Microscopic Assessment of the Enamel Etching Pattern According to Different Etching Times Using Orthophosphoric Acid Gels

RUXANDRA SAVA ROSIANU[1], COSMIN SINESCU[1], MEDA LAVINIA NEGRUTIU[1], TIBERIU HOSSZU[2]*, ANCA TUDOR[1], ANGELA CODRUTA PODARIU[1]

[1] Victor Babes University of Medicine and Pharmacy, Faculty of Dentistry, Department I, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania

[2] Vasile Goldis" Western University of Arad, 94 Revolutiei Blvd., 310025, Arad, Romania

The aim of our study was to compare micro-morphological changes in enamel surfaces after etching for 60 or 120 s by 37% phosphoric acid gels. Eighteen extracted, not carious human teeth with sound buccal surfaces were obtained, thoroughly cleaned with a water slurry of pumice powder, with a soft rubber prophylaxis cup rotating at low speed and stored in saline at room temperature. After the teeth were sectioned longitudinally in mesio-distal direction using a low-speed bur, the enamel was etched with 37% phosphoric acid (Lucstar, Romania) for 60s and 120s with applicator sponges, rinsed with water and sprayed with air for 15 s, and dried for another 15 s. Each section was evaluated under the microscope and photomicrographs were taken of representative areas that were most often observed using statistical analysis. The enamel surface etched with 37% phosphoric acid gels for 60 and 120 s showed similar etching patterns. No evident morphological differences were observed in the type of enamel etching patterns when treated with 37% phosphoric acid gels for 60 and 120 s. However, etching with 37% phosphoric acid gel for 60 s resulted in a partial removal of the prism core and the micro-morphological characteristics of the enamel surface approached those produced by 37% phosphoric acid gels used for 120 s. Phosphoric acid gel used at concentrations of 37% produced similar micro-morphological etching patterns on enamel surfaces after 60 or 120 s of application.

Keywords: enamel, orthophosphoric acid, etching, etching pattern

Phosphoric acid etching is a good method of preparing tooth enamel for bonding resins and orthodontic attachments [1]. However, one potential disadvantage is the possibility of decalcification, which leaves the enamel susceptible to caries attack, especially under orthodontic attachments [2].

Efforts have been made to develop or introduce a simplified alternative, but enamel acid etching remains the most effective procedure for stable enamel bonding. Although acid etching is considered the most popular procedure in dentistry, there are characteristics that deserve special attention because of how crucial they can be in many clinical situations.

A clinician's ability to bond a restoration to enamel has influenced changes in prosthetic and cavity preparations, restorative approaches for esthetical corrections, bonding techniques for orthodontic devices, and the treatment of caries [3-5]. In the beginning the research aimed to find an adhesive system that could interact with efficacy in dentin, and more recently, the research has focused on simplifying the systems. Enamel is the hardest tissue in the human body. Its mineral portion is approximately 96% of its weight, the rest contains organic components and water. The mineral elements include hydroxyapatite crystals, approximately 0.03 to 0.2 μm large, surrounded by a thin film of firmly bound water. In prismatic enamel, which constitutes the main fraction, the crystals are densely grouped and arranged in 3 directions. With this arrangement, lengthy prisms (a diameter of about 5 μm), from the dentin- enamel junction to the outermost surface of enamel, are formed. The prisms maintain their integrity and support because of their transverse arrangement, irregular morphology and overlapping patterns. At the moment of enamel instrumentation, the prisms are exposed in several planes according to their direction [5].

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. The difference of angulation of the prism crystals causes the acid to have higher demineralization potential at certain micro regions. Depending on the angulation of the prisms, demineralization can be greater at the prism head or at the periphery. These features are respectively known as type I and type II acid-etching patterns. This feature is important in understanding the fundamentals of adhesion though it is not clinically relevant [5].

An important clinical factor in bonding to enamel is the tissue to be bonded. The surface instrumentation, the patient's age, and environmental factors can lead to subtle differences in enamel characteristics and influence the ability of an acid conditioner to properly demineralize [5]. The aim of our study was to compare micro-morphological changes in enamel surfaces after etching for 60 or 120 s by 37% phosphoric acid gels.

Experimental part

Material and methods

Eighteen extracted, not carious human teeth with sound buccal surfaces were obtained, thoroughly cleaned with a water slurry of pumice powder with a soft rubber

* Tel.: (+40) 0257259853

Fig. 1 A377 Digital camera used for the photomicrographs

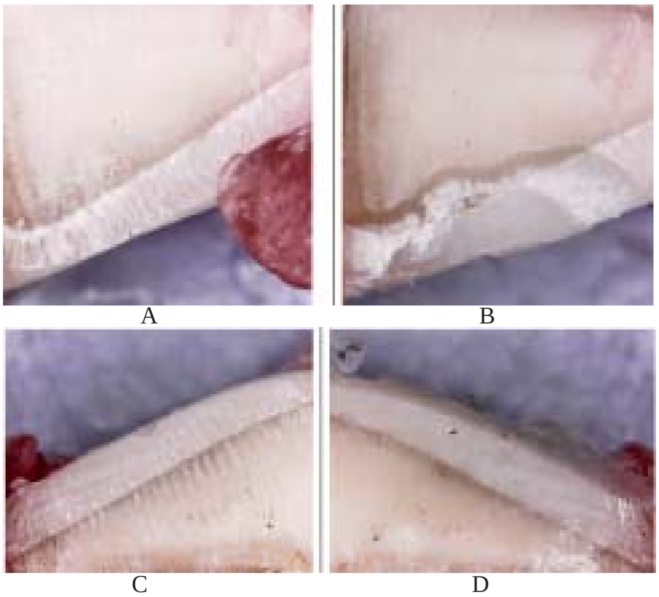

Fig. 2 A. Enamel surface before demineralization; B: Enamel surface after 60 s demineralization; C: Enamel surface before demineralization; D: Enamel surface after 60 s demineralization

prophylaxis cup rotating at low speed and stored in saline at room temperature. The roots of the teeth were removed and each crown was then mounted horizontally with the buccal surface exposed.

After the teeth were sectioned longitudinally in mesio-distal direction using a low-speed bur, the enamel was etched with 37% phosphoric acid (Lucstar, Romania) for 60s and 120s with applicator sponges, rinsed with water and sprayed with air for 15s, and dried for another 15 s. The etched enamel had a uniform, dull, frosty, appearance. A qualified researcher examined all the sections under a microscope. Each section was evaluated and photo-micrographs were taken of representative areas that were most often observed. For the photomicrographs, an A377 digital microscope camera with USB port was used. The camera has a CMOS sensor of 2 MPX and a magnification of 20X-800X, manual focus from 0 mm-40 mm and allows a measurement of 0.03 mm. It also has 10 LED lights, the intensity of the light can be manually adjusted. The PC interface was achieved by the USB 2.0 port, and the operating system was Windows XP.

Then the depth of demineralization of the tooth was statistically analysed by using one-way ANOVA at 95% conûdence interval followed by lavage. Teeth were divided into two groups according to the etching time, group 1 – 60 s etching, group 2 – 120 s etching. Tukey's post-hoc comparison test.

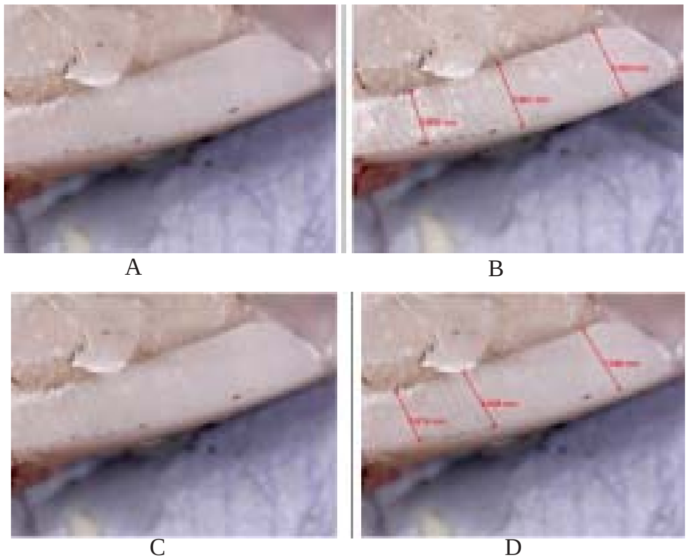

Fig. 3 A: Longitudinal section of the enamel after 60 s demineralization; B: Thickness of the enamel layer after 60 s demineralization; C: Longitudinal section of the enamel after 120 s demineralization; D: Thickness of the enamel layer after 60 s demineralization

Results and discussions

The etching changed the micro-morphological appearance of enamel surfaces independent of the etching time.

The enamel surface etched with 37% phosphoric acid gels for 60 and 120 s showed similar etching patterns. The prism core was preferentially removed leaving the prism peripheries relatively intact (fig. 2).

No evident morphological differences were observed in the type of enamel etching patterns when treated with 37% phosphoric acid gels for 60 and 120 s.

However, etching with 37% phosphoric acid gel for 60 seconds resulted in a partial removal of the prism core and the micro-morphological characteristics of the enamel surface approached those produced by 37% phosphoric acid gels used for 120 s (fig. 3).

After the statistical analysis, for the enamel surface, the lowest penetration scores were found in group 1 (1.27 ± 0.519) (table 1) and no statistical significance was found between groups.

Independent of the etching time, acid etchant used in this study affected the enamel surface morphology, creating micro-porosities by selective removal of the prism material. Enamel etching with phosphoric acid created an etch pattern characterized by a deep, uniform, area of demineralization [2]. In the presence of intact enamel, because of higher inorganic contents, the intact enamel surface presents some unique features. First, young patients' teeth have an aprismatic layer of approximately 30 μm that covers the entire crown. This layer is lost with time; however hard tissue of the teeth becomes more mineralized when exposed to the oral environment in patients with equilibrium in the demineralization process. This causes the surface layer of enamel to present hypermineralization features when compared with the innermost enamel. These 2 differences can influence the feature of the etching pattern and result in less homogeneous etching patterns, compromising the quality of bonding.

The presence of resin tags formed at the enamel surface by acid etching is an important factor in the fight against micro leakage [6]. A deeper etching pattern ensures better resin penetration, but it does not guarantee a sealant–

Table 1
COMPARISON OF THE PENETRATION SCORES OF THE ENAMEL SURFACES ON THE INCISAL AND GINGIVAL SIDES AND THE OVERALL EVALUATION USING THE KRUSKAL–WALLIS TEST (SD STANDARD DEVIATION) AND MULTIPLE-GROUP COMPARISONS BETWEEN THE DIFFERENT ETCHING TIMES FROM THE INCISAL AND GINGIVAL SIDESAND THE OVERALL EVALUATION USING THE MANN–WHITNEY U-TEST WITH BONFERRONI CORRECTION

Group	Incisal Mean ± SD	Gingival Mean ± SD	Overall Mean ± SD
Group 1	1.27 ± 0.519	1.30 ± 0.404	1.28 ± 0.250
Group 2	1.47 ± 0.477	1.82 ± 0.457	1.65 ± 0.327
Comparison 1-2	Not sign.	P<0.05	Not sign.

enamel interface that is free of micro leakage, or better sealant retention [7]. This is supported by an in vivo study that found no differences between sealants applied over a self-etching adhesive or phosphoric acid-etched teeth after 24 months [8]. However, several in vitro studies have not advocated the use of self-etching adhesives on intact enamel because of the significantly lower bond strengths, greater micro leakage, and an etching pattern that is not deep enough to obtain good penetration of the bonding resin [9, 10]. Our results are consistent with those in the literature.

Several studies report that the removal of the surface layer of enamel enhances the etching result, and consequently, the bond strength. A recent study showed that optimal bond strength to aprismatic enamel is achieved by increasing the time of acid etching suggested by the manufacturer from 15 to 30 s (with 35% phosphoric acid). It is the authors' belief that chemical and morphological characteristics of intact enamel would influence this difference. Further studies must be performed to identify the response of enamel with different mineral features when acid etched [5].

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. The difference of angulation of the prism crystals causes the acid to have higher demineralization potential at certain micro regions. Acid etching removes approximately 10μm of enamel surface and creates a morphologically porous layer (5 μm to 50 μm deep) [11, 12]. The surface free energy is doubled [13, 14] and as a result, the low-viscosity fluid resin contacts the surface and is attracted to the interior of these micro porosities created by conditioning through capillarity (capillary attraction) [15-17]. Therefore, resin tags are formed into micro porosities of conditioned enamel that after adequate polymerization, provide a resistant, long-lasting bond by micromechanical interlocking with this tissue [18-23].

A relevant factor in bonding to enamel is the cleaning of the substrate to be bonded. Some advantages clinicians attribute to acid etching are bactericidal action and cleansing potential. Phosphoric acid has an antibacterial effect [24]. To potentiate this effect, some acid conditioners with antibacterial agents (eg, 3% cetypyridinium chloride) have been made available. Without questioning the confidence of this fact, this is not the objective of the acid etching when taking into consideration exclusively bonding to enamel. For a proper etching, the surface of enamel must be clean. This cleaning must be accomplished before etching using cotton pellets soaked in agents such as chlorhexidine gluconate and benzalkonium chloride. Alternatively, the air/water spray of a triple syringe is an option in easy access locations, and the Prophylactic pastes and Robinson brushes are useful on not instrumented enamel surfaces. A suitable alternative is a bicarbonate jet. Our study showed that cleaning the teeth with a water slurry of pumice powder with a soft rubber prophylaxis cup rotating at low speed gives satisfying results.

Conclusions

Phosphoric acid gel used at concentrations of 37% produced similar micro-morphological etching patterns on enamel surfaces after 60 or 120 s of application. Additional studies that include different laser parameters are required to evaluate the level of penetration and the design of the conditioned enamel surface.

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