Comparative SEM Analysis of the Effect of Acidic Monomers and Phosphoric Acids on Dental Enamel

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The aim of this study was to compare the etching pattern of two of the most commonly used etching techniques: the self-etching primers and phosphoric acid. 24 extracted premolars extracted for orthodontic reasons were assigned in two groups: group A – treated with self-etching primer, group B – treated with 37% phosphoric acid. Conditioners were applied according to the manufacturer’s instruction. Specimens treated with self-etching primer were washed with alcohol and acetone, the ones treated with phosphoric acid were rinsed with water. Each specimen was examined under a scanning electron microscope. Results: the etching pattern of self-etching primer showed a moderate demineralization with holes and scratches. A more aggressive demineralization of the prism cores were observed on specimens treated with phosphoric acid, both interprismatic and prismatic enamel being dissolved.

Key words: dental acid etching, self-etching primer conditioning, scanning electron microscopy

Bonding systems used in orthodontics in order to attach brackets on the labial surface of the teeth are based on resin composite materials and glass ionomer cements. The first category needs micromechanical retention on the enamel surface caused by surface conditioning.

Buonocore (1955) reported that bonding to enamel surface could be increased by conditioning the surface with 85% phosphoric acid for 30 seconds [1]. Since this study, acid etching is used in everyday practice, removing approximately 10 μm of the enamel surface, creating a porous layer and increasing the surface energy and wettabiity [2-4].

Right after tooth eruption, the surface of the enamel is constantly covered by plaque. The continuous metabolic activity within this microorganic film leads to a cyclical change in pH and demineralization-remineralization mechanisms. SEM analysis can discover the earliest signs of human enamel demineralization and teeth surface looks porous.

There are two types of enamel dissolving:
- type I is a subsurface lesion (white spot lesions) - H3PO4 dissolves the head of the prism, with the peripheral material or interprismatic substance remaining intact;
- type II is enamel erosion, which can damage the prism center and periphery as well - the acid dilutes the peripheral zone of the prisms, leaving the prism head relatively intact.

There is also a combination of the two models, type III, meaning the complete destruction of enamel prisms after acid etching - the surface change has no specific features but displays generally some superficial dissolution that does not alter the deeper strata where the enamel prisms are located (fig.1).

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**Fig.1** Representative SEM topography images for primary groups (A) with Type-1 etching pattern, (B) with Type-2 etching pattern and (C) with Type-3 etching pattern, and permanent groups, (D) with Type-1 etching pattern, (E) with Type-2 etching pattern and (F) with Type-3 etching pattern.
These 3 etching patterns appear randomly at any point on the enamel and can be found together in the same enamel zone. Clinically, however one can only see a white, opaque surface, exhibiting the quantity but not quality of the affected surfaces [5, 6]. Silverstone [7] later showed that the most retentive etching patterns were types 1 and 2, because the porous surface offered retentive areas of greater size and depth. The type 3 etching pattern did not present a defined and deep morphology and lacked the micromechanical retention, offered by the previous two.

After acid etching, physical and chemical procedures increase the active surface necessary for retention of composite and adhesive materials. It is good to dissolve strictly the non organic part of the enamel, the monocalcium monohydrate salts from the centers and edges of crystal prismatic structures.

After demineralization, new crystals of the apatite enamel can be created by remineralization. The level and the result of these reparative processes depend on the type of etching material, on acid concentration, crystal orientation in relation to teeth surface, but mostly on time of acid treatment.

The self-etching systems combine the etching and priming steps. This eliminates the need for rinsing and possible damage of gingival tissue. The active ingredient of self-etching primers is a methacrylate phosphoric ester. Phosphoric acid and a methacrylate group are combined into a molecule that etches and primes simultaneously. The phosphate group of the methacrylated phosphoric acid ester dissolves the calcium and removes it from hydroxyapatite. The primer molecules penetrate the enamel rods concurrently with etching. The etching process is effectively stopped because the phosphoric acid forms a complex with the dissolved calcium [5]. These products cause less harm to the enamel surface than conventional acid-etching techniques [6, 7].

The purpose of this study was to investigate by scanning electron microscopy the effect of the two most common etching agents (37% phosphoric acid and self-etching primer) on the surface of the enamel.

Experimental part

For comparative analyses of enamel surfaces 24 human premolars extracted for orthodontic reasons were used. Teeth were obtained from patients from 13 to 17 years old and stored in 0.5% chloramines solution in a refrigerator at 4°C no longer than 3 months. Criteria for including the teeth in this study were: intact buccal surface, no treatment of these surfaces with any kind of previous chemical or mechanical agent, no fissures and lack of caries.

The labial surface of the teeth were polished with a rubber cap and fluoride-free pumice, sprayed with water and dried with a compressed oil-free air stream.

The teeth were divided in two groups of 12 teeth each group and each group was exposed to different etching agents.

The specimens in group A were etched with self-etching primer TransbondTM Plus Self Etching Primer (3M Unitek) (a 2-propenoic acid, 2-methyl-phosphinocibis (oxy-2,1ethandiyl) ester) according to the directions recommended by manufacturers by rubbing the surface with a tip of an applicator for 3-5 s/tooth and then gently dry the primer into a thin film with using an oil and moisture-free air source. Specimens from group B were etched with 35% phosphoric etching gel (Transbond XT Etching gel, 3M Unitek) applied with a minisponge to the enamel surfaces for 30 s, rinsed under running water for another 30 s and then dried with an oil-free air stream.

Specimens treated with self-etching primer were thoroughly rinsed with acetone and ethanol solutions in order to dissolve and remove the self-etching primer. All teeth were dried, dehydrated in a dessicator for 10 h and treated enamel surfaces were sputter-coated with silver in vacuum evaporator. The entire surface was examined under a scanning electron microscope (JEOL - JEM - 5510 - LV, Tokyo, Japan) and photomicrographs were registered at different magnifications.

Results and discussions

Figures 2, 3 and 4 represent the morphology of enamel surface treated with self-etching primer (group A). At 25x magnification the whole treated area is visible (fig. 2). At larger magnification (fig. 3, 4) mild demineralization can be seen, almost normal enamel structure can be detected. Focal holes, scratches, enamel crystals and primer masking the enamel surface can also be seen (fig. 5). The etching patterns ranged from mild demineralization (fig. 6) to an aspect of surfaces etched with phosphoric acid. Ground enamel allowed the self-etching primers to condition the subsurface enamel, exposing crystallites when the etching pattern was considered at least moderate. The pH values of self-etching system tested was higher than that for phosphoric acid. In general, the demineralization effects of these systems are proportional to the acidity of the acidic primers or self-etching adhesive solutions [2]. The self-etching primers were less aggressive than phosphoric acid etchants and the conditioning effects were also reduced in ungrounded enamel surfaces. This result is in agreement with other studies that demonstrated that self-etching adhesives do not form a proper and defined acid etching pattern in intact surfaces. SEM observations
indicated that only shallow pits were produced after some self-etching treatments in intact enamel.

Labial surfaces treated with phosphoric acid present the well known white, frosty appearance (fig. 7, magnification 25X). The etching pattern was not uniform, dissolution of prism cores and boundary regions can be observed. The well-known honeycomb pattern, alternating with scratches, poorly etched zones was observed (fig. 8, 9). Dissolution of prism cores and boundary regions can be observed, however, the conditioning of enamel surface was not uniform along the ungrounded surfaces. Although phosphoric acid etchants present pH below 1, ungrounded enamel surfaces were not totally attacked or conditioned by acid etching. The etching effect of phosphoric acid etchants was similar to that previously described by Retief (1973) and Silverstone, et al. (1975). The prism cores and boundaries were etched by 34% and 35% phosphoric acids, causing dissolution of both inter and intraprismatic areas. The predominant etching pattern was type 2, which has the peripheral region of prisms removed and prism cores relatively unaffected. The ungrounded enamel treated with phosphoric acids also showed formation of a porous surface, exhibiting the exposed enamel crystallites along the entire surface. However, the etching pattern was not uniform throughout the surfaces. Some areas showed little etching effects, whereas other areas exhibited extensive demineralization [2]. Figure 8 shows remnants of aprismatic or prismless layer that was partially dissolved by phosphoric acid etching. In this current study, as enamel surfaces were obtained from young premolars, it was possible that the treated outer enamel layer would be prismless.
Brackets are bonded for a limited time on a labial surface of a tooth. To have sufficient and effective bond strength, easy debonding and limited enamel damage is critical in everyday orthodontics. In order to have a proper bond strength, teeth should be prepared for bonding and the need of etching enamel surface is obvious.

Self-etching systems are aqueous mixtures of polymerizable acidic monomers and methacrylate components. Acidic monomers have been developed containing esters from phosphoric acid, carboxylic acid or derivatives. Their etching efficacy depends on acidic monomer, pH of adhesive solution, etching time and application method. They are responsible for etching the dental substrates, whereas methacrylate components, such as HEMA, are available for monomer infiltration and polymerization of the bonding agent. As the application of self-etching adhesives comprises simplified bonding procedures, there have been concerns regarding the longevity of bonding, according to some in vitro and in vivo results. Studies have shown that most of the self-etching adhesives do not etch enamel as deeply as the phosphoric acid etchants did and the shallow etching pattern could compromise the bonding to enamel. Pashley and Tay (2001) reported that the efficacy of self-etching primers in intact enamel does not depend solely upon their etching aggressiveness, but also on monomer composition of each material. It is also possible that the low enamel bond strengths might be caused by the high amount of unpolymerized acidic monomers remaining after curing. Thus, no correlation among degree of primer aggressiveness, enamel etching pattern and bond strength to ungrounded enamel has been reported for self-etching adhesives [2].

The same difference in etching pattern was observed by certain studies. Typical honeycomb aspect and a rough, etched surface were created by acid etching. Less pronounced etching of the surface enamel was obtained by self-etching primer systems [2, 8 - 10].

The self-etching primer mechanism of bonding to enamel is based on nanoretentive interlocking between crystallites and adhesive resin [22]. These morphological features of the resin-enamel bonds are different from that formed with etch & rinse adhesive systems. This thin hybridized complex of resin in enamel produced by self-etching without the usual micrometer-size resin tags can be responsible for lower bond strength and questionable effectiveness on enamel surfaces [23]. Based on scientific evidence, some authors have recommended the instrumentation of enamel before bonding, in attempt to increase the bond strength [6]. Thus, resin bond strength achieved with self-etching systems is sometimes comparable to those achieved with phosphoric acid, despite the differences between enamel etching patterns [2]. However, the controversy about the effectiveness still remains, since other studies did not show the same results of bond strength.

The shear bond strengths of bracket bonding after enamel conditioning is influenced by the etching pattern and etching efficacy. By comparing the conventional multi-step adhesive system, the self-etching primer and the self-adhesive system, conventional multi-step system produced the greatest bond strength, followed by the self-etching primer and the self-adhesive system. The self-adhesive system produce less debonding failures and more calcium particles on the bracket base remained after debonding. Due to the moderate etching pattern, the simplified bonding procedures caused an undesirable decrease in tensile bond strength [11].

The results showed significant differences in demineralization patterns and are in disagreement with some in vitro investigations, which demonstrated comparable bond strengths when using self etching primers and phosphoric acid as the conditioners [12 - 14]. In order to measure the shear bond strength of stainless steel brackets bonded to enamel in vitro with self-etching primer (Transbond Plus Self Etching Primer, 3M Unitek, Monrovia, Calif) on previously extracted human teeth, some of the studies followed the enamel demineralization pattern in different time duration etching. Teeth were randomly divided into 4 groups of 12: (1) control group with a conventional etchant and separate primer, (2) experimental group with the self-etching primer, left for 15 seconds before bonding, (3) same as group 2, but with the primer left for 2 minutes before bonding, (4) same as group 2, but with the primer left for 10 minutes before bonding. For each group, stainless steel brackets were mounted onto the prepared enamel, stored for 24 h at 37°C, and tested in a testing machine with a crosshead speed of 1 mm/min. There was no significant difference in the bond strength between the 4 groups as determined by analysis of variance (ANOVA) (P < .05). Under the conditions of these experiments, a 10-minute delay in bonding after application of the self-etching primer might not be deleterious to adhesion [15].

Adhesion to enamel depends on achieving the maximum retentive capacity of the surface from the effect of acid etching. This retentive morphology should be homogeneous over the entire treated surface. Notwithstanding, the topographic quality of enamel etching with H3PO4 is not achieved over the entire adhesion surface. Espinoza’s study showed more than 50% of the treated surface was not etched. This result is in agreement with the work of Hobson, where he found more than 60% of intact surface [16].

In order to assess the effect of water and saliva contamination on the shear bond strength and bond failure site of different orthodontic primers, in vitro studies were carried out on bovine permanent mandibular incisors. Each primer-adhesive combination was tested under 7 different enamel surface conditions: (1) dry, (2) water application before priming, (3) water application after priming, (4) water application before and after priming, (5) saliva application before priming, (6) saliva application after priming, and (7) saliva application before and after priming. Stainless steel brackets were bonded in each test group with composite resin [17]. After bonding, all samples were stored in distilled water at room temperature for 24 h and then tested for shear bond strength. No contaminated enamel surfaces had the highest bond strengths for conventional, hydrophilic, and self-etching primers, which produced the same strength values. In most contaminated conditions, the self-etching primer had higher strength values than either the hydrophilic or conventional primers [18]. The self-etching primer was the least influenced by water and saliva contamination, except when moistening occurred after the recommended 3-second air burst. No significant differences in debonding locations were found among the groups bonded with the self-etching primer under the various enamel conditions [19 - 21].

After debonding, the previously etched enamel surface should be remineralized. It can be possible by all the intra- or extraoral agents which can deliver the proper level of mineral agents. The hardness and elastic modulus of the enamel surface region is decreased by enamel etching with 35% phosphoric acid or priming with self-etching primers. The influence is minimal for self-etching primer.
adhesive; those with fluoride-releasing ability may increase the hardness and elastic modulus of the enamel surface region by remineralization of the enamel. The fluoride-releasing ability of the composite resin adhesive and glass-ionomer adhesive prevents enamel demineralization and the deterioration in mechanical properties of enamel around brackets [24]. In order to obtain the almost perfect restitution of affected enamel surfaces, less aggressive agents should be used in enamel preparation for orthodontic reason. Studies have demonstrated that partial rehardening or remineralization occurs after acid etching, but the original level of mineralization cannot be restored. Even with partial enamel remineralization or surface hardening, the unsightly visual appearance of a clinical white spot lesion will remain [25].

Conclusions

In this study we presented two of the most common etching techniques recommended for orthodontic purpose. Our results showed that conditioning enamel surface with 37% phosphoric acid give a pronounced etching pattern, both interprismatic and prismatic enamel is dissolved. Etching with a self-etching primer cause a moderate demineralization of the enamel surface.

References


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