Comparing different enamel pretreatment options for resin-infiltration of natural non-cavitated carious lesions

ABDELAZIZ, Marwa, et al.

Abstract

To compare two different enamel pretreatments and their effect on the efficiency of penetration of a one-component adhesive into natural carious lesions.

Reference


PMID: 27093769
Comparing different enamel pretreatment options for resin-infiltration of natural non-cavitated carious lesions

MARWA ABDELAZIZ, DMD, ADELÉ LODI RIZZINI, DMD, TISSIANA BORTOLOTO, PD, DMD, PhD,
GIOVANNI TOMASSO ROCCA, DMD, ALBERT J. FEILZER, DDS, PhD, FRANKLIN GARCIA-GODOY, DDS, MS, PhD, PhD
& IVÓ KREJCI, DMD, PhD

ABSTRACT: Purpose: To compare two different enamel pretreatments and their effect on the efficiency of penetration of a one-component adhesive into natural carious lesions. Methods: Eight extracted human molars and premolars with non-cavitated interproximal lesions were selected. ICDAS code 1-2 was assessed by visual, microscopic, X-ray and Diagnocam record analysis. Samples were cut vertically across the demineralization to obtain two symmetrical lesions, (n=16). After isolating the cut surfaces with nail varnish, paired lesion halves’ surfaces were pretreated with two different techniques: Group 1: surfaces were firstly abraded with fine diamond-coated metallic strips (Steelsearbo) and then etched with 37% H₃PO₄ acid (Omini-etch, 120 seconds); Group 2: lesion surfaces were etched with 15% HCl acid (Icon-etch, 120 seconds). All teeth were stained with rhodamine isoindocyanate (RTIC) solution (12 hours) and subsequently stored in dry chamber (3 hours). All samples were penetrated with a one-component adhesive (Scotchbond Universal) for 180 seconds and coated with a thin layer of flowable composite (Tetric Flow). After light curing, unencapsulated dye was bleached by immersion in 30% hydrogen peroxide for 12 hours at 37°C. Remaining lesion pores were stained with sodium fluorescein solution. Thin cuts of the teeth were observed with confocal microscopy and computer image analysis was performed (ImageJ). The percentage of penetration (area of resin penetration/area of total demineralization ×100) was calculated. Results: Pretreatment with fine aluminum oxide-coated metallic strip followed by 37% H₃PO₄ acid showed a larger infiltration area (51.7% ± 12.2) in almost all samples compared to pretreatment with 15% HCl acid alone (22.1% ± 13.2). Statistical analysis using t-test showed a significant difference between the two groups (P= 0.011. (Am J Dent 2016;29:3-9).

CLINICAL SIGNIFICANCE: Enamel pretreatment with aluminum oxide-coated metallic strip and 37% H₃PO₄ acid is a valid alternative pretreatment to 15% HCl acid to enhance one-component adhesive penetration into natural caries lesions.

κ: Dr. Marwa Abdelaziz, Division of Cariology and Endodontology, University of Geneva, Rue Barthélémy-Menn 19, CH-1205 Geneva, Switzerland. E-κ: marwa.abdel@unige.ch

Introduction

Interproximal areas are difficult to access for daily hygiene procedures making them susceptible for caries development. The impaired access to such lesions also hampers their detection and treatment and the sacrifice of large amounts of sound enamel and dentin is often required to access the lesion.

The first clinical signs of dental caries are white spot lesions in enamel; they are characterized by a relatively unaltered enamel surface and a substantial mineral loss in the underlying lesion body. When an initial proximal caries lesion is detected, the principles of minimally invasive dentistry suggests postponing the first operative treatment as long as possible and to arrest the progression of the lesion by preventive measures only. In fact, traditional preventive measures aim to enhance remineralization by application of fluoride varnishes and improving the patients’ oral hygiene by plaque control and dietary counselling. Unfortunately, in many cases, these measures only slow down the progression of carious lesions without arresting or reversing it. Furthermore, patients’ lack of compliance may contribute to the progression of the lesion and eventually lead to enamel cavitation. Once a cavity is established, non-invasive measures are often ineffective because patients are unable to clean the dental plaque accumulated in that cavity by mechanical oral hygiene. The biofilm is protected within a microcavity and the process of demineralization continues. The presence of a cavitation is therefore often regarded as a threshold for operative intervention.

Caries infiltration is a non-invasive operative treatment for non-cavitated proximal lesions; it aims to arrest caries lesion progression by occluding the enamel porosities with low viscosity light curing resins. After the first studies on resin infiltration in the seventies, the concept was further developed in Germany, from in vitro studies on penetration of resin into cavies and marketed under the brand name of Icon. This technique involves the erosion of the relatively impermeable surface layer of the lesion with 15% hydrochloric acid gel, desiccation of the lesion with ethanol and subsequent application of a so-called infiltrant which penetrates into the lesion.

The principle of the technique is to occlude the pores with resin, thus impeding the active carious process of demineralization and stabilizing the carious lesion. It provides mechanical support to the fragile enamel by replacing the lost minerals that form the porosities with light curing resin without any invasive procedures. On the contrary to caries sealing, excess resin on the lesion surface is removed before the material is light cured. Consequently the caries-inhibiting effect is primarily achieved by occlusion of the pores within the lesion body and not by creating a composite barrier on the surface.

Several in vivo studies have shown that when this technique was used, progression was significantly lower compared with preventive measures alone. Thus, this technique
seems to be more effective than prevention in controlling proximal caries in the short and medium term\textsuperscript{11} and represents a promising non-invasive operative approach.\textsuperscript{2,9,18}

A limiting factor for proper diffusion of the resin into the body of the lesion is the hyper-mineralized superficial layer present on the surface of natural caries lesions.\textsuperscript{9,21,22} Because of alternating de- and remineralization cycles in the oral cavity, the superficial layer of a natural lesion is inhomogeneous, showing high mineral content\textsuperscript{4} and low pore volume.\textsuperscript{5,8} Its relative impermeability might hamper resin penetration and therefore the surface layer should be properly removed prior to the infiltration procedures.\textsuperscript{4,9,21}

Several studies\textsuperscript{4,7,10,23,24} have investigated how to remove the outer superficial layer with acid etching. Chemical treatment by acid etching, so called erosion, enhanced the topography of enamel, changing it from a low-reactive surface to a surface that is more susceptible to adhesion.\textsuperscript{24} It increases surface porosity and thus allows access to the inner more porous enamel.\textsuperscript{5,6,26}

Etching gels in different concentrations, such as 35-37% H\textsubscript{3}PO\textsubscript{4}, 5% and 15% HCl and varying application times have been tested.\textsuperscript{4,5,7,12,24} Orthophosphoric acid is routinely used for conditioning of enamel and dentin in adhesive dentistry.\textsuperscript{24,25} On the other hand, hydrochloric acid has been used in esthetic dentistry to remove superficial discolorations by enamel microabrasion procedures.\textsuperscript{25,27} Some studies\textsuperscript{3,8,17} showed that 15% HCl acid was more suitable to remove the hypermineralized enamel layer because of its erosive properties. Others\textsuperscript{2} showed no significant difference of maximum or average penetration depth between active lesions pretreated with either one of the acids and confirm that both acids allow for a significant increase of surface roughness of enamel.\textsuperscript{1}

The present study investigated the efficiency of resin infiltration into a lesion pretreated with a fine aluminum oxide coated metallic strip then with 37% orthophosphoric acid prior to the resin infiltration procedure to “expose” the porous structure of a natural initial caries lesion. The hypothesis was that pretreatment with a metallic abrasive strip followed by application of 37% H\textsubscript{3}PO\textsubscript{4} acid was comparable to pretreatment with 15% HCl acid to promote resin penetration in natural non-cavitated caries lesions.

Materials and Methods

Teeth selection - Eight extracted human molars and premolars were chosen from a pool of extracted teeth. The chosen teeth showed initial white/brown non-cavitated spot lesions on a proximal surface. The teeth were carefully cleaned and then photographed using a digital camera with a macro lens (Nikon D5300,\textsuperscript{8} Nikkor AF-S 105mm f/2.8 VR Micro\textsuperscript{9}). To assess the ICADAS code of the lesions,\textsuperscript{28} two trained examiners observed teeth independently, with the aid of different diagnostic tools: a stereomicroscope (Leica MZ6\textsuperscript{8}) with up to \times12 magnification combined with cold light source (Leica CLS 100\textsuperscript{8}), Radiographs, DIAGNOCam\textsuperscript{2} and DIAGNOdent.\textsuperscript{3} Only lesions that were unanimously scored as ICADAS code 1 or 2 and that presented no cavitation were included into the study.

Specimen preparation - The roots of the teeth were embedded in methacrylate resin (Technovit 4071\textsuperscript{5}) and fixed on object holders. The teeth were cut vertically perpendicular to the lesion surface (Diamond cut-off wheel, MOD 13,\textsuperscript{7} 0.4 mm) to obtain two halves. The cut surfaces were photographed using a digital camera (DS5000, Nikkor 105 micro\textsuperscript{8}). Only teeth showing two similar lesion depths in the paired lesion halves were included. One specimen broke during the procedure and was therefore excluded. One tooth presented two lesions (mesial and distal). A total of 16 lesions was evaluated in this study, eight for each group. The cut surfaces were varnished and paired lesion halves were randomly allocated to either one of two etching procedures (Group 1 and Group 2).

Surface pretreatment, first staining and infiltration - On lesion surfaces of ICADAS Group 1 (ICADAS code 1 or 2), fine aluminum oxide-coated metallic strip (Steelcarbo\textsuperscript{8}) were used to abrade the most mineralized outer layer. Then the 37% H\textsubscript{3}PO\textsubscript{4} gel (Omni-Etch\textsuperscript{9}) was applied for 120 seconds.

In ICADAS Group 2 lesions (ICADAS code 1 or 2), halves were etched with 15% HCl (Icon-Etch\textsuperscript{9}) for 120 seconds. In both groups the acidic gel was gently applied with a microbrush. Subsequently, the acid gel was washed away using a water-air spray dental syringe for 60 seconds.

Indirect staining technique\textsuperscript{29} - All teeth were stored in an ethanolic solution of rhodamine B isothiocyanate (RITC 0.1%) for 12 hours. The lesions were dried by storage in a desiccator containing silica gel for 3 hours. A one-component adhesive (Scotchbond Universal\textsuperscript{30}) was applied on the lesion surface using a microbrush and allowed to penetrate for 3 minutes with reapplication after 1.5 minutes. Excess material was gently wiped away with dry microbrushes before light curing for 40 seconds using an LED high-power device, which delivered a power density of 1,200 mW/cm\textsuperscript{2} (Bluephase\textsuperscript{31}). A second application of Scotchbond Universal was performed and it was allowed to penetrate for 1 minute. Excess material was wiped away and then polymerized for 40 seconds. Finally a layer of a flowable resin composite (Tetric EvoFlow) was applied and after removing the excess it was polymerized for 40 seconds. To bleach all red fluorophores from RITC that were not enclosed by the resin, specimens were stored in 30% hydrogen peroxide solution for 12 hours at 37°C. Subsequently, specimens were washed with tap water for 10 seconds.

Preparation of thin slices and second staining - The half-cut surfaces with the nail varnish were gently polished with abrasive papers (2,400, 4,000 grit, Labo Pol-2\textsuperscript{32}). Then, the specimens were fixed in the diamond saw (Diamond cut-off wheel, MOD 13, 0.4 mm) and thin sections (0.8 mm-1.0 mm) were obtained. To visualize porous structures such as non-infiltred lesion parts, specimens were immersed in a 50% ethanol solution of 100 μm sodium fluorescein (NaFl); for 3 minutes. Subsequently, specimens were washed in water for 10 seconds.

Confocal laser scanning microscopy and image analysis - Specimens were observed using a confocal scanner microscope (CLSM, Leica SP5-2P) with \times10 objective in dual fluorescence mode to detect RITC (red) and NaFl (green) fluorescence simultaneously as previously described.\textsuperscript{33} The area of demineralization appeared green and the resin-penetrated area appeared red. RGB (stack) images were recorded with a size of 512 × 512 pixels, 1,542.47 × 1,542.47 μm in size and if lesions exceeded the size of one microscopic field of view, multiple images were taken. Image analysis was performed.
using ImageJ® software. The area of demineralization in green was outlined and measured (LAdemin). Subsequently each stack image was split into the three grayscale images containing the red, green and blue components of the original RGB image: in the red image the threshold was adjusted and used to calculate the area of the resin penetration (InfAdemin). The area measurements were based on maximum projections of z-stacks of about 100 μm thickness of the lesion.

Results

In CLSM analysis, porous structure like the lesion body was displayed in green due to staining with fluorescein; the resin penetration was displayed red due to RITC staining and resin penetration (Figs. 2-4). In the final sample of eight paired lesions (n = 16 in total), seven out of eight showed higher values of area penetration percentage when pretreated with metallic strip and H$_3$PO$_4$ acid (Table). Group 1 (pretreatment with abrasive metallic strip + 37% H$_3$PO$_4$ acid) showed penetration areas ranging from 34.3% to 64% of the total lesion size while penetrated area for Group 2 (pretreatment with 15% HCl acid) ranged from 4.8% to 39.2%.

Pretreatment with a fine aluminum oxide-coated metallic strip and 37% H$_3$PO$_4$ acid showed a larger penetration area on average (51.7% ± 12.2) compared to pretreatment with 15% HCl acid alone where the mean penetrated area was (22.1% ± 13.2). Statistical analysis using t-test showed a significant difference between the two groups (P = 0.011).

Lesions in Group 1 showed a more dense penetration pattern than lesions of Group 2 (Fig. 2,3). Lesions clinically classified as active showed comparable penetrated areas in both groups, whereas inactive lesions showed less penetrated areas in Group 2 (Fig. 4).

Discussion

The results described above confirmed the hypothesis of this study and demonstrated that when compared to pre-treat-

<table>
<thead>
<tr>
<th>Group 1: Stripping + H$_3$PO$_4$</th>
<th>Group 2: HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of infiltration</td>
<td>% of infiltration</td>
</tr>
<tr>
<td>62.153%</td>
<td>12.681%</td>
</tr>
<tr>
<td>50.328%</td>
<td>31.061%</td>
</tr>
<tr>
<td>63.381%</td>
<td>4.861%</td>
</tr>
<tr>
<td>47.674%</td>
<td>32.095%</td>
</tr>
<tr>
<td>64.031%</td>
<td>19.496%</td>
</tr>
<tr>
<td>34.493%</td>
<td>31.893%</td>
</tr>
<tr>
<td>34.320%</td>
<td>39.274%</td>
</tr>
<tr>
<td>57.561%</td>
<td>5.983%</td>
</tr>
</tbody>
</table>

ment with 15% HCl etching to prepare the lesion surface for resin penetration, enamel pre-treatment with a fine aluminum oxide metallic strip followed by the application of 37% H$_3$PO$_4$ acid for 120 seconds had comparable if not better results of the resin infiltration area.

The technique for confocal microscopy using multiple fluorescent dyes with well-separated excitation and emission wavelengths allows selective visualization of the materials and the remaining porous structures; it is therefore suitable for detailed structural analysis of porous hard tissues.5

The penetration of a liquid (uncured resin) into a porous structure (enamel lesion) can be described by the Washburn equation.8,9,31 Physically, this equation assumes that the porous structure is a bundle of parallel cylindrical tubes of uniform radius. The penetration of the liquid is driven by capillary forces30 and the equation relates the distance (d) that is traversed by the liquid in time (t) to the surface tension (γ), the contact angle (θ), the viscosity of the liquid (penetration coefficient (η)), and the capillary radius (porous structure) (r):

$$d^2 = \frac{(γ \cos θ + 2η)}{t}$$

Given the same liquid (adhesive resin), the parameters that can influence the penetration in natural caries lesions are: removal of organic material contaminants,32,34 effective removal of the outer mineralized layer,5,10,17,32 proper drying of the porous lesion16 and resin application time.8,10,31

The Washburn equation simplifies the real conditions during penetration of enamel subsurface lesions. The bottom of enamel lesions is not open, thus entrapped air or water may have an influence on penetration because it may remain at the bottom of the lesion, impeding resin penetration into this region.5 Furthermore, the pore diameters within the lesions vary in the various lesion zones, influencing the penetration speed.6 Thus, before resin application, drying of the lesion is required to remove water from the porosities and therefore enable capillary action to soak the resin into the pores. Desiccation favors resin penetration by increasing the surface free energy.16 This is why in this study the specimens were stored in a desiccator for 3 hours to allow complete water removal from enamel porosities.

The superficial layer of enamel lesions is a very densely mineralized thin layer of enamel and it represents an obstacle for proper diffusion of the resin into the more porous underlying body of the lesion.5,21,22 According to an X-ray microtomography study,22 the surface layer thickness of white spot lesions has great variability and ranges from 35 to 130 μm.
The maximum mineral content of the lesions is in the superficial layer and it varies between 74% and 100% of that of sound enamel. The present results suggest that low resin penetration can be mainly attributed to remaining highly mineralized surface parts. Therefore, a complete erosion of the surface layer and exposure of the lesion body is considered essential for a conditioning procedure prior to infiltration of low-viscosity resins.

Several studies have compared conditioning procedures such as H₃PO₄ and HCl acid etching. Orthophosphoric acid is widely used in concentrations of 35-37% for conditioning of enamel and dentin surfaces in adhesive dentistry. In previous studies, it was used for the reduction of the surface layer prior to resin penetration of artificial and natural lesions. Hydrochloric acid, due to its erosive properties, has been used in esthetic dentistry to remove superficial discolorations by enamel microabrasion and to erode the superficial layer prior to the so-called infiltration technique. Analysis of the literature showed various results regarding the use of these etchants as penetration pre-treatments. According to some in vitro studies, surface etching with 30-40% phosphoric acid was inferior to pretreatment with 15% hydrochloric acid with respect to surface layer reduction. Also, the application of 37% H₃PO₄ for 30 seconds resulted in less permeable enamel compared to 15% HCl applied for 120 seconds. Another study reported that both the 37% orthophosphoric acid and the 15% hydrochloric acid applied for 120 seconds led to a statistically significant increase in surface roughness of enamel affected by acute and chronic non-cavitated carious lesions. Other results showed that in active lesions, application time of 120 seconds either with 35% orthophosphoric acid or 15% hydrochloric acid resulted in no significant difference in the maximum percentage of penetration depth and average percentage of penetration depth. These results suggest the need to combine 37% orthophosphoric acid gel with an additional pretreatment to guarantee more uniform results.
An important aspect to consider for a correct enamel penetration is a proper decontamination of the surface to treat. In the oral cavity the enamel lesion is exposed to several contaminants, such as biofilm and proteins, which tend to decrease the surface energy of enamel. These contaminants limit the penetration and the efficacy of etching agents, leading to significantly impaired resin penetration. The remaining debris and biofilm might not be removed by conventional prophylaxis or etching process alone. Therefore, additional pretreatment procedures have been proposed. In pit and fissure sealing, air abrasion with aluminum oxide particles was shown by Yazici et al. to eliminate organic material at the bottom and walls of fissures and to remove the superficial aprismatic and fluoride-rich enamel layer leading to a more reactive tooth enamel. This procedure induced a more retentive etching pattern and enhanced etchant penetration into the enamel, strongly suggesting the need to add a further pretreatment for the proximal penetration technique to assure an efficient decontamination of the surface prior to the etching procedure.

In the present in vitro study, the biofilm removal was achieved by cleaning the samples and pretreating them with a fine aluminum oxide metallic strip prior to the application of phosphoric acid gel. Metallic strips are commonly used in orthodontic procedures to adjust tooth width by stripping. The different grits can offer a controlled interproximal reduction. Fine metallic strips of 40 µm grit may be suitable to remove the 35/130 µm-thick enamel layer. Furthermore, the instrumentation of the enamel tissue by a mechanical preparation can change its response to the acid etching: the removal of the surface layer of enamel enhances the etching results and consequently the adhesive technique. A mechanical pretreatment was demonstrated to remove the relatively etch resistant layer of enamel thus allowing a better action of the etching allowing for a more efficient resin penetration.

Therefore, given the impaired accessibility of proximal surfaces to hygiene procedures, treatment with a fine aluminum oxide coated metallic strip can help remove the organic contaminants while removing at the same time the outer hypermineralized enamel layer, leading to a more retentive etching pattern and a more permeable enamel surface after phosphoric acid etching.

The application of the etching gels alone and in addition to the metallic strip were analyzed in an unpublished study in our laboratory. In Fig. 5, scanning electronic microscopy (SEM) images show that the superficial pattern of sound enamel treated with either etching gel could only partially condition the enamel surface. On the other hand, the application of a metallic Al₂O₃ strip followed by H₃PO₄ gel application led to an enamel pattern more favorable for resin adhesion and lesion penetration.

It may be argued that interproximal stripping could weaken the proximal contact area. The exact location and shape of the white spot lesion is determined by the localization of the biofilm. Thus, on a proximal surface, the lesion formed beneath the biofilm is a kidney-shaped area between the contact area and the gingival margin, thus below the contact area. In an in vivo situation a dental wedge can be placed to slightly separate the surfaces and ease the procedures. Therefore, the use of the metallic strip on the interproximal surface will not weaken the proximal contact.

It could also be argued that the removal of the surface layer might additionally weaken the lesion structure. In the present study, however, no cavitation occurred after the pretreatment procedure. It was also previously discussed that using abrasives could leave grooves on the surface. There is, therefore, some concern that undetected microwravities in the roughened enamel surface when not effectively filled by the resin might hamper the long-term success of the treatment. To further safeguard the clinical outcome of the infiltration, an additional
is a Senior Lecturer; Dr. Rocca is a Senior Lecturer and Head Clinical Supervisor; and Dr. Krejci is Professor and Chair. Division of Cardiology and Endodontology, University Clinics of Dental Medicine, University of Geneva, Geneva, Switzerland. Dr. Feizler is Professor, Department of Dental Materials Science, and Dean, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and VU University Amsterdam, Amsterdam, The Netherlands. Dr. Garcia-Godoy is Professor and Senior Executive Associate Dean for Research, College of Dentistry, University of Tennessee Health Science Center, Memphis, Tennessee, USA.

References
Enamel pretreatment & resin infiltration


