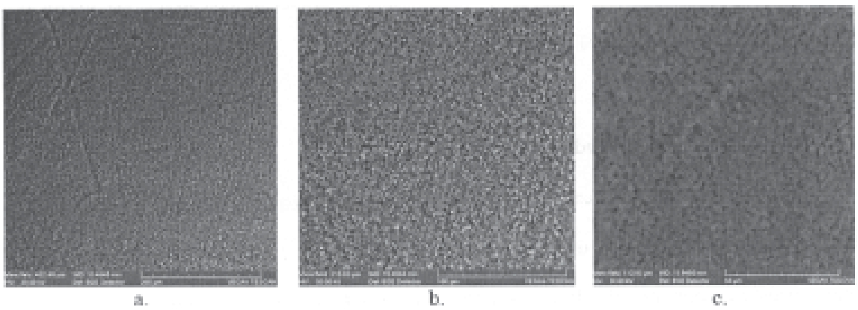

Fig. 3. SEM aspects of enamel carious lesion after etching using 37% phosphoric acid for 60 s: a. 500 X, b. 1000X, c. 2000X

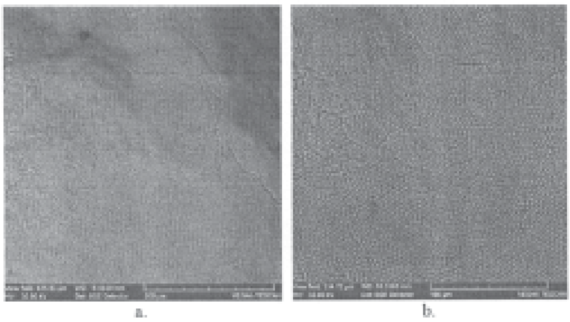

Fig. 4. SEM aspects of enamel carious lesion after etching using 15% hydrocloric acid for 2 min: a. 500 X, b. 1000X

	Mean calcium ion concentration (wt%)±SD	Mean phosphorous ion concentration (wt%)±SD
control	54.87±0,3	27.36±0,2
37% phosphoric acid action, 15 seconds	53.65±0,3	26.48±0,4
37% phosphoric acid action, 30 seconds	53.47±0,2	26.36±0,2
37% phosphoric acid action, 60 seconds	52.87±0,3	25.81±0,2
15% hydrocloric acid action, 2 minutes	48.68±0,2	23.75±0,1

Table 1
MEAN CALCIUM AND PHOSPHOROUS IONS CONCENTRATIONS (WT%) ± STANDARD DEVIATION IN THE STUDY GROUPS

The results of EDX chemical samples analysis demonstrated an evident loss of minerals, on both calcium and phosphorous ions, after 37% phosphoric acid etching for 15, 30 and 60 s and after 15% hydrocloric acid etching for 2 min when compared to control group (table 1). The highest loss in the average concentration of calcium ions was registered after etching of the enamel for 2 min using hydrocloric acid (from 54, 87 wt% in non-etched samples to a mean of 48.68 wt% in etched samples). The concentrations of calcium ions after acid etching for 15 and 30 s recorded very similar average values (53.47 wt%, respectively 53,65 wt%). For phosphorous ions, the same decreasing tendency of the average values after acid etching was manifested (from 27.36 wt% in non-etched samples to 26.48 wt% in the phosphoric acid etched ones for 15 s, 26.36 wt% in those etched for 30 s, 25.81 wt% in those etched for 60 seconds and 23.75 wt% in those etched with hydrochloric acid) (table 1).

Phosphoric acid and hydrochloric acid used in this study affected the surface morphology of the carious enamel by creating microporosities by selective removal of the prismatic material. Increases in the free surface energy and in the porosity of the tissue seem to have a great importance in obtaining the adhesion and in depth of resin penetration into the etched enamel layer [15, 16]. The retentive characteristics of the etched enamel depend on the type of acid, on its action time and on the enamel chemical composition. Selection of a certain etching agent should consider the constants of its dissociation, as well. Microretentions may be obtained only at the level of prismatic enamel. Dissolution of the hydroxyapatite crystal begins at its extremity, advancing towards the center, as a result of the higher concentration in carbonate ions, the lateral sides of the rods being more resistant. Demineralization is selective, due to the different morphological arrangement of the enamel rods. Different aspects in sound enamel morphology when phosphoric acid was used for different periods of time were recorded in previous studies [17, 18]. The morphology of the sound enamel etched with phosphoric acid for 15 or 30 s seemed to be very similar [19, 20]. A limitation of this study is represented by the fact that superficial enamel layer of artificial carious lesion is more homogeneous and presented less thickness and mineral content when compared to natural lesion [21]. Except that, in morphological analyses, the outer surface of the sound enamel appears as a tissue having some area with prismless structure. This aprismatic layer is more frequently seen in primary teeth, where the width of the zone is larger compared to permanent teeth [22]. That could explain the SEM aspects in some etched enamel samples, where interprismatic dissolution alternated with no dissolution area.

In our study was proved that even when phosphoric acid was used for 15 and 30 s on artificial lesion surface of primary teeth, the carious enamel showed similar morphology. The enamel surface was the most affected by acid action when hydrochloric acid was used for 2 minutes. Previous studies was demonstrated that etching with phosphoric acid and other acid solution leaded to an incomplete reduction of the surface layers of carious lesion in enamel [23-31]. 15% hydrochloric acid etching for 90-120 s leaded to an effective erosion of the surface layer of natural enamel caries in primary teeth.

The enamel of primary teeth contains hydroxyapatite crystals as inorganic material (96% by weight and over 86% by volume), [32] remnants of proteins from the period of development, water (3.5% by weight) and organic matter (0.6% by weight) [33]. Acid etching causes demineralization of hydroxyapatite and release of the elements present on the enamel surface. In this study a decrease of calcium and phosphorous ions content were recorded after etching with phosphoric acid and hydrochloric acid, as a result of enamel dissolution.

Conclusions

Phosphoric acid and hydrochloric acid affect the surface morphology of the carious enamel in primary teeth. Etching with 37% phosphoric acid for 15 or 30 s lead to similar morphological changes on artificial enamel carious lesion. 15% hydrochloric acid etching for 2 minutes causes uniform, pronounced dissolution of enamel carious lesion surface. A decrease of calcium and phosphorous ions content were recorded after etching with phosphoric acid and hydrochloric acid, as a result of enamel dissolution.

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