©Operative Dentistry, 2015, 40-5, 492-502

In Vitro Effects of Resin Infiltration on Enamel Erosion Inhibition

GC Oliveira • AP Boteon • FQ Ionta
MJ Moretto • HM Honório • L Wang
D Rios

Clinical Relevance

While patient compliance is key to preventive measures related to dental erosion, the application of resin-based materials could serve as an alternative treatment for inhibiting erosion progression.

SUMMARY

Resin-based materials that show promising effects for preventing the progression of erosion have been studied. This *in vitro* study evaluated the effects of applying resin-based materials, including resin infiltration, on previously eroded enamel subjected to erosive challenges. The influence of enamel surface etching prior to application of the material was also studied. Bovine enamel blocks were immersed in hydrochloric acid (HCl), 0.01 M (pH 2.3), for 30 seconds in order to form a softened erosion lesion. The blocks were then randomly divided into nine groups (n=12) and treated as follows: C = control without treatment; Hel = pit & fissure resin sealant (Helioseal Clear); Adh = two-step self-etching adhesive system (AdheSe); Tet = two-step conventional adhesive system (Tetric N-bond); and Inf = infiltrant (Icon). The Helno, Adhno, Tetno, and Infno groups received the same materials without (or with no) surface conditioning. The depth of the material's penetration into softened erosion lesions was qualitatively analyzed using reflection and fluorescence confocal microscopy. After application of the materials, the blocks were immersed in HCl for two minutes; this step was followed by immersion in artificial saliva for 120 minutes four times a day for five days (erosive cycling). Both the enamel alteration and material thickness were analyzed using

Gabriela Cristina de Oliveira, DDS, MS, PhD student, Bauru School of Dentistry, University of São Paulo, Pediatric Dentistry, Orthodontics and Public Health, Bauru, São Paulo, Brazil

Ana Paula Boteon, DDS, MS student, Bauru School of Dentistry, University of São Paulo, Operative Dentistry, Endodontics and Dental Materials, Bauru, São Paulo, Brazil

Franciny Querobim Ionta, DDS, MS, Bauru School of Dentistry, University of São Paulo, Pediatric Dentistry, Orthodontics and Public Health, Bauru, São Paulo, Brazil

Marcelo Juliano Moretto, DDS, MS, PhD, Bauru School of Dentistry, University of São Paulo, Pediatric Dentistry, Orthodontics and Public Health, Bauru, São Paulo, Brazil

Heitor Marques Honório, DDS, MS, PhD, Bauru School of Dentistry, University of São Paulo, Pediatric Dentistry, Orthodontics and Public Health, Bauru, São Paulo, Brazil

Linda Wang, DDS, MS, PhD, Bauru School of Dentistry, University of São Paulo, Operative Dentistry, Endodontics and Dental Materials, Bauru, São Paulo, Brazil

*Daniela Rios, DDS, MS, PhD, Bauru School of Dentistry, University of São Paulo, Pediatric Dentistry, Orthodontics and Public Health, Bauru, São Paulo, Brazil

*Corresponding author: Alameda Octavio Pinheiro Brisolla 9-75, Bauru, São Paulo 17012-901, Brazil; e-mail: danirosop@yahoo.com.br

DOI: 10.2341/14-162-L

profilometry, and the results were submitted to Kruskal-Wallis and Dunn tests (p>0.05). Images from the confocal microscopy showed minimal penetration of Adh/Adhno and deep penetration of Inf/Infno into the erosive lesions. The groups Hel, Adh, Inf, Tetno, and Infno resulted in the formation of a layer of material over the enamel, which was effective in inhibiting the progression of erosion. In conclusion, the infiltrant, with or without etching, was able to penetrate and protect the enamel against dental erosion. The other resin-based materials, except for the two-step conventional adhesive, were able to penetrate and inhibit the progression of erosive lesions only when they were applied after enamel etching.

INTRODUCTION

Dental erosive wear has become a more prevalent and increasing clinical concern.[1-4] The ideal treatment for arresting the development of erosion is to eliminate the cause, which is not always practical or achievable.[5] For this reason, most studies related to the prevention and treatment of dental erosive wear have focused on various fluoride compounds.[6-10] However, there are controversial findings in the literature related to the effectiveness of fluoride in terms of reducing or preventing erosive tooth wear.[11-13] Another proposed therapy is the use of resin-based materials over the dental tissue, which serves as a mechanical barrier between the enamel/dentin and the acidic attack.[14] In a series of *in vitro*, *in situ*, and clinical studies,[15-19] a research group investigated the protective effects of resin-based sealants and adhesives against dentin erosive wear. In summary, coating the dentin with a resin-based bonding agent resulted in a protective effect that lasted for up to three months.[18] On the other hand, the use of a fissure sealant to protect palatal dentin surfaces showed the prevention of tooth wear for up to nine months.[19] Recently, Wegehaupt and others[20] evaluated *in vitro* the long-term protective effects of surface sealants against enamel erosive wear by hydrochloric and citric acids. The resin-based surface sealants tested reduced the enamel loss under long-term (28-day) acid exposition.[18]

Resin infiltration is a new approach that was developed to counteract incipient enamel caries lesions.[19-27] In contrast to conventional sealants, in which the material adheres to the enamel surface, resin infiltration penetrates into the porous lesion body of enamel's initial carious lesions using a special low-viscosity resin that blocks the diffusion of acids into the lesion, thereby slowing or arresting the progression of caries.[21-29] Reflecting back, resin-based materials, such as pit & fissure sealants and adhesives, were not developed to seal erosive lesions, and since resin infiltration blocks the demineralizing effects of cariogenic acids, it is important to note the effects of resin infiltration on erosive lesions. However, the manufacturer contraindicates its use for erosion, regardless of whether anything has been reported in the literature related to the use of resin infiltration to treat erosive lesions. To promote resin infiltration in the resin infiltration system, hydrochloric acid (HCl) is used to remove the hypermineralized superficial layer of the carious lesion.[30] Nevertheless, there remains a concern about the possibility of removing the softened eroded enamel, which could impair the resin infiltrant adhesion, compromising its possible effects against erosion.

Therefore, the present study evaluated the effects of the application of resin-based materials, including resin infiltration, on previously eroded enamel subject to erosive challenges. The influence of eroded enamel surface conditioning prior to material application was also studied. The hypothesis of this study was that all of the resin-based materials that were evaluated would be able to protect eroded enamel against erosion and that enamel etching would not interfere with this effect.

METHODS AND MATERIALS

Experimental Design

This blinded study evaluated the preventive effects of four resin-based materials (pit & fissure resin sealant, Helioseal Clear; self-etching adhesive, AdheSE; conventional adhesive, Tetric N-bond; and the infiltrant Icon) against the progression of dental erosion. Each material was applied with and without enamel superficial conditioning and compared to the control (enamel without resin-based material application). The enamel samples were initially eroded and randomly divided into the studied groups (each group, n=12) for resin-based materials application. The erosive challenge was conducted for five days. The response variable was profilometry (blind analysis for the studied materials). Two additional specimens per group were used to illustrate penetration of the resin-based materials into the eroded enamel using confocal microscopic visualization.

Sample Preparation

Freshly extracted bovine teeth were sectioned at the cementum-enamel junction with a water-cooled,

Table 1: *Resin-based Materials Group, Composition, and Application Steps According to the Manufacturer's Instructions*

Material	Group	Composition	Application Steps
Helioseal Clear (Hel)	Pit & fissure resin sealant	Bis-GMA, TEGDMA, and additives	37% phosphoric acid (30 s), rinsing, and drying; helioseal clear (15 s) and polymerization
AdheSE (Adh)	Self-etching adhesive system	AdheSE Primer: acrylate acid phosphoric, bis-acrylamide derivate, and additives; AdheSE Bond: dimethacrylate, HEMA, silicic dioxide, initiators, and stabilizers	AdheSe-primer (30 s); drying; AdheSe-bond (5 s); drying and polymerization
Tetric N-bond (Tet)	Conventional adhesive system	Bis-GMA, ethanol, HEMA, acrylate phosphonic acid, glycerin dimethacrylate, urethane dimethacrylate, di-tri-phenyl-phosphine-methyl benzoyl, and additives	37% phosphoric acid (30 s), rinsing and drying; tetric N-bond (5 s); drying and polymerization
Icon (Inf)	Infiltrant	Icon-etch: hydrochloric acid, pyrogenic silicic acid, surface-active substances; Icon-dry: 99% ethanol; Icon-infiltrant: methacrylate-based resin matrix, initiators, and additives	Etching with 15% hydrochloric acid (120 s), rinsing and drying; 95% ethanol- and air-drying; resin infiltration with a syringe (180 s); polymerization; and infiltrant reapplication (60 s) plus polymerization

Abbreviations: Bis-GMA, bisphenol A diglycidyl ether dimethacrylate; HEMA, 2-hydroxyethyl methacrylate; TEGDMA, triethylene glycol dimethacrylate.

diamond-coated disc (Extec Corp, Enfield, CT, USA) using an ISOMET low-speed saw cutting machine (Buehler Ltd, Lake Bluff, IL, USA). The crowns were individually placed, enamel surface down, in a silicone cylindrical mold with an inner diameter of 5.6 cm, and these crowns were embedded in acrylic resin (Jet Ltd, Campo Limpo Paulista, SP, Brazil). After removing the samples from the mold, they were ground flat with water-cooled silicon carbide discs (320, 600, and 1200 grades of Al_2O_3 paper; Buehler Ltd) and polished with felt paper wet by diamond spray (1 μm; Buehler Ltd). The loss of enamel during the grinding steps was controlled with a micrometer (Mitutoyo, Tokyo, Japan) to be approximately 200 μm. The samples were cleaned using an ultrasonic device for 10 minutes and were then checked microscopically (40×; Carl Zeiss Micro-imaging GmbH 37081, Göttingen, Germany) for the presence of white spots and cracks.

A surface Knoop hardness (KHN) test was performed (five indentations in the center of the slab spaced 200 μm apart, 25*g*, five seconds; HMV-2000; Shimadzu Corporation, Tokyo, Japan) to select 200 bovine enamel blocks (SHi) with hardness values between 317 and 388 KHN (mean surface hardness of 353 ± 17 KHN). The bovine enamel samples were then subjected to short-term acidic exposure by immersion in 0.01M HCl (pH 2.3) for 30 seconds (17.6 mL per block), resulting in surface softening without tissue loss.[31] The surface hardness determination was performed again (SHd) with five measurements localized at a distance of 100 μm in relation to the initial indentations for the final selection of 108 enamel samples with initial erosive lesions (hardness values between 149 and 193 KHN [mean surface hardness of 171 ± 11 KHN]).

After selection, the materials were applied according to the manufacturers' instructions (Table 1). On groups without superficial enamel etching for both the pit & fissure resin sealant and the conventional adhesive system, the enamel was not etched with 37% phosphoric acid gel. On samples from the self-etching adhesive system group, the enamel was not conditioned with AdheSE Primer. Finally, on the infiltrant group, the enamel was not conditioned with 15% HCl gel.

Erosive Cycling

The samples were subjected to five days of erosive cycling by immersion in 0.01M HCl, pH 2.3 (17.6 mL per sample), for two minutes at 37°C under constant motion, followed by immersion in artificial saliva (17.6 mL per sample) for two hours. This cycle was repeated four times per day, and at the end of each day, the samples were stored overnight (14 hours) in artificial saliva.[32] The composition of the artificial saliva that was used was 0.33 g KH_2PO_4; 0.34 g Na_2HPO_4; 1.27 g KCl; 0.16 g NaSCN; 0.58 g NaCl; 0.17 g $CaCl_2$; 0.16 g NH_4Cl; 0.2 g urea; 0.03 g glucose; 0.002 g ascorbic acid; 2.7 g mucin in 1000 mL of distilled water; and pH 7.0.[33]

Profilometric Analysis

Prior to treatment, identification marks were made on the sample surfaces using a scalpel, which allowed for accurate repositioning of the stylus. Subsequently, five baseline surface profiles were

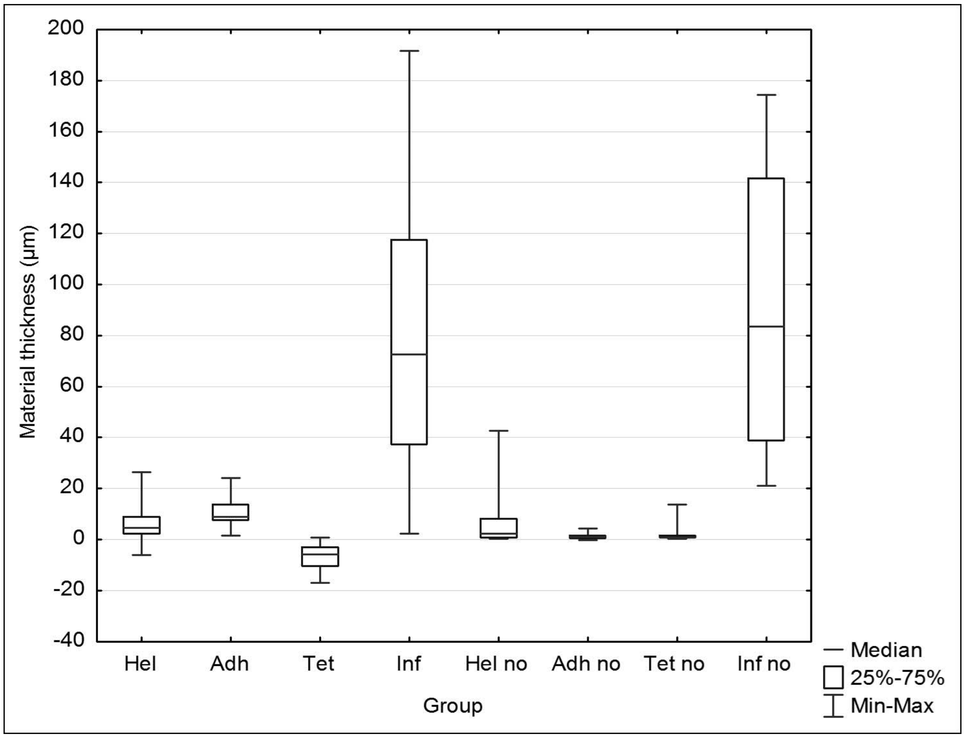

Figure 1. *Median, interquartile range, minimum and maximum values of the resin-based material thickness after application (µm). Nomenclature (Hel = pit & fissure resin sealant, Helioseal Clear; Adh = two-step self-etching adhesive system, AdheSE; Tet = two-step conventional adhesive system, Tetric N-bond; and Icon = infiltrant, Icon). Only the name of the material = application according to manufacturer's instruction. The name of the material + no = application without enamel etching. Median followed by distinct lowercase letters represents the significant difference among the groups, considering material thickness (Kruskal-Wallis and Dunn test,* $p<0.05$*).*

obtained from all of the samples as references using a profilometer (MarSurf GD 25, Göttingen, Germany) at a certain distance: 2.25, 2.0, 1.75, 1.5, and 1.25 µm. The marks and two-thirds of the enamel surface were covered with nail varnish and the resin-based materials were applied. The nail varnish was removed, and profilometric analysis was performed again at the same sites used for the baseline measurements. Then, after recovering the marks with nail varnish, the samples were subjected to erosive cycling. The nail varnish was subsequently removed to enable another profilometric analysis. The resin-based material thickness after application and material and/or enamel loss after erosive cycling were quantitatively determined using specific software (MarSurf XCR 20) by calculating the average thickness of the materials and the depth of the eroded surface relative to the baseline surface profiles, respectively. Since the enamel samples could be precisely repositioned in the wells of the profilometer, it was possible to match the respective baseline and final profiles.[34]

Confocal Microscopy Analysis

A 0.05 mg/mL ethanolic solution of tetramethylrhodamine isothiocyanate (Sigma-Aldrich, Steinheim, Germany), was used to label the materials under study by adding 0.02 mL of this solution in 0.5 mL of the material.[35,36] The treatments were performed according to each group (two eroded enamel specimens per group), following the manufacturers' instructions. Resin penetration was observed using confocal laser scanning microscopy (CLSM; LEICA TCS SPE, Leica Microsystems CMS, Mannheim, Germany); the microscope was equipped with four solid-state lasers from 488 to 635 nm. The specimens were observed using a 40× objective in fluorescence (wavelength λ=532 nm) and reflection (wavelength λ=488 nm) modes.

Statistical Analysis

Statistical analysis was performed using SigmaPlot version 12.3 (2011 Systat Software GmbH, Erkrath, Germany). The assumptions of normal distribution of errors (Shapiro-Wilk test) and equality of variances were checked. Since the assumptions were not satisfied, the Kruskal-Wallis test and the Dunn post hoc test were applied. The significance level was set at 5%.

RESULTS

The thickness of the studied materials over the enamel specimens is provided in Figure 1. The thickest layer resulted from the application of resin infiltrant; however, there was no significant difference compared to the self-etching adhesive and pit & fissure sealant

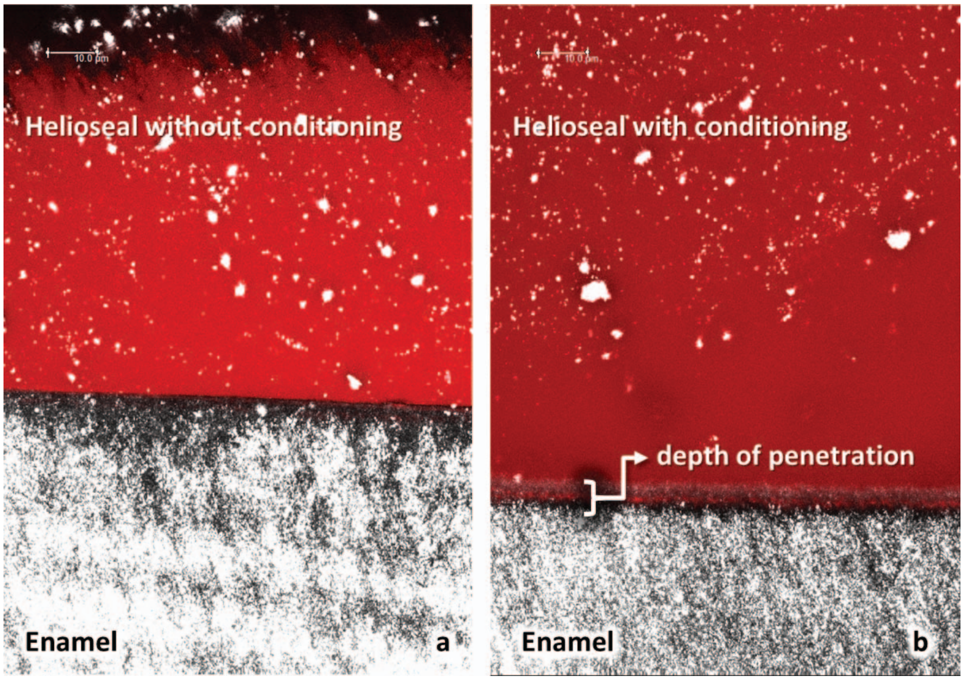

Figure 2. *CLSM pictures of penetration of pit & fissure sealant, Hel. (a) Without enamel etching; (b) with enamel etching.*

when enamel etching was performed. After application of the conventional adhesive with previous enamel etching, negative values, which represent the absence of material over enamel and even enamel loss, were observed. This group showed no significant differences between the same material and the self-etching adhesive without conditioning, since both materials showed a very thin layer of material.

CLSM pictures showed the presence of pit & fissure sealant over enamel regardless of the enamel etching (Figure 2a,b); however, material penetration was observed only when the enamel was etched (Figure 2b). In Figures 3a and 4a it was possible to observe the absence of enamel etching in the self-etching adhesive and conventional adhesive groups, which resulted in the absence of penetra-

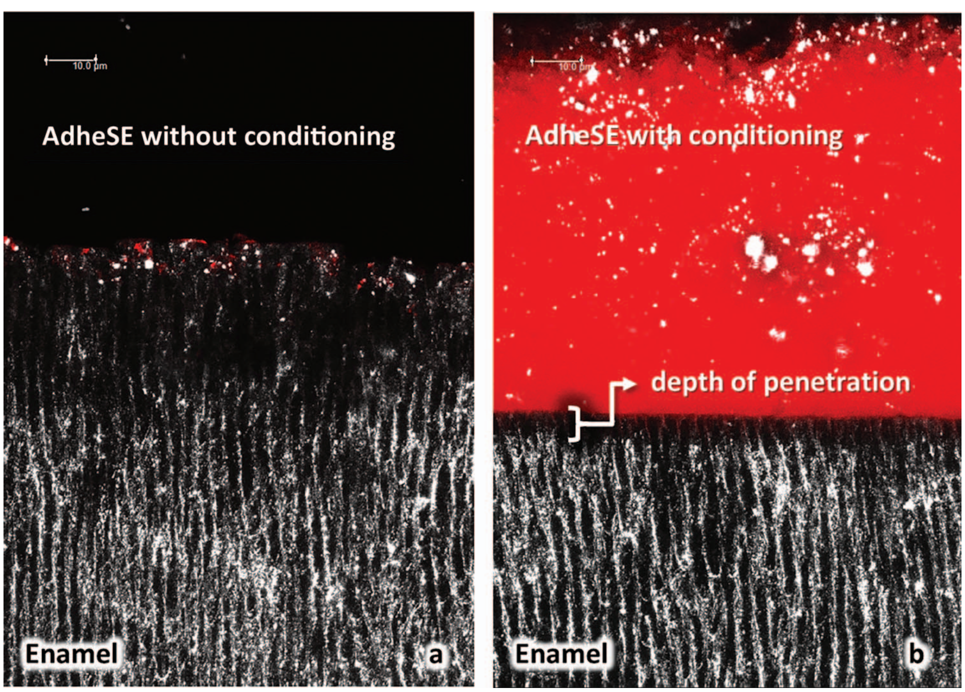

Figure 3. *CLSM pictures of penetration of two-step self-etching adhesive system, Adh. (a) Without enamel etching; (b) with enamel etching.*

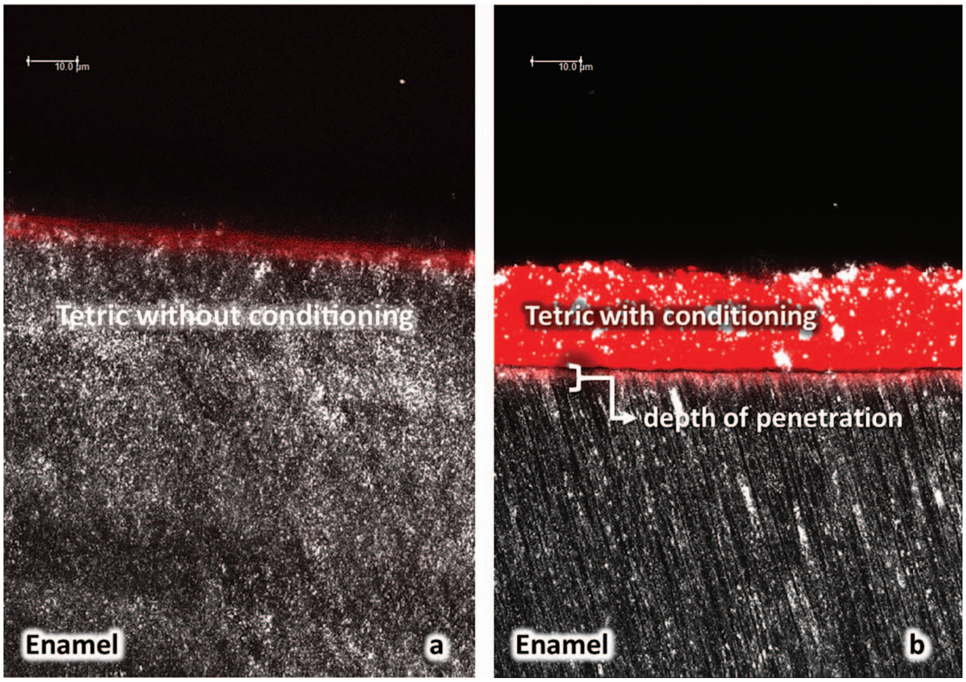

Figure 4. *CLSM pictures of penetration of two-step conventional adhesive system, Tet. (a) Without enamel etching; (b) with enamel etching.*

tion and material. On the other hand, groups in which primer or phosphoric acid was applied showed a thin layer of material penetration into the enamel (Figures 3b and 4b). The resin infiltrant showed the deepest material penetration (Figure 5a,b), especially when hydrochloric acid was used (Figure 5b).

All of the resin-based materials applied with previous conditioning provided enamel protection against erosive cycling, except for the conventional adhesive (Figure 6). On specimens in which the enamel was not conditioned, enamel loss similar to that of the control group ($p>0.05$) could be seen; only on the resin infiltrant and conventional adhesive

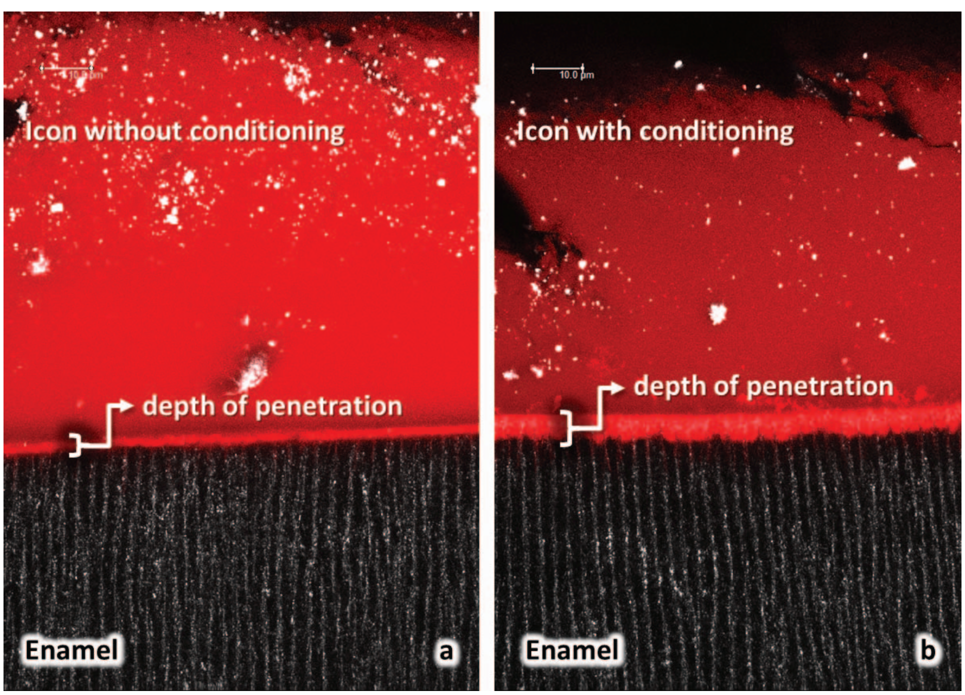

Figure 5. *CLSM pictures of penetration of infiltrant, Icon. (a) Without enamel etching; (b) with enamel etching.*

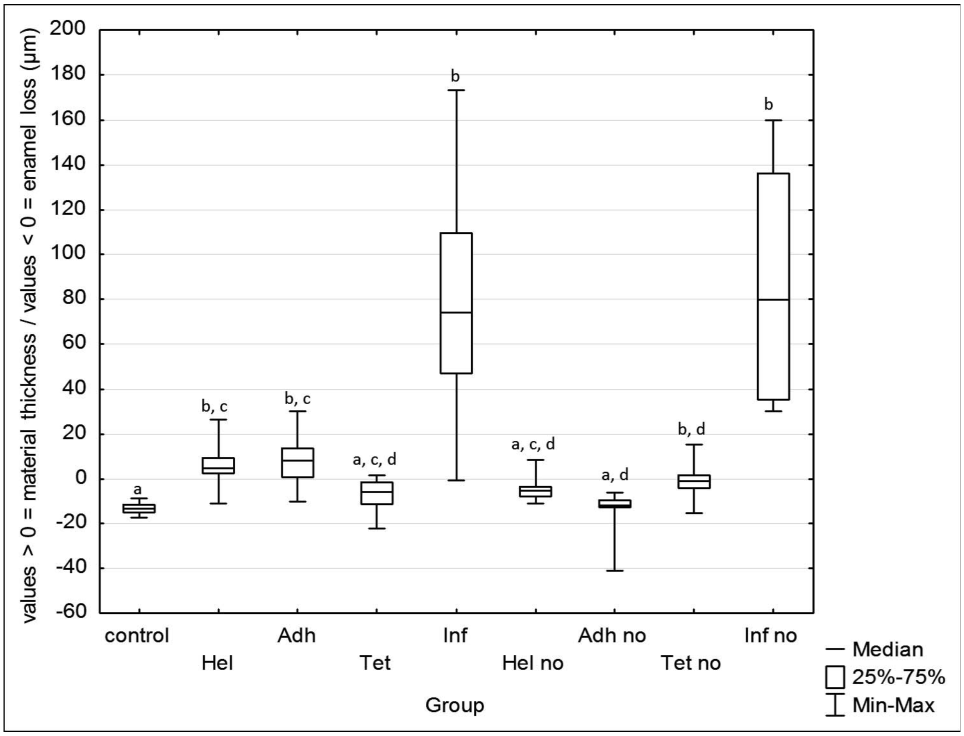

Figure 6. *Median, interquartile range, minimum and maximum values of the material and/or enamel loss after erosive cycling (µm). Nomenclature (Hel = pit & fissure resin sealant, Helioseal Clear; Adh = two-step self-etching adhesive system, AdheSE; Tet = two-step conventional adhesive system, Tetric N-bond; and Icon = infiltrant, Icon). Only the name of the material = application according to manufacturer's instruction. The name of the material + no = application without enamel etching. Median followed by distinct lowercase letters represents the significant difference among the groups, considering enamel loss (Kruskal-Wallis and Dunn test,* $p<0.05$*).*

groups was the material maintained on the enamel, preventing enamel wear (Figure 6).

DISCUSSION

The application of resin-based materials preceded by enamel etching, except for the conventional adhesive, protected the enamel against the progression of erosion. However, it is important to note that this result was obtained from an *in vitro* protocol with a five-day erosive challenge. According to Wegehaupt and others,[20] an erosive time of six minutes with HCl (pH 3.0) simulates one day of an intraoral clinical situation, since, in gastroesophageal reflux patients, the pH drops below 5.5 for 4.3 minutes during 24 hours.[37] In the present study, the HCl pH was 2.3 and the blocks were immersed for 40 minutes; thus, the cycling protocol might simulate 10 or more *in vivo* days. However, it is not known how these materials might act clinically on erosive challenges of a longer duration, especially with regard to the infiltrant. There are clinical studies that demonstrate the need for reapplication of the adhesive and pit & fissure sealant after three and nine months, respectively, to maintain their preventive effects in relation to dentin wear. Those clinical studies were conducted with other commercial brands, and the materials were applied over dentin, not enamel.[18,19]

The adhesives were not designed to be exposed to the oral environment, since they were developed to enhance the adhesion of resin composites. When used over dental substrate to form a mechanical barrier against the action of acids, similar to resin pit & fissure sealants, their effectiveness is related to their retention and durability. Those characteristics depend on two factors: penetrability into the acid-etched enamel and wear resistance in the oral environment.[38]

Resin infiltration was developed for the conservative treatment of initial carious lesions,[29] which are characterized by a subsuperficial structure of demineralization.[39,40] To promote resin penetration, HCl is used to remove the hypermineralized superficial layer of the carious lesion. On the other hand, the initial erosive lesion corresponds to a superficial softened area[39-41] that might be penetrated by resin infiltration. However, an additional effect of HCl as the infiltrant conditioner on an eroded and softened area could be enamel wear, lower mineral content, or a mechanically less-stable surface. Considering these aspects, resin infiltrant without acid etching was tested. For standardizing purposes, the other materials were also tested without enamel conditioning even though phosphoric acid promotes less enamel alteration when compared to HCl. In the groups where the enamel was etched, and mainly in the infiltrant group, it is likely that the softened layer of the initial erosion lesion was removed. Other studies have reported that enamel etching with phosphoric acid and HCl resulted in enamel loss of approximately 10 and 15 μm, respectively,[23,38] which corresponds to a thicker layer compared with initial erosion.[39] Even with the possible removal of the erosion lesion, the images obtained with confocal microscopy showed that all materials penetrated into the previously etched enamel, with an emphasis on the infiltrant, which presented a thick, homogeneous, and deep penetration compared to the other studied materials. This greater penetration showed that the remaining enamel presented appropriate characteristics for adhesion (Figure 5). According to Lussi and others,[41] persistent acidic attacks result in substance loss in which the outermost superficial enamel is eliminated and the remaining tissue is softened. This softened tissue reaches equilibrium and there is no further progress, not even with prolonged acidic impact when bulk mineral undergoes further dissolution.[42] Thus, this equilibrated, softened tissue might also show constant characteristics related to enamel adhesion.

Penetration of the infiltrant was an unexpected result that was obtained, even when there was no enamel etching. A possible explanation for this is the type of acid used to develop the initial lesion of erosion—HCl—which corresponds to the indicated acid for enamel etching for the infiltrant. Note that the length of time for which the acid was applied, which can influence the surface characteristics of enamel, was lower in this study (30 seconds × 120 seconds), since the objective was to form an initial erosion lesion (softened surface) without wear. A pilot study was conducted to determine the amount of time required for enamel to soften without enamel loss. This characteristic was assessed by the loss of surface hardness. The parameter used was the loss of sharpness of the indentations' limits after acidic attack at each 15-second interval compared to the indentations performed on sound enamel. In the etching technique for the infiltration of caries, HCl is used for two minutes.

In terms of wear in the oral environment, resin-based materials are subject to two major challenges: acids and mechanical forces. Laboratory studies indirectly and directly found an erosive resistance to adhesives and fissure sealants.[43,44] Even under prolonged *in vitro* erosive challenge, resin-based materials remained on the dental substrate, protecting the dentin against erosion.[43] Different types and brands of adhesives might show different behaviors in terms of adhesion and their ability to protect against enamel erosion. The results of this study show enamel wear when conventional adhesive was applied (Figure 4). It is hypothesized that there were enamel sites that were acid etched but not covered with adhesive. The application of two adhesive layers could compensate for this failure; however, in this instance, one cover layer was applied for standardization purposes. In addition, despite enamel loss after application of the conventional adhesive, substantial additional enamel loss was not observed when the specimens were subjected to erosive challenge, suggesting a protective effect. When comparing thickness of the material after application (Figure 1) with thickness of the same material subjected to erosive challenge (Figure 6), minimal wear was noted for the groups with enamel conditioning. For the pit & fissure resin sealant and self-etching adhesive system without enamel conditioning, the material thickness was significantly less after the acid attack. Nevertheless, this phenomenon was due to the entire material loss, not material wear.

There are no data related to infiltrant resistance to acids. In the present study, the infiltrant was able to protect the enamel. And even after the erosive challenge, the thickness of the infiltrant that covered the enamel surface was nearly the same, regardless of enamel conditioning. In Figure 1, the thick layer of the infiltrant can be seen, which resulted from the mode of application and apparatus provided by the manufacturer. A layer of infiltrant of lesser thickness could have been produced if a microbrush was used, as was the case in the other groups. On the other hand, after 20,000 abrasion cycles, when the infiltrant vs the adhesive applied over the caries lesion was compared with the original enamel (42.6±20.7 μm vs 40.4±18.5 μm, $p>0.05$), nonsignificant differences in vertical wear loss were measured.[45] However, the infiltrant material showed surface and morphological aspects that pointed to improved surface stability and infiltration quality.[45] Thus, a thicker layer of infiltrant might be beneficial for wear resistance to toothbrush abrasion and might be tested in future studies. Furthermore, color stability of the infiltrant merits further study to assess its potential as a preventive layer for smooth enamel surfaces in esthetic areas.[46]

The resistance of resin-based materials to abrasion from tooth brushing is poorly reported in the literature.[47] Adhesives are mainly studied as composite surface coatings.[48,49] When considering erosion associated with brush abrasion, the results are controversial.[50,51] An *in vitro* study[51] showed that after one year of tooth brushing, significant surface deterioration with deleterious loss of enamel and discoloration was observed in all four of the tested sealants. The authors emphasized the need for revision of the application of sealants on smooth enamel surfaces.[51] In addition to the promising results of the present study, more studies are necessary to clarify retention and durability of the studied resin-based materials, especially in terms of prolonged erosive and abrasive challenges. Furthermore, before resin infiltration can be tested in clinical situations, it is also important to know the effects of the material on dentin erosion inhibition, since erosive lesions are frequently diagnosed at an advanced stage when the dentin is compromised.

CONCLUSIONS

Infiltrant applied with or without enamel etching was able to penetrate and protect enamel from dental erosion. Other resin-based materials, except for two-step conventional adhesives, were able to penetrate and inhibit the progression of erosive lesions only when applied after enamel etching. However, especially in the case of infiltrant, this was the first step to evaluating the infiltrant's ability to prevent the progression of erosion, and further studies will be necessary before these results can be extrapolated for clinical use.

Acknowlegement

The authors acknowledge the financial support of FAPESP (Processes 2011/12395-4; 2011/16208-4).

Human Subjects Statement

This study was conducted at the Bauru School of Dentistry, University of São Paulo.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

(Accepted 27 October 2014)

REFERENCES

1. Huysmans MC, Chew HP, & Ellwood RP (2011) Clinical studies of dental erosion and erosive wear *Caries Research* **45(Supplement 1)** 60-68.
2. Dugmore CR, & Rock WP (2003) The progression of tooth erosion in a cohort of adolescents of mixed ethnicity *International Journal of Paediatric Dentistry* **13(5)** 295-303.
3. El Aidi H, Bronkhorst EM, Huysmans MC, & Truin GJ (2010) Dynamics of tooth erosion in adolescents: A 3-year longitudinal study *Journal of Dentistry* **38(2)** 131-137.
4. Jaeggi T, & Lussi A (2006) Prevalence, incidence and distribution of erosion *Monographs in Oral Science* **20** 44-65.
5. Lussi A, & Carvalho TS (2014) Erosive tooth wear: A multifactorial condition of growing concern and increasing knowledge *Monographs in Oral Science* **25** 1-15.
6. Ganss C, Schlueter N, Hardt M, Schattenberg P, & Klimek J (2008) Effect of fluoride compounds on enamel erosion in vitro: A comparison of amine, sodium and stannous fluoride *Caries Research* **42(1)** 2-7.
7. Ren YF, Liu X, Fadel N, Malmstrom H, Barnes V, & Xu T (2011) Preventive effects of dentifrice containing 5000 ppm fluoride against dental erosion in situ *Journal of Dentistry* **39(10)** 672-678.
8. Wegehaupt FJ, Sener B, Attin T, & Schmidlin PR (2011) Anti-erosive potential of amine fluoride, cerium chloride and laser irradiation application on dentine *Archives of Oral Biology* **56(12)** 1541-1547.
9. Kato MT, Lancia M, Sales-Peres SH, & Buzalaf MA (2010) Preventive effect of commercial desensitizing toothpastes on bovine enamel erosion in vitro *Caries Research* **44(2)** 85-89.
10. Schlueter N, Duran A, Klimek J, & Ganss C (2009) Investigation of the effect of various fluoride compounds and preparations thereof on erosive tissue loss in enamel in vitro *Caries Research* **43(1)** 10-16.
11. Austin RS, Stenhagen KS, Hove LH, Dunne S, Moazzez R, Bartlett DW, & Tveit AB (2011) A qualitative and quantitative investigation into the effect of fluoride formulations on enamel erosion and erosion-abrasion in vitro *Journal of Dentistry* **39(10)** 648-655.
12. Amaechi BT, & Higham SM (2005) Dental erosion: Possible approaches to prevention and control *Journal of Dentistry* **33(3)** 243-252.
13. Wegehaupt FJ, & Attin T (2010) The role of fluoride and casein phosphopeptide/amorphous calcium phosphate in the prevention of erosive/abrasive wear in an in vitro model using hydrochloric acid *Caries Research* **44(4)** 358-363.
14. Buzalaf MA, Magalhães AC, & Wiegand A (2014) Alternatives to fluoride in the prevention and treatment of dental erosion *Monographs in Oral Science* **25** 244-252.
15. Azzopardi A, Bartlett DW, Watson TF, & Sherriff M (2001) The measurement and prevention of erosion and abrasion *Journal of Dentistry* **29(6)** 395-400.
16. Azzopardi A, Bartlett DW, Watson TF, & Sherriff M (2004) The surface effects of erosion and abrasion on dentine with and without a protective layer *British Dental Journal* **196(6)** 351-354.
17. Sundaram G, Wilson R, Watson TF, & Bartlett DW (2007) Effect of resin coating on dentine compared to repeated topical applications of fluoride mouthwash after an abrasion and erosion wear regime *Journal of Dentistry* **35(10)** 814-818.
18. Sundaram G, Wilson R, Watson TF, & Bartlett D (2007) Clinical measurement of palatal tooth wear following coating by a resin sealing system *Operative Dentistry* **32(6)** 539-543.

19. Bartlett D, Sundaram G, & Moazzez R (2011) Trial of protective effect of fissure sealants, in vivo, on the palatal surfaces of anterior teeth, in patients suffering from erosion *Journal of Dentistry* **39(1)** 26-29.

20. Wegehaupt FJ, Tauböck TT, Sener B, & Attin T (2012) Long-term protective effect of surface sealants against erosive wear by intrinsic and extrinsic acids *Journal of Dentistry* **40(5)** 416-422.

21. Soviero VM, Paris S, Leal SC, Azevedo RB, & Meyer-Lueckel H (2013) Ex vivo evaluation of caries infiltration after different application times in primary molars *Caries Research* **47(2)** 110-116.

22. Paris S, Soviero VM, Seddig S, & Meyer-Lueckel H (2012) Penetration depths of an infiltrant into proximal caries lesions in primary molars after different application times in vitro *International Journal of Paediatric Dentistry* **22(5)** 349-355.

23. Paris S, Dörfer CE, & Meyer-Lueckel H (2010) Surface conditioning of natural enamel caries lesions in deciduous teeth in preparation for resin infiltration *Journal of Dentistry* **38(1)** 65-71.

24. Paris S, Hopfenmuller W, & Meyer-Lueckel H (2010) Resin infiltration of caries lesions: An efficacy randomized trial *Journal of Dental Research* **89(8)** 823-826.

25. Paris S, & Meyer-Lueckel H (2010) Inhibition of caries progression by resin infiltration in situ *Caries Research* **44(1)** 47-54.

26. Paris S, Meyer-Lueckel H, & Kielbassa AM (2007) Resin infiltration of natural caries lesions *Journal of Dental Research* **86(7)** 662-666.

27. Paris S, Meyer-Lueckel H, Cölfen H, & Kielbassa AM (2007) Penetration coefficients of commercially available and experimental composites intended to infiltrate enamel carious lesions *Dental Materials* **23(6)** 742-748.

28. Meyer-Lueckel H, Chatzidakis A, Naumann M, Dörfer CE, & Paris S (2011) Influence of application time on penetration of an infiltrant into natural enamel caries *Journal of Dentistry* **39(7)** 465-469.

29. Meyer-Lueckel H, & Paris S (2008) Improved resin infiltration of natural caries lesions *Journal of Dental Research* **87(12)** 1112-1116.

30. Meyer-Lueckel H, Paris S, & Kielbassa AM (2007) Surface layer erosion of natural caries lesions with phosphoric and hydrochloric acid gels in preparation for resin infiltration *Caries Research* **41(3)** 223-230.

31. Young A, & Tenuta LM (2011) Initial erosion models *Caries Research* **45(Supplement 1)** 33-42.

32. Rios D, Honório HM, Magalhães AC, Wiegand A, de Andrade Moreira Machado MA, & Buzalaf MA (2009) Light cola drink is less erosive than the regular one: An in situ/ex vivo study *Journal of Dentistry* **37(2)** 163-166.

33. Klimek J, Hellwig E, & Ahrens G (1982) Effect of plaque on fluoride stability in the enamel after amine fluoride application in the artificial mouth *Deutsche Zahnärztliche Zeitschrift* **37(10)** 836-840.

34. Attin T, Becker K, Roos M, Attin R, & Paqué F (2009) Impact of storage conditions on profilometry of eroded dental hard tissue *Clinical Oral Investigations* **13(4)** 473-478.

35. D'Alpino PH, Pereira JC, Svizero NR, Rueggeberg FA, & Pashley DH (2006) Use of fluorescent compounds in assessing bonded resin-based restorations: A literature review *Journal of Dentistry* **34(9)** 623-634.

36. D'Alpino PH, Pereira JC, Svizero NR, Rueggeberg FA, & Pashley DH (2006) Factors affecting use of fluorescent agents in identification of resin-based polymers *Journal of Adhesive Dentistry* **8(5)** 285-292.

37. Bartlett DW, Evans DF, Anggiansah A, & Smith BG (1996) A study of the association between gastro-oesophageal reflux and palatal dental erosion *British Dental Journal* **181(4)** 125-131.

38. Irinoda Y, Matsumura Y, Kito H, Nakano T, Toyama T, Nakagaki H, & Tsuchiya T (2000) Effect of sealant viscosity on the penetration of resin into etched human enamel *Operative Dentistry* **25(4)** 274-282.

39. Honório HM, Rios D, Santos CF, Magalhães AC, Delbem AC, Buzalaf MA, & Machado MA (2010) Cross-sectional microhardness of human enamel subjected to erosive, cariogenic or combined erosive/cariogenic challenges *Caries Research* **44(1)** 29-32.

40. Honório HM, Rios D, Santos CF, Magalhães AC, Buzalaf MA, & Machado MA (2008) Effects of erosive, cariogenic or combined erosive/cariogenic challenges on human enamel: An in situ/ex vivo study *Caries Research* **42(6)** 454-459.

41. Lussi A, Schlueter N, Rakhmatullina E, & Ganss C (2011) Dental erosion—An overview with emphasis on chemical and histopathological aspects *Caries Research* **45(Supplement 1)** 2-12.

42. Rakhmatullina E, Beyeler B, & Lussi A (2013) Inhibition of enamel erosion by stannous and fluoride containing rinsing solutions *Schweizerische Monatsschrift für Zahnmedizin* **123(3)** 192-198.

43. Wegehaupt FJ, Tauböck TT, & Attin T (2013) Durability of the anti-erosive effect of surfaces sealants under erosive abrasive conditions *Acta Odontologica Scandinavica* **71(5)** 1188-1194.

44. Van Bebber L, Campbell PM, Honeyman AL, Spears R, & Buschang PH (2011) Does the amount of filler content in sealants used to prevent decalcification on smooth enamel surfaces really matter? *Angle Orthodontist* **81(1)** 134-140.

45. Belli R, Rahiotis C, Schubert EW, Baratieri LN, Petschelt A, & Lohbauer U (2011) Wear and morphology of infiltrated white spot lesions *Journal of Dentistry* **39(5)** 376-385.

46. Paris S, Schwendicke F, Keltsch J, Dörfer C, & Meyer-Lueckel H (2013) Masking of white spot lesions by resin infiltration in vitro *Journal of Dentistry* **41(Supplement 5)** 28-34.

47. Gando I, Ariyoshi M, Ikeda M, Sadr A, Nikaido T, & Tagami J (2013) Resistance of dentin coating materials against abrasion by toothbrush *Dental Materials Journal* **32(1)** 68-74.

48. Cilli R, de Mattos MC, Honorio HM, Rios D, de Araujo PA, & Prakki A (2009) The role of surface sealants in the

roughness of composites after a simulated toothbrushing test *Journal of Dentistry* **37(12)** 970-977.

49. Zimmerli B, Koch T, Flury S, & Lussi A (2012) The influence of toothbrushing and coffee staining on different composite surface coatings *Clinical Oral Investigations* **16(2)** 469-479.

50. Schmidlin PR, Göhring TN, Roos M, & Zehnder M (2006) Wear resistance and surface roughness of a newly devised adhesive patch for sealing smooth enamel surfaces *Operative Dentistry* **31(1)** 115-121.

51. Korbmacher-Steiner HM, Schilling AF, Huck LG, Kahl-Nieke B, & Amling M (2013) Laboratory evaluation of toothbrush/toothpaste abrasion resistance after smooth enamel surface sealing *Clinical Oral Investigations* **17(3)** 765-774.