

The Effect of Phosphoric Acid Etching Application Time on the Enamel Morphology – a Comparative SEM Study

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Our objective was to evaluate the etching pattern and the depth of demineralization during enamel etching with phosphoric acid applied on un-instrumented enamel surfaces in different time duration. 40 human premolars extracted for orthodontic reason were divided in four groups and after preparing them for conditioning, were treated with 37% phosphoric acid for 15, 30 and 45 s. The fourth group was the control group. All teeth were analyzed with scanning electron microscopy. Control teeth showed transversally oriented, superimposed prisms and small, polygonal or round holes where the prisms were perpendicular to the examined surface. When etching was made for 15 s, a sponge-like moderate demineralization can be seen which affect mostly the interprism spaces. In the case of a longer acid treatment (30 s) the demineralization was greater at the prism head and at the periphery, demineralization of interprismatic areas can also be seen. When we etched for 45 s, we observed that both inter- and intraprismatic areas were affected, the head of prisms were almost completely razed. Our results showed different micro morphological changes depending on the time duration of acid treatment.

Key words: enamel etching, phosphoric acid, SEM, time duration

Prior bonding brackets directly to labial surface of the teeth, enamel should be conditioned. When composite adhesive are used for bracket bonding, one of the most commonly used conditioner is the phosphoric acid in various concentrations.

The first step of the acid etch technique involves the application of the orthophosphoric acid (H_3PO_4) etching solution to the enamel surface. Rubbing the enamel during conditioning adversely affects the bonding of composite resins to these surfaces. Hormati and al. reported, however, that the shear bond strength of a composite resin to enamel surfaces which were etched by a dabbing or a rubbing action were not significantly different despite the marked differences in etch patterns as observed by scanning electron microscopy (SEM) [1].

Phosphoric acid demineralizes the enamel surface at depths ranging from 5 μm to 25 μm [1- 3]. Chemical treatment by acid etching enhances the topography of enamel, changing it from a low-reactive surface to a surface that is more susceptible to adhesion. The demineralization is selective because of the morphological disposition of the prisms. Acid-etched enamel can remineralize [4-6], but the amount of time required for this to occur is variable, and the extent of recovery is incomplete [7,8]. Importantly, the acid-etched surface allows the less mineralized underlying enamel to be exposed to a potentially acidic microenvironment [9, 10]. Etched enamel exposed to cariogenic solutions has been repeatedly shown to be more severely affected than is unetched enamel [11].

While manufacturers of bonding systems typically recommend specific conditioning protocols, the effect of changing the conditioning time on the etching pattern has been investigated previously, yielding differing results. Some

studies have shown that a reduced etching time does not result in lower bond strengths; other authors have reported significant reductions in bond strengths when decreasing etching time. In addition, Olsen et al. specifically investigated the effect of enamel etching time on the shear bond strength (SBS) of orthodontic brackets. Their study concluded that while 0- and 5-second etch time were insufficient to successfully bond brackets, no significant difference in SBS was seen when the etch time was reduced from the recommended 30 seconds to only 10 seconds. Thus, it was determined that this shorter etching time provided clinically acceptable SBSs when used to bond orthodontic brackets [21].

After debonding, previously etched enamel surfaces can remineralise mostly from saliva. The more aggressive etching was before bonding, remineralisation will be incomplete [12]. Because all hard tissues are in continuous ionic change with the environment, it could be expected that human enamel will respond to acid conditioning differently, depending on age and factors related mainly to saliva and diet. Further research should be carried out to verify whether enamel changes over time might have clinical relevance when acid etched [6].

The purpose of this study was to evaluate the pattern of acid etching and the morphology after acid etching depending of the time duration enamel surfaces were treated with phosphoric acid.

Experimental part

For comparative study of enamel surfaces 40 human premolars extracted for orthodontic reason were used. The selected teeth should not be treated with any kind of agent in the past; they should not present restorations, caries or fissures due to the pressure of the pliers during the

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extraction. Teeth were stored in distilled water at room temperature to prevent dehydration.

The labial surface of all teeth were cleaned and polished with fluoride-free paste using a rubber cup mounted on a low-speed contra-angle handpiece for 15 s. The surfaces were rinsed with water and dried with air. Etching was done in different time duration with 37% phosphoric within all the experiment groups, according to the manufacturer's instructions.

Specimens were divided in four groups with ten teeth each according to the following protocol:

I.Group – control group, without etching

II.Group - teeth conditioned for 15 s with 37% phosphoric acid

III.Group – samples conditioned for 30 s with 37% phosphoric acid

IV.Group – samples conditioned for 45 s with 37% phosphoric acid

After acid treatment all teeth were rinsed 20 s, dried with moisture-free compressed air and dehydrated in a desiccator for 10 h. Then treated enamel surfaces were sputter-coated with silver in vacuum evaporator. The entire etched surface was examined under a scanning electron microscope (JEOL - JEM - 5510 - LV, Tokyo, Japan) and photomicrographs were registered at different magnifications (25x, 3500x, 5000x)

Results and discussions

SEM analysis of control teeth showed transversally oriented, superimposed prisms. It can be also detected prisms with different orientation; the ones which are perpendicular to the surface appear like small, polygonal and round holes (fig.1.).

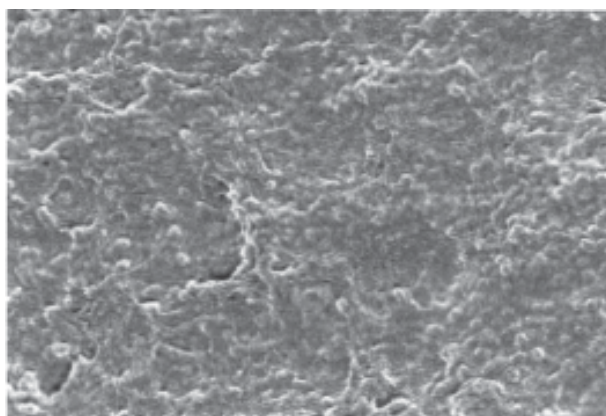


Fig.1. SEM micrograph of intact enamel surface, control group. (magnification 5000x)

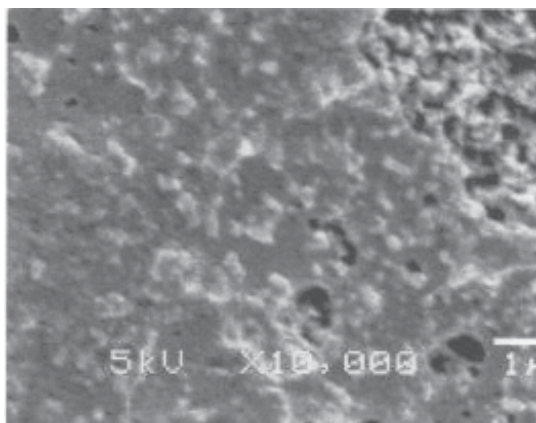


Fig.2. SEM image of 15 seconds acid etched enamel. Slight demineralization of interprism spaces can be observed. (magnification 10000x)

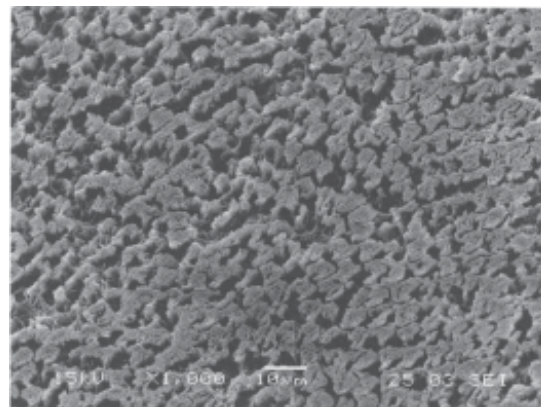


Fig.3. SEM micrograph of 30 seconds etched enamel surface. Acid razing mostly on the periphery of prisms, shape of "fish-scales" (magnification 1000x)

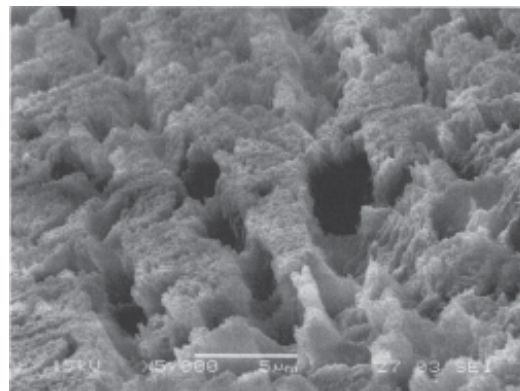


Fig.4. SEM image of perpendicular prisms etched 30 seconds with 37% phosphoric acid. Erosions of prisms head can be observed. (magnification 5000x)

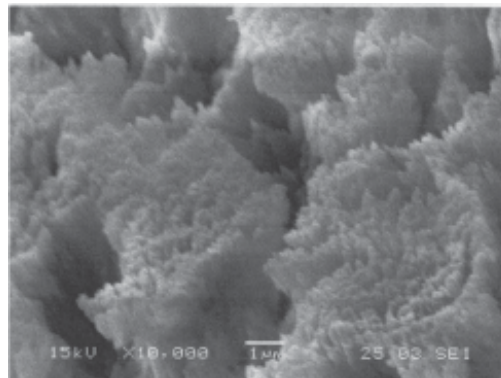


Fig. 5. At a higher magnification of 30 seconds etching, both inter- and intraprismatic dissolution can be observed.

When conditioning with 37% phosphoric acid was made for 15 s (group II.) the beginning of a sponge-like moderate demineralization can be seen which affect mostly the pores among the prisms, the interprism spaces (fig.2.). Intact areas can also be seen, mostly on the margins of etched enamel surfaces.

When acid conditioning was longer (30 seconds– group III.) the depth and morphology of demineralized zones has increased. Demineralization is greater at the prism head and at the periphery. On zones where prisms are oriented perpendicular to the surface, the demineralization of interprismatic areas can also be seen (figs. 3-6.).

Group IV specimens were treated with 37% phosphoric acid for a longer time (45 seconds) which explains the roughness of overall enamel. Both inter- and intraprismatic areas are affected, the head of prisms are almost completely razed. This gives to the SEM image a honey-comb like aspect. (figs.7-9)

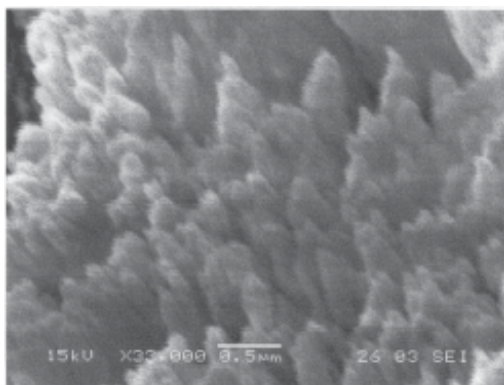


Fig.6. SEM image of 30 seconds etched enamel. 0,5-5 μm demineralized interprismatic zones can be seen. (magnification 33000x)

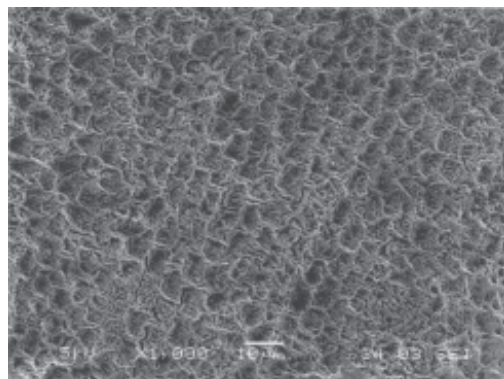


Fig.7. SEM image of enamel surface after phosphoric acid conditioning for 45 seconds. The honey-comb aspect is due to intra- and interprismatic erosion. (magnification 1000x)

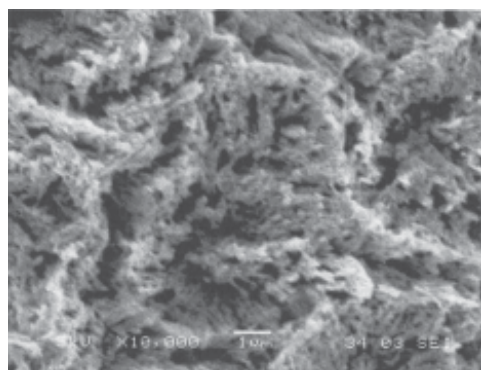


Fig.8. SEM image of enamel surface after phosphoric acid conditioning for 45 seconds. An aggressive interprismatic demineralization can be observed. (magnification 10000x)

The flat surface of dental enamel has to be razed in order to create micro-retentions prior bonding brackets. This treatment will make enamel surface retentive and more susceptible for adhesion.

Some of the studies compared the enamel etch patterns of different etching agents. Gardner's et al. ex vivo study compared the etch pattern achieved on the orthodontic bonding area of extracted mandibular premolars treated with 37% phosphoric acid and 2.5% nitric acid applied for 15, 30, or 60 seconds. The etch patterns were viewed with a scanning electron microscope and assessed with a 5-point etch scale. Statistical analysis showed that by increasing the duration of acid application (irrespective of acid type) significantly increased the amount of better quality etches. Phosphoric acid at 37% concentration was more effective at producing a good-quality etches than was nitric acid at 2.5% for all 3 application times. The quantity of good-quality etch produced by phosphoric acid

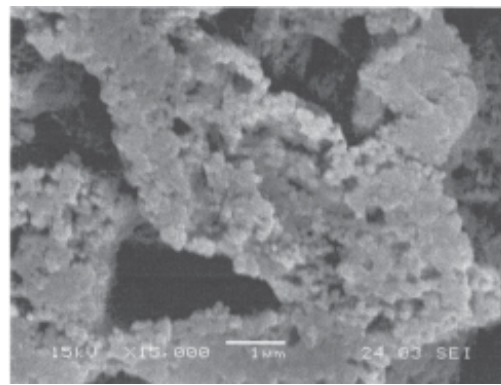


Fig.9. After 45 seconds of acid treatment, demineralization is rather aggressive. All the interprismatic substance is damaged.

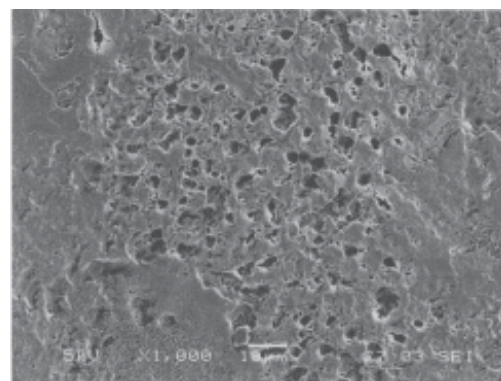


Fig.10. The etched surface is not homogenous due to the more mineralized enamel surface of teeth used in this study

at 37% was time specific, with 15 seconds being significantly less effective than 30 or 60 s. However, 60 seconds was not significantly better than 30. Comparing the upper half of the orthodontic bonding area with the lower half showed no statistical significant differences for either acid type or length of application ($P > .005$). These findings support the use of 37% phosphoric acid and indicate an optimum application time of 30 seconds[23].

When enamel etching was introduced in clinical use (1960s) the recommendations were 60 seconds etching with 85% phosphoric acid [14, 15]. Ever since, many authors recommend reducing the etching time to 30 or 15 s. Manufacturers have recommended 15 s because it saves time without compromising the adhesive performance [13, 16, and 17].

In the early 1990 s, it was common to find etchants such as 10% phosphoric acid, 10% maleic acid, 10% citric acid, 2.5% oxalic acid, and 2.5% nitric acid. Some of these acids do not result in a dull, frosty-white appearance typical of conditioned enamel, but a few studies show that this does not negatively affect immediate adhesive bonding to instrumented enamel. However, other research shows a significant decrease of the bond strengths. Because of the lack of long-term clinical evidence on the durability of bonding to enamel using these less aggressive conditioners, the previous use of 32 to 40% phosphoric acid conditioner is still the best option to achieve predictable bonding to enamel.

Some authors recommend reducing the etching time to 15 seconds when a 32 to 40% phosphoric acid is used [18]. Most of the manufacturers of adhesive systems have recommended 15 s because it saves time without compromising the adhesive performance.

Reducing times have been suggested because acid conditioning causes superficial tissue loss, it is desirable

that minimal tooth structure be dissolved; therefore, minimal acid-application time should be used. The difference between 15-second and 30-second application time of phosphoric acid on enamel dissolution is very small. The chemical reaction of the conditioning occurs quickly and, as mineral components are lost, the acid potential decreases by buffering. It has been suggested that the conditioning time be reduced to 15 seconds, which is considered adequate for creating a retentive enamel surface with no difference in the enamel etching pattern or decrease of the bond strengths to instrumented enamel. In vitro studies have demonstrated that a 15-second conditioning time is also adequate for orthodontic adhesive procedures [19, 20]. Another advantage of reducing etching duration is saving chair time.

The enamel surfaces used in our study were of young teeth, with no restorative treatment or any kind of instrumentation before. Therefore they can be considered intact surfaces. The hard tissue can be more mineralized, even an aprismatic enamel layer can be present. This can explain the less of homogeneous etching pattern (fig.10.).

According to Busscher et al. (1987) and Legler et al. (1990) the increase in the surface free energy of the enamel surface and the increase in the porosity of already porous tissue produced by the acid conditioning are probably more important than the depth of resin penetration into the etched enamel surface. Zidam and Hill (1986) reported that the amount of enamel surface lost was dependent on the concentration of acid and that, to a certain point, the surface loss increased as the acid concentration increased. Nevertheless, a significant difference in surface roughness of tooth enamel did not result in a significant difference in the shear bond strengths (Legler et al., 1989). Gottlieb et al. (1982) also used concentrations of phosphoric acid ranging from 10% to 60 for 60 seconds and found no significant differences in resin-to-enamel tensile bond strengths. However, they did find significantly lower tensile bond strength when a 70% phosphoric acid solution was used. It has also been suggested that reduction of etching time may produce effective retention characteristics and may be less destructive to enamel (Barkmeier et al., 1986) [22].

Even though the antibacterial capacity of phosphoric acid is well-known, enamel surface must be cleaned before etching. Using a noninstrumented surface for bonding, prophylactic paste and brushes are enough and useful [21]. After etching, the surfaces were dried and desiccated so etched surfaces were contamination-free.

Goes's and al. [22] micromorphological study did not reveal any difference in the etching patterns between 35% and 10% phosphoric acid after 15 or 60 second application on enamel. At these etching times and concentrations, the pattern most often observed was Silverstone's type I etching (Silverstone et al., 1975), in which the prism core material was preferentially removed leaving the periphery intact. Previous studies also confirm that these etching times did not have a significant effect on bond strength (Silverstone et al., 1975; Barkmeier et al., 1986; Triolo et al., 1993; Guba et al., 1994). On the other hand, etching with 10% maleic acid gel for 15 seconds partially removed the apatite crystals from the prism core and an etching time of 60 s resulted in more dissolution of prism core material. Although there was no micromorphological difference in the type of etching pattern produced in enamel with either etching time, after 60 seconds of etching the topographical view of the prism core structure approached that produced by 35 and 10% phosphoric acid gels. This study has also shown that the acid conditioning of a dentin

surface with 35 and 10% phosphoric acid gels and 10% maleic acid gel for 15 and 60 seconds produced similar micromorphological effects on dentin surface. With both concentrations, application times and types of acids, the etching removed the smear layer and opened the dentinal tubule orifices, as previously shown (Perdigão and Swift, 1994). However, the dentin surfaces treated with 35% phosphoric acid gel for 15 and 60 s showed a particulate residue left after rinsing. According to Perdigão et al. (1994), the surface residue is silica used to thicken the etching gel and is not entirely removed by rinsing with water; however the presence of a silica residue did not interfere with bonding of resin to the tooth structure [22].

The presence of prismatic and aprismatic enamel affects the efficacy of phosphoric acid. While a more uniform surface appearance is achieved by etching in the presence of prismatic enamel, bond strength is reduced in the presence of aprismatic enamel. Due to moisture contamination during respiration and the presence of aprismatic enamel, more bracket failures are observed in the lower second premolars of patients in permanent dentition. (Mattick & Hobson, 2000) Uniform prism structures on the outer enamel layer of deciduous teeth are not observed. Therefore, phosphoric acid application after sandblasting with 50 µm aluminum oxide is needed for deciduous teeth. (Zachrisson & Büyükyılmaz, 2005) Etching procedures with phosphoric acid differ in terms of microtopographic etching patterns over enamel surfaces. (Mattick & Hobson, 2000; Hosein & Sherriff, 2004) The intended etching pattern was only observed in 1/20 of enamel etched with phosphoric acid. This was attributed to the presence of aprismatic enamel and partial contact between phosphoric acid and the enamel surface. (Mattick & Hobson, 2000) Microtopographic evaluation depending on the etching procedure revealed a non-uniform depth. It was reported that a depth of 3-15µm or more is necessary to provide optimum shear bond strength and penetration. (Hosein & Sherriff, 2004) However, in literature, surface depths between 10 µm and 175 µm were presented. (Diedrich, 1981; Gwinnett, 1973; Silverstone, 1977) The differences in the depth (Hobson & McCabe, 2002; Daronch & DeGoes, 2003) are thought to be caused by aprismatic enamel and remineralization of Ca-P to enamel surface. (Chow & Brown, 1973).

Our study exemplifies that both 15 and 30 seconds acid conditioning affect interprismatic zones. Demineralisation of prism cores appears only when acid attack is longer. These changes may be a benefit in increasing the bond strength of brackets bonded on the etched surfaces but they can be insufficiently remineralized after debonding.

The field has been also discussed elsewhere [24, 25].

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