Scanning electron microscopy evaluation of the effect of etching agents on human enamel surface

Caio G. Zanet* / Victor E. Arana-Chavez** / Marcelo Fava***

Acid etching promotes microporosities on enamel surface, which provide a better bonding surface to adhesive materials. The purpose of this study was to comparatively analyze the microstructure of enamel surface after etching with 37% phosphoric acid or with two self-etching primers, Non-rinse conditioner (NRC) and Clearfil SE Bond (CSEB) using scanning electron microscopy. Thirty sound premolars were divided into 3 groups with ten teeth each: Group 1: the buccal surface was etched with 37% phosphoric acid for 15 seconds; Group 2: the buccal surface was etched with NRC for 20 seconds; Group 3: the buccal surface was etched with CSEB for 20 seconds. Teeth from Group 1 were rinsed with water; teeth from all groups were air-dried for 15 seconds. After that, all specimens were processed for scanning electron microscopy and analyzed in a Jeol 6100 SEM. The results showed deeper etching when the enamel surface was etched with 37% phosphoric acid, followed by NRC and CSEB. It is concluded that 37% phosphoric acid is still the best agent for a most effective enamel etching.

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INTRODUCTION

The majority of protocols for dental adhesion in restorative dentistry include acids to dissolve the hydroxyapatite crystals of hard dental tissues. Although acid etching was proposed for enamel using phosphoric acid[1] in concentrations ranging 30%-40%, it is currently employed for both enamel and dentin with the same concentration and time of application.[2] However, whereas the removal of hydroxyapatite exposes the organic components in dentin, the effect of acid etching on enamel is strikingly different. Acid etching promotes the complete removal of some microns of the outer enamel because of its high mineral and small amounts of organic content.[3]

A large number of studies have been carried out over the last years with the purpose to analyze the effects of acid etching on dentin but contrary little attention has been drawn to those on enamel. In relation to dentin, a debate regarding how strong should the decalcifying effect be to remove the mineral from dentin without affecting the remaining organic components, in special collagen. For this reason, a number of weak acids have been tested on dentin with the intent to substitute phosphoric acid.[4]

Phosphoric acid creates a superficial microporosities in enamel by dissolving the hydroxyapatite crystals either at the rod core or at the rod periphery of a given enamel rod.[5] The microporosities originated by the acid etching allow a better bonding of adhesive and restorative materials to enamel, due to the adhesive monomers penetrating into them, forming resin tags that yield micromechanical retention.[6] However, the effects of weak acids on enamel are not fully known.

Among the new currently available adhesive systems we find two main groups, the first one includes weak acids, and the second that contains acidic monomers.[2] Even though many authors have stated that these two conditioning agents promote a slight superficial etching of enamel which can be disadvantageous for the success of adhesive procedures,[7] used routinely when both enamel and dentin structures are simultaneously conditioned.[8] Moreover, some conditioning agents do not require rinsing whereas others already include the adhesive system.

* Caio G. Zanet, Post Graduate student

** Victor E. Arana-Chavez, Professor of the Department of Cell and Developmental Biology, Institute of Biomedical Sciences, University of São Paulo

*** Marcelo Fava, Professor of Pediatric Dentistry, Department of Preventive and Social Dentistry, School of Dentistry of São José dos Campos, UNESP

Send all correspondence to Dr. Victor E. Arana-Chavez, Laboratory of Mineralized Tissue Biology, Department of Cell and Developmental Biology, Institute of Biomedical Sciences, University of São Paulo. 05508-900 São Paulo, SP, Brazil.

E-mail: vearana@usp.br

With the purpose to analyze the effect of a weak acid (maleic acid) and of an acidic monomer (10-MDP, 10-methcryloyloxdecyl di-hydrogen phosphate) on the enamel surface, two weak conditioning agents (NRC, Non-Rinse Conditioner – Dentsply and CSEB, Clearfil SE Bond – Kuraray) were compared to 37% phosphoric acid (Dentsply) by using scanning electron microscopy (SEM).

MATERIALS AND METHODS

Thirty sound human maxillary first premolars orthodontically indicated for extraction from 14 to 16-year old teenagers of both sexes were used. All patients and their parents or legally responsible person were fully informed about the procedures and their written consent was obtained. The study was authorized by the Ethical Committee of the School of Dentistry, UNESP, Brazil. To avoid dehydration, the teeth were stored in physiological solution of 0.9% sodium chloride at room temperature until their use.

All the teeth had their root sectioned out and their crowns cleaned and polished with pumice stone and water using a Robinson bristle brush mounted on a slow-speed handpiece. The specimens were rinsed with an air/water spray and left to air-drying.

The specimens were randomly divided into three groups of ten teeth each. All teeth were etched on their buccal surface with 37% phosphoric acid (Dentsply) for 15 seconds (Group 1), with the self-etching primer Non-Rinse Conditioner –NRC (Dentsply) for 20 seconds (Group 2), or with the self-etching primer Clearfil SE Bond –CSEB (Kuraray) for 20 seconds (Group 3). The etching time for the groups 1, 2, and 3 followed the manufacturer's instruction. Only the teeth from group 1 were rinsed with water spray. Then, all specimens from the three groups were air-dried for 15 seconds.

After that, the samples were dehydrated through an increasing ethanol series (70% to absolute) and air-dried. Specimens were mounted on aluminum stubs with their treated surfaces face up, using a colloidal silver adhesive, and sputter-coated with gold in a Bal-Tec SDC-050 apparatus before examination under a JEOL 6100 scanning electron microscope, operating to 10-15 kV.

RESULTS

The etched area at the buccal surface of the tooth crowns appeared as an opaque surface clearly discernible from the smooth surrounding non-etched enamel in all the specimens, even when they were examined at low power magnifications. (Figure 1).

The specimens etched with phosphoric acid revealed a clear difference between the treated area and the surrounding intact enamel. Although several regions presented the outer enamel surface with different aspects, the most common was the removal of the rod cores. Thus, the rod peripheral regions appeared prominent while the rod cores were concave (Figure 2). In other regions, the rod periphery regions were removed and therefore they appeared as grooves surrounding the prominent rod cores.

In the specimens etched with NCR, the outer enamel surface exhibited less depth than that of phosphoric acid. The predominant etching pattern observed in this group was the removal of the rod periphery that yielded the prominence of rod cores (Figure 3).

Although the etched area was discernible when specimens etched with CSEB were examined at low magnifications, it was evident that the etching effect on the outer enamel surface was very small. Even though the examination at high magnifications was unable to identify exposed enamel crystals and therefore the

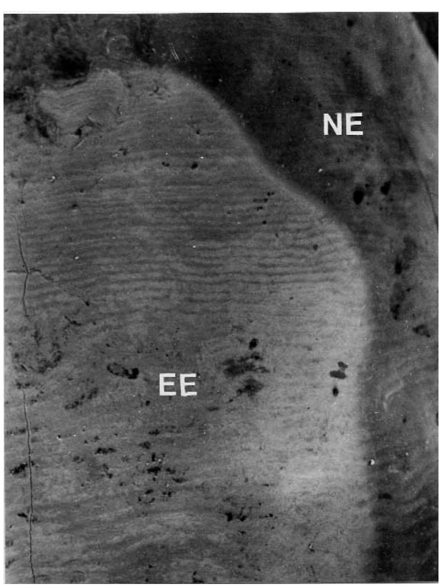

Figure 1. Scanning electron micrograph showing a low power view of the buccal face of a tooth in which acid etching has been applied. Observe that the etched area (EE) and the surrounding non-etched enamel (NE) are clearly discerned. 45x.

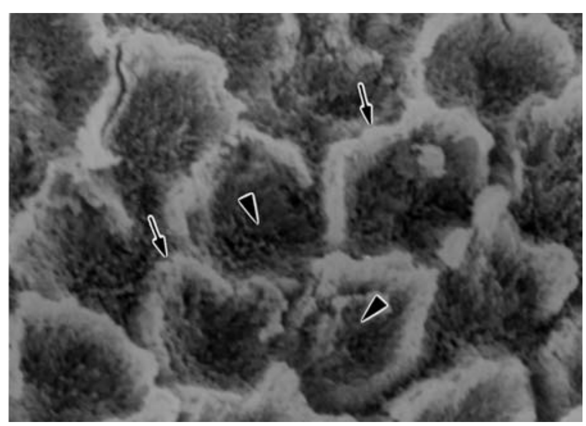

Figure 2. Scanning electron micrograph showing a specimen in which the enamel was etched with phosphoric acid. The rod peripheries appear prominent (arrows), while the rod cores are concave (arrowheads). 6000x.

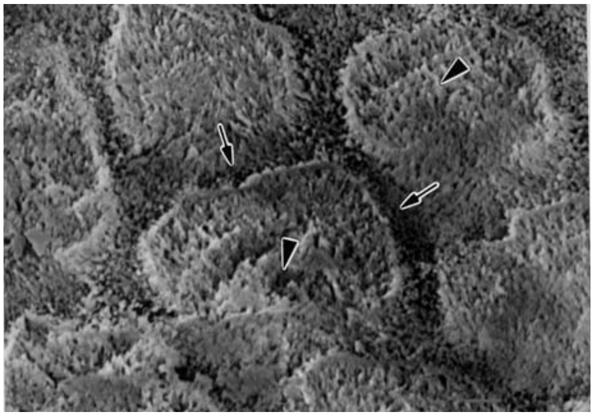

Figure 3. Scanning electron micrograph showing the enamel surface etched with NRC. The rod cores that are prominent (arrowheads) appear surrounded by grooves (arrows) that correspond to the rod peripheries. 8750x.

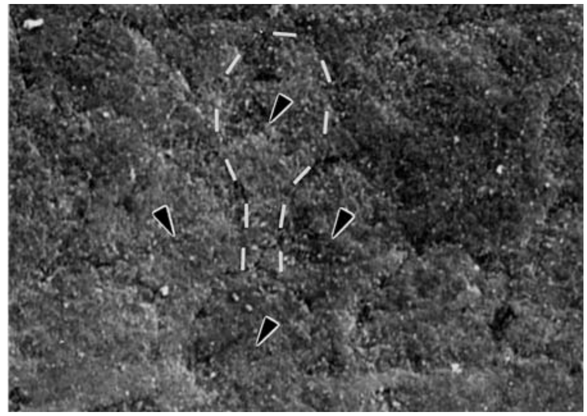

Figure 4. Scanning electron micrograph showing a specimen etched with CSEB. Observe that the effect of this conditioning agent was very low and therefore the outer enamel surface appears somewhat smooth. Slight grooves representing the boundaries between the cores and the peripheries of rods may be identified revealing a keyhole aspect (white lines). 5600x.

etched enamel surface was smooth. The slight demineralizing effect of this conditioning agent only allowed the discernment of the contour of rod cores and rod peripheries that together exhibited a keyhole appearance (Figure 4).

DISCUSSION

This SEM study showed different patterns of etching on the enamel surface with the three acids studied. The microporosities were deeper with phosphoric acid, somewhat shallower with the system containing maleic acid (NRC), and very slight with the system that contains 10-methcryloyloxdecyl di-hydrogen phosphate (CSEB).

The deep microporosities obtained with 37% phosphoric acid were expected because its use as conditioning agent is currently standard in the majority of protocols on adhesion to dental hard tissues. Two clearly identified enamel etching patterns were observed after treatment with phosphoric acid: in the first one, the rod cores appeared removed and was surrounded by relatively intact peripheries; in the second one, the peripheral regions were removed while the rod cores remained intact. Indeed, most regions exhibited the central cores removed with the rod peripheries prominent. Both etching patterns that were defined by Silverstone[5] as type I and type II, respectively, are generally considered to yield the desirable etched surface for an adequate bonding to enamel. Thus, the etching patterns promoted by this acid that requires rinsing were considered as a control for comparing the effect of the two non-rinsing agents studied.

Although it is well established that phosphoric acid provides a sufficiently roughen enamel surface for guaranteeing an efficient adhesion, the possibility of the use of a conditioning agent that does not require rinsing may represent an interesting advantage in clinical procedures. It is owing to the time employed during etching is relatively long in some clinical cases, self-etching adhesive systems have been released. They are based on hydrophilic acid monomers, do not require the rinsing step and are capable of acting as bonding agents to both enamel and dentin substrates.[9]

The etching patterns obtained with the other two agents were difficult to discern, in contrast with the typical Silvertone's etching patterns type I and type II observed in the phosphoric acid-etched specimens. The low etching capability possessed by the non-rinsing agents applied may be the reason for their slight etching effect. The ~pH 1.4 of the maleic acid (NRC) and of 10-MDP (CSEB)[10] appear to be unable to efficiently etch the enamel to a satisfactory depth, when compared with the lower pH 0.6 of phosphoric acid. In addition, it has been shown that increasing concentration of calcium and phosphate during the etching process tends to decrease the apatite dissolution.[11]

Although the findings showed herein would be expected only when enamel is etched, they higlight for attention in most protocols of adhesion due to the current tendency in restorative dentistry to use the same adhesive system for both enamel and dentin structures. Both are two clearly different dental substrates: whereas enamel contains few amounts of organic components, dentin possesses abundant collagen fibrils in intimate association with a variety of noncollagenous proteins.[12] Thus, since adhesion to enamel is mainly micromechanical, the formation of microporosities is mandatory for yielding an adequate bonding. Therefore, the results from the present study point out that phosphoric acid is still the best conditioning agent for enamel either to etch it simultaneously with dentin during cavity preparations in restorative dentistry or to etching the outer enamel surface for the use of fissure sealants, esthetics or to bond orthodontic brackets.

SUMMARY AND CONCLUSIONS

The results showed deeper etching when the enamel surface was etched with 37% phosphoric acid, followed by NRC and CSEB. It is concluded that 37% phosphoric acid is still the best agent for a most effective enamel etching.

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