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Abstract
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Reference

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Non-invasive proximal adhesive restoration of natural non-cavitated proximal lesions

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ABSTRACT: Purpose: To investigate the infiltration potential of different self-etch adhesives into natural non-cavitated proximal lesions and the effect of dehydration protocol on the infiltration of a self-etch adhesive. Methods: 29 extracted molars and premolars with natural proximal lesions (ICDAS 1-2) were sectioned through the lesion providing two samples from each lesion. To compare the different adhesives, three groups of eight lesions were abraded with fine metallic strips and then etched with 37% H₃PO₄ acid for 120 seconds. All teeth were stained with rhodamine isothiocyanate. After drying with compressed air and ethanol application, lesions were infiltrated with Scotchbond Universal, Clearfil SE Protect or OneCoat 7 Universal for 180 seconds and then coated with a thin layer of flowable composite (Tetric Flow). To compare the effect of dehydration protocol on infiltration, two groups of nine paired lesions were pretreated as described above. One group was dried using compressed air alone and the second group was dried using compressed air and ethanol, both groups were then infiltrated with Scotchbond Universal then coated with a thin film of flowable composites. After light curing, un-encapsulated dye was bleached by immersion in hydrogen peroxide. 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). Results: ANOVA and Duncan post-hoc tests showed no significant differences of the infiltrated area between the three adhesives (P= 0.835), no significant difference was found between the group dried with air compared to the one dried with air and ethanol. It can be concluded that the tested adhesives may be used for infiltration of natural lesions following the described pretreatment. (Am J Dent 2018;31:243-248).

CLINICAL SIGNIFICANCE: Enamel pretreatment with metallic strip and 37% H₃PO₄ acid promotes the infiltration of different adhesives into natural non-cavitated caries lesions.

Introduction

Sealing and infiltration seem to arrest proximal lesions better than fluoride applications and flossing. Sealing proximal lesions has been described in many studies and although it provides the external protection, the lesion is not mechanically reinforced in-depth as is the case with resin infiltration. The concept of lesion infiltration provides a non-invasive possibility to partly reinforce the non-cavitated lesion reaching the first third of enamel on bitewing radiographs with a low viscosity resin.

To enhance resin infiltration, it was important to develop a material with a high penetration coefficient (PC). The penetration coefficient of multiple products revealed wide variations that ranged from 4.0 to 474.9 cm/second. The addition of ethanol significantly increased the PCs due to a decrease of viscosity and contact angle. Highest PCs were found for mixtures containing TEGDMA, HEMA and 20% ethanol.

After 2011, the development of the infiltrant (ICON”), and its application technique continued; the penetration coefficient (PC) was improved by a mixture of ethanol and a resin, mainly consisting of triethylene glycol dimethacrylate (TEGDMA).

Yet an infiltrant composed mainly from triethylene glycol dimethacrylate (TEGDMA) has many disadvantages on the long-term due to high water sorption and degradation by acid challenges. When compared to an unfilled Bis-GMA based bonding resin (HelioBond®), the infiltrant exhibited a higher extent of oxygen inhibition, lower hardness, lower elastic modulus and higher plastic to elastic indentation energy. Rahiotis et al explained these inferior qualities by the fact that TEGDMA is selectively released from homo- or co-polymers with rigid aromatic dimethylacrylate monomers after immersion in water or in polar solvents implying a polymer network prone to chemical degradation. This may explain other findings where the protective effect of HelioBond exerted on enamel exposed to an acidic environment was better than that of the infiltrant. Providing a protective resin layer over the infiltrated lesion reduces the risk of degradation over time.

The question about the use of adhesives for infiltration of initial carious lesions is not recent; the use of classic adhesives for infiltration has been described repeatedly in the literature. In 2001 and 2002 two studies described the infiltration of adhesives into enamel lesions. They used artificially demineralized bovine enamel and showed that 95% of the lesion’s body was filled and that sealants reduced the pore volumes within the lesion. In 2004 a study measured the penetration depth of an unfilled resin (HelioBond) into artificial lesions after 120 seconds of etching measured a mean penetration depth of 68 microns. In 2006 the penetration ability of five dental adhesives and a fissure sealant into artificial initial enamel lesions for an application time of either 15 or 30 seconds was evaluated and it was demonstrated that the longer application time allowed for deeper infiltration.

The removal of the hypermineralized superficial layer in natural initial carious lesions seems to be a crucial factor to enhance the infiltration of the infiltrant. An investigation compared the infiltration of the infiltrant into natural lesions etched for 120 seconds by either HCl or H₃PO₄ and since the results showed that infiltration was enhanced in lesions etched...
with 15% hydrochloric acid (HCl), this acid was chosen due to its erosive properties that enables the removal of the hypermineralized layer. In subsequent experiments, the timing and the frequency of application of this experimental resin infiltrant was tested and optimized.16-18

Even though it was demonstrated that the use of 15% HCl was more reliable and effective to remove the outer hypermineralized layer in natural lesions,19 phosphoric acid in concentrations of 30-40% is widely used for routine conditioning of enamel and has been applied to expose the subsurface of non-cavitated lesions prior to sealing them20 or prior to the infiltration of artificial lesions with resins.12,14

Another study21 proved that the active application of 37% H3PO4 with a brush for 30 seconds increased the porosity volume of surface layer and the percentage of infiltrated areas in comparison to the application of 15% HCl for 120 seconds.

Recently, one study22 showed that passive HCL gel application may form a CO2 gas bubble that may negatively affect the removal of the outer layer and hence the infiltration. On the other hand, active application seemed to improve the homogeneity of the etching pattern.

Enamel pretreatment to enhance the removal of the hypermineralized layer seems to be an essential step to enable infiltration of resins into the subsurface lesion. A recent study from our group23 compared the infiltration of one component adhesive (Scotchbond Universal) after removing the hypermineralized layer using a metallic strip and then etching for 2 minutes with H3PO4, demonstrating a deeper infiltration of the adhesive compared to enamel pretreatment with HCl for 120 seconds.

The aim of the present study was (1) to compare three commercial adhesives for infiltration of natural lesions after removing their outer layer with a metallic strip followed by 120 seconds etching with H3PO4, and (2) to compare also the effect of the drying protocol on the infiltrated area of these natural lesions.

The null hypothesis stated that there was no difference of the infiltrated area among the three adhesives, and that there was no difference between drying with compressed air and drying with compressed air followed by ethanol application prior to infiltration.

**Materials and Methods**

Extracted human molars and premolars were chosen from a pool of cleaned extracted teeth. The chosen teeth showed initial white/brown spot lesions on a proximal surface. The teeth were carefully cleaned and then photographed using a digital camera with a macro lens (Nikon D5300, Nikon AS-F Micro 105 mm). To assess the ICDAS codes of the lesions, examiners assessed the teeth independently, with the aid of different diagnostic tools: a stereomicroscope up to ×4 magnification (Leica CLS 100, Leica MZ6), digital radiography, and DIAGNOcam.1 Only lesions that were unanimously scored as ICDAS code 1 or 2 and that presented no cavitation were included in this study.

For the first part of the study, 21 teeth were used while for the second part, eight teeth were used. Sample size was calculated based on an average infiltration area of 25% (SD 10), difference of 16%, power adjusted at 80%, and with the level of confidence set to 95%, (P< 0.05). The sample size required at least eight samples in each group.

The roots of the teeth were embedded in methacrylate resin (Technovit 4071) and fixed on object holders. The teeth were cut vertically perpendicular to the lesion surface (Diamond cut-off wheel, MOD 13, 0.4 mm). The cut surfaces were photographed using a digital camera (Nikon D5300, Nikon AS-F Micro 105 mm). Only teeth showing distinct lesion on cut surfaces were included. Five teeth presented two lesions (mesial and distal); a total of 21 lesions were evaluated for the first part (eight per group) while for the second part paired lesions were used and each group had nine lesions. The cut surfaces were protected by two layers of transparent varnish.

On all the lesions' surfaces, fine diamond coated metallic strips were used to remove the most mineralized outer layer of the lesion. Then 37% orthophosphoric acid was applied for 120 seconds, un-waxed floss was used to activate and distribute the acid.

Subsequently, the gel was washed away using a water-air spray dental syringe. All teeth were then stored in an ethanol solution of rhodamine B isothiocyanate (RTIC 0.1%) for 12 hours as described by Paris et al.24

For the first part of the study, lesion surfaces were firstly air dried for 10 seconds, then 96% ethanol was applied for 30 seconds and subsequently air dried for another 10 seconds. The lesions were then infiltrated with three different self-etch adhesive systems: Group 1.1: Scotchbond Universal, Group 1.2: Onecoat 7 Universal,1 and Group 1.3: Clearfil SE Protect Bond.25

For the second part, paired lesion halves were allocated to either one of two drying procedures. Lesion surfaces of Group 2.1 were dried using compressed air for 30 seconds; the air was blown tangential to the surface as to simulate air drying through the contact point. In Group 2.2 lesion surfaces were firstly air dried for 10 seconds, then 96% ethanol was applied for 30 seconds and subsequently air dried for another 10-15 seconds.

In all groups, the application procedure was as follows: The
adhesive was applied on the lesion surface using a fine microbrush and allowed to penetrate for 3 minutes with re-application after 1.5 minutes. Excess resin material was gently wiped away with a dry microbrush before light curing for 40 seconds. A second application of adhesive was performed and it was allowed to penetrate for 1 minute. Excess material was wiped away and then polymerized for 40 seconds. A thin film of a flowable resin composite (TetricEvo Flow®) was applied and distributed using un-waxed dental floss and after removing the excess it was polymerized for 40 seconds using a high-power LED device, which delivered a power density of 1.200 mW/cm² (Bluephase®). To bleach all red fluorophores from RTC which were not enclosed by the adhesive, specimens were stored in 30% hydrogen peroxide solution for 12 hours at 37°C. Subsequently, specimens were washed with tap water for 10 seconds.

The surfaces with the nail varnish were gently polished (2,400, 4,000 Struers, Labo Pol-2®) before remounted in the saw (Struers®) to obtain sections of around 1 mm thickness. To visualize porous structures such as non-infiltrated lesion parts, specimens were immersed in a 50% ethanol solution of 100 μM sodium fluorescein (NaF) for 3 minutes. Subsequently, specimens were washed in water for 10 seconds. Specimens were observed using confocal laser scanning microscope (CLSM) (Leica SP5-2P) with ×10 objective in dual fluorescence mode to detect RTC (red) and NaFl (green) fluorescence simultaneously as described by Paris et al. The area of demineralization appeared green and the resin infiltration appeared red. Images were 512 × 512 pixels in size and if lesions exceeded the size of one microscopic field of view, multiple images were taken.

Image analysis was performed using Image® software. In the microscopic images, the areas of demineralized enamel (LAdemin) in green and the extent of the infiltrated area in the demineralized enamel (InfAdemin) in red were measured with the threshold tool. To calculate how the infiltrant penetrated into the lesion the following variable was calculated: percentage of infiltration = area of infiltration/area of total demineralization × 100 (Inf%Ademin = InfAdemin/LAdemin × 100) as described in a previous study.

The null hypothesis was verified by applying ANOVA and Duncan post-hoc tests for the first part and the student t-test for the second part using SPSS® for Mac, software version 21, with level of confidence set to 95% (P< 0.05).

Results

In CLSM analysis, porous structures like the lesion body were displayed in green due to staining with fluorescein. The resin penetration was displayed in red due to RTC staining retained in resin after polymerization (Fig. 1).

The Table provides an overview of the results of both parts of the study. For the first part of the study the percentages of the infiltrated areas were: OneCoat 7 Universal (Group 1.1): 27.7% (SD 14.9); Scotchbond Universal (Group 1.2): 28.8% (SD 14.6); and Clearfil SE Protect (Group 1.3): 24.7% (SD 12.9). ANOVA and Duncan post-hoc tests showed no significant differences between groups (P= 0.835). Figure 2a provides a boxplot to show the differences among the three groups.
For the second part, average resin infiltration area for the lesions dried with air (Group 2.1) was 30.2% (SD 13.4) while for lesions dried with ethanol and air (Group 2.2) it was 27.45% (SD 13.3). Student t-test showed no significant difference between the groups treated with air or ethanol (P= 0.653). Figure 2b provides a boxplot to visualize the differences between the two groups. Of the nine paired lesions of the second part of the experiment, seven lesions were in enamel while two reached dentin. When looking at enamel lesions alone (n=7), the average of lesions dried with air or ethanol were very close (29.7 SD 14.4) and (29.2 SD15.3), respectively. While the two remaining dentin lesions seemed to be better infiltrated when dried with air only (Fig. 2).

**Discussion**

This study investigated the infiltration of three commercially available adhesives into natural lesions and compared the effect of dehydration protocol on the quality of resin infiltration. The results showed no significant difference between the three adhesives tested and no significant difference between compressed air alone and combination of air and ethanol. Infiltration with the three different adhesives tested and with the two drying protocols did not influence the infiltrated area when done after the removal of the hypermineralized layer with an abrasive strip and subsequent etching with 37% orthophosphoric acid.

The average of infiltrated area from all five groups combined in the present study was 27.7%, which is similar to the reported area infiltrated with the resin infiltrant ICON which reached up to 25%.15,26

The results are in agreement with a study77 that compared three different adhesives for the infiltration and sealing of non-cavitated proximal lesions: Excite (E), Prime & Bond NT (PB), and Single Bond (SB). It was concluded that all three adhesives could be used for infiltration and that the maximum average of adhesive penetration into the lesions was 697 ± 412 μm, with these reported average values per group: 612.5 ± 354 μm (SB), 693.3 ± 471.6 μm (PB), and 786 ± 393.5 mm (E) and no significant differences among the groups (P> 0.05).

Regarding the drying protocols, the literature12,28-30 showed that dehydration of enamel caries lesions with ethanol or acetone prior to caries infiltration enhanced resin penetration into deeper enamel lesions. This effect is attributed to the fact that organic solvents such as ethanol lead to increased displacement of water from enamel porosities, enhancing resin penetration.28,29 On the other hand, other studies12,30 demonstrated that when all lesions were analyzed without taking into consideration the lesion depth, no significant differences between various pretreatments could be observed. One explanation might be due to the preparation of the samples, since all teeth were kept in the ethanol rhodamine solution to color the lesions for 12 hours. The long exposure to ethanol might have rendered the second ethanol application irrelevant. Further studies should investigate the dehydration procedure using another protocol, avoiding the ethanol exposure prior to infiltration.

Multiple factors influence sealing and resin infiltration into non-cavitated enamel lesions. These factors have been studied and continuously improved since the introduction of adhesive systems.33,34 They can be mainly divided into tooth related and procedure related factors. Tooth related factors include: (1) lesion depth:22,29 Superficial lesions tend to be better infiltrated. Besides the fact that resin had to penetrate less enamel to fill the lesion, this may also be explained by deeper and better dehydration due to reduced depth; (2) Lesion activity:22,29 Active lesions have open pores and the outer layer is less mineralized than the one found in arrested lesions, which may affect the speed and depth of infiltration as it varies through the depth of the lesion; (3) Surface contamination:18,30,31 Organic protein layer covering the enamel can alter the treatment if not removed properly before the infiltration procedure.

Procedure related factors involve: (1) Surface pretreatment: Mechanical pretreatment can be achieved by abrasive strips (proximal surfaces), or by air abrasion (occlusal or buccal surfaces) while chemical pretreatment is usually obtained using some kind of acid. The type of acid, how it is applied (passive or active) and its application time influence the removal of the hypermineralized layer.18,30,31,37,39 (2) Dehydration of the treated enamel: Dehydration of enamel caries lesions with ethanol or acetone prior to caries infiltration enhanced resin penetration into deeper enamel lesions.12,28,30 This effect is attributed to the fact that organic solvents such as ethanol lead to increased displacement of water from the enamel porosities which enhances resin penetration.31,32 (3) Resin viscosity (penetration coefficient): This factor appears to facilitate the penetration of the resin into deeper lesions.4,10 as well as (4) Application time and frequency, which seems to be optimal at 3 minutes according to the results reported using the resin infiltrant (ICON).7,40

The results from this study might suggest that if the superficial layer is removed efficiently, lesions can be infiltrated with regular adhesives, even if they are slightly charged by filler particles, as was the case in the present study. The superficial layer is a very densely mineralized thin layer of the enamel surface and it represents an obstacle for proper diffusion of the resin into the more porous underlying body of the lesion.19 According to an x-ray microtomography study,41 the superficial 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. Other results2 suggest that negligible resin penetration can be mainly attributed to the remaining highly mineralized surface parts. Therefore, a complete erosion of the surface layer and exposure of the lesion body have been the aim of a conditioning procedure prior to the infiltration with low-viscosity resins, mostly using 15% HCl applied for 120 seconds to erode the surface layer.18,42 Studies showed that using 37% H3PO4 was equally effective if the surface layer is abraded using a rotating brush to activate the acid for 30 seconds21 or by stripping/abrating the lesion with a fine metallic strip.39

Scanning electron microscope (SEM) images showed that both HCl and H3PO4 only partially conditioned sound enamel surfaces.39 This could be explained by CO2 gas bubbles observed under the HCl etching gel as described in a recent study.27 The use of an abrasive metallic strip to remove the hypermineralized layer of a natural lesion followed by 120 seconds H3PO4 gel application79 showed more resin infiltrated
area probably due to an enamel pattern more favorable for resin adhesion and infiltration. 

The enhanced homogenous removal of the hypermineralized enamel surface layer and the exposure of the porous subsurface enamel used in the protocol presented in this study might explain the similar average of infiltrated areas in all groups. In the present study, due to the small sample size, no differentiation between active or inactive lesions was investigated. Active lesions have a lower mineral content and a more porous surface layer compared to inactive ones. This might explain the elevated standard deviation value in the present results and other previous studies on natural lesions.

The flowable composite layer applied after the infiltration provides an extra protection against further acid challenge and transforms the mere infiltration into a restoration that has more potential for arresting the lesion. 

Within its limitations, this study showed that natural non-cavitated proximal lesions pretreated with an abrasive strip prior to etching with 37% orthophosphoric acid can be infiltrated using different available self-etch adhesives. The drying protocols do not seem to influence the infiltrated area of such lesions. Clinical studies are needed to confirm the findings of this in vitro evaluation.

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c. 3M ESPE, St. Paul, MN, USA.

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e. Leica, Heidelberg, Germany.

f. KaVo, Biberach/Riß, Germany.

g. Kulzer, Wahrheim, Germany.

h. Siven, Ballerup, Denmark.

i. HORICO, Berlin, Germany.

j. OMNIDENT Dental, Rodgau, Germany.

k. Sigma-Albrich, Steinheim, Germany.

l. Coltene/Whaledent AG, Alstätten, Switzerland.

m. Kuraray Noritake Dental Inc., Okayama, Japan.

n. Microbrush, Grafton, Wisconsin, USA.

o. Sigma-Albrich, Brondby, Denmark.

p. NIH, Bethesda, MD, USA.

q. IBM SPSS, Armonk, NY, USA.

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