

Resin Infiltration of Fissure Caries with Various Techniques of Pretreatment *in vitro*

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Key Words

Enamel · Fissure caries · Infiltration · Micro-abrasion

Abstract

The resin infiltration technique might be used for occlusal caries lesions in order to arrest their progression. This *in vitro* study evaluated the influence of various modifications of the infiltration technique on the penetration abilities of the infiltrant into occlusal lesions. Extracted human molars and premolars ($n = 140$) with non-cavitated white spot lesions were randomly allocated to 7 groups. As control, specimens were etched with 15% hydrochloric acid (HCl) gel for 120 s and resin infiltrated for 180 s (Icon; DMG). As modification HCl gel reduced in surface tension and viscosity with and without abrasives was applied using 3 different types of brushes either to oscillate or rub the HCl gel onto the enamel surface. The median maximum lesion depth was 1,232 μm (interquartile range 882–1,513). Compared with the control procedure [23% (16/50)] a higher percentage penetration was observed if the HCl gel was mixed with a small amount of abrasives were rubbed into the fissures using a modified

brush with stiff bristles that were adjusted to the fissure relief for either 120 s [100% (64/100)] or 30 s [98% (61/100); $p < 0.05$; Mann-Whitney test]. All other experimental treatments resulted in penetration results in-between. It can be concluded that the use of an abrasive HCl gel in conjunction with a modified brush mostly enhances resin infiltration into fissure caries lesions.

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The morphology of the fissure relief of human molars and premolars consists of adjacent grooves and fossae [Rohr et al., 1991] that represent the most vulnerable locations to develop dental caries [König, 1963; Macek et al., 2003]. Daily tooth brushing with fluoride toothpaste aims to prevent caries initiation and progression. However, patients often show a lack of compliance with biofilm removal, and self-cleaning of fissures, e.g. by the tongue, does not play a central role as for other tooth surfaces [Splieth et al., 2010]. Thus, fissure sealants are recommended for caries-prone fissures in permanent molars, particularly for patients with increased caries risk [Ahovuo-Saloranta et al.,

Table 1. Protocol of the various treatments

Group	Etchant	Time of etching, s	Additional procedure
Etch + Inf	Icon etch	120	none
Etch _{NB} + Inf	modified etchant	120	none
Etch _{OB} + Inf	modified etchant	120	oscillating brush for etching
Etch _{OB} + Inf _{OB}		120	oscillating brush for etching + infiltration
Etch _{FB} + Inf	modified etchant + abrasives	120	flat brush for etching
Etch _{MB120} + Inf		120	modified brush for etching
Etch _{MB30} + Inf		30	modified brush for etching

The modified etchant included Icon etch/HCl solution (15%) (ratio 1:1) and sodium dodecyl sulphate (1%).

2008; Berger et al., 2010; Simonsen, 2002]. After a pretreatment with phosphoric acid sealants are applied to fill up the fissure relief. They function as a superficial barrier to isolate the enamel surface from the pathogenic biofilm [Griffin et al., 2008; Mejare et al., 2003; Mertz-Fairhurst et al., 1998]. The caries-preventive effect of sealants ranges from a relative risk reduction of 87% after 12 months to about 60% after 48–54 months [Ahovuo-Saloranta et al., 2008] depending on the retention of the sealant [Mejare et al., 2003]. Furthermore, sealants function as a barrier for fermentable carbohydrates to avoid glycolysis by bacteria within the lesion [Mertz-Fairhurst et al., 1998] and seem to delay lesion progression if placed on caries lesions [Splieth et al., 2010]. However, they usually penetrate only superficially into caries lesions [Paris et al., 2014].

In contrast to fissure sealing with the resin infiltration technique, deep penetration of infiltrants into enamel caries lesions can be achieved. Hereby, a diffusion barrier not on the enamel surface but inside the lesion body is created [Meyer-Lueckel and Paris, 2008]. In proximal lesions a relatively high mineralized surface layer ($\leq 50 \mu\text{m}$ thickness) covers the porous lesion body and therefore inhibits resin penetration [Meyer-Lueckel et al., 2007]. It has been shown that pretreatment with 15% hydrochloric acid (HCl) gel removed up to 70% of surface layers [Meyer-Lueckel et al., 2007]. It has been argued that also 37% phosphoric acid (H_3PO_4) gel frequently used for adhesive purposes could be effective to erode the surface layer of rather active proximal lesions [Neuhaus et al., 2012]. Nonetheless, pretreatment with 15% HCl gel for 2 min allows a more complete erosion of the surface layer [Meyer-Lueckel et al., 2007] resulting in an almost complete penetration of the infiltrant into proximal lesions [Meyer-Lueckel and Paris, 2010].

In contrast to proximal caries only a negligible penetration of infiltrants into fissure caries lesions could be achieved after etching with 37% H_3PO_4 gel [11% (0/20)]. The use of 15% HCl gel resulted in a higher but still incomplete penetration of occlusal lesions [41% (30/78)] [Paris et al., 2014]. Possibly, due to air bubbles, calculus remnants and residual biofilm, the acid was not capable of reaching the lesion parts situated in pits and fissures. Consequently, the highly mineralized surface layer was not eroded sufficiently, resulting in rather low penetration depths [Paris et al., 2014].

Thus, the aim of the present study was to compare the penetration depths of an infiltrant after different pretreatment procedures using 15% HCl gel. We hypothesized that these modifications would lead to a significantly deeper and more complete penetration of the infiltrant into non-cavitated caries lesions situated in fissure areas compared with the standard infiltration technique.

Materials and Methods

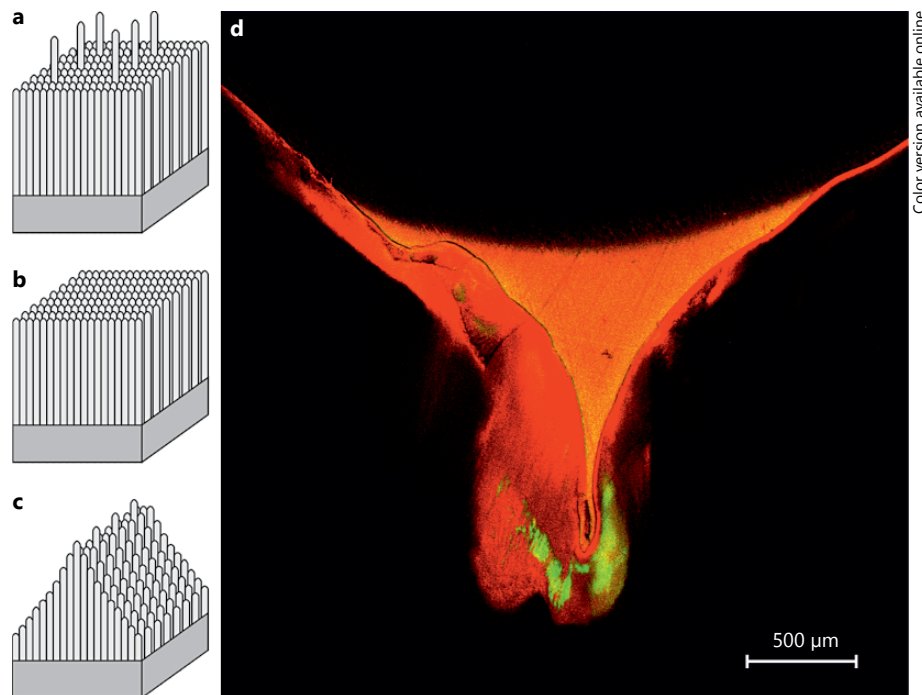
Specimen Selection

Extracted human molars and premolars ($n = 140$) with active, non-cavitated white spot lesions (ICDAS-2; dull surface, chalky opacity) were graduated by 2 trained and calibrated examiners (S.P., J.L.) according to the International Caries Detection and Assessment System (ICDAS) [Ismail et al., 2007]. In case of disagreement a consensus rank was agreed. The study protocol conformed to the principles outlined in the German Ethics Committee's statement for the use of human body material in medical research [Central German Ethics Committee, 2003].

Treatment

The groups as well as the different treatment protocols are shown in table 1. As control, the fissure systems were etched with 15% HCl gel [Icon etch (Etch); DMG, Hamburg, Germany] for

Fig. 1. Pictures of the 3 different brushes (a–c) that were used in this study: relatively long and flexible bristles of a battery-driven oscillating brush (a) vibrated the HCl gel or the infiltrant. The conventional flat brush (b) seemed not to reach deeper parts of the fissure relief, whereas the rather stiff bristles (differing in length) of the modified brush (c) seemed to rub the HCl gel on the enamel surface even in the fundus area. **d** Representative image of an infiltrated fissure caries lesion (Etch_{MB120} + Inf). The infiltrant (red) penetrated into the porous lesion body (green = non-infiltrated; colours in the online version only).



Color version available online

120 s and rinsed for 30 s (group: Etch + Inf). Furthermore, a modified etchant [addition of 1% sodium dodecyl sulphate and a mixture with 15% HCl solution (1:1)] was used (group: Etch_{NB} + Inf). The modified etchant was moved within the fissures using an oscillating brush (Dr. Best Vibration Zwischenzahn; GSK, Bühl, Germany; fig. 1a; groups: Etch_{OB} + Inf, Etch_{OB} + Inf_{OB}) or the modified etchant, additionally mixed with 2% pumice (abrasives), was rubbed (manually by the examiner J.L.) preferably within the fissure relief on the enamel surface. Thereby, a flat brush (group: Etch_{FB} + Inf; Dr. Best Plus; GSK; fig. 1b) or a modified toothbrush were used (group: Etch_{MB120} + Inf; Dr. Best X-Zwischenzahn; GSK; fig. 1c). Additionally, in one group the time of etching was shortened from 120 to 30 s (group: Etch_{MB30} + Inf). After pretreatment all specimens were stored in 0.1% ethanolic solution of tetramethylrhodamine isocyanate (RITC; Sigma Aldrich, Steinheim, Germany) for 24 h and afterwards carefully dried with compressed air (10 s). The infiltrant [Icon infiltrant (Inf); DMG] was labeled with RITC and applied for 180 s either with (Etch_{OB} + Inf_{OB}) or without (all other groups) the oscillating brush. Afterwards, the infiltrant was hardened with blue light for 60 s (530 mW/cm²; Astralis 5; Ivoclar Vivadent, Ellwangen, Germany).

The roots of the teeth were removed (Band Saw 300cl; Exact Apparatebau, Norderstedt, Germany) and the specimens were embedded (Technovit; Heraeus Kulzer, Wehrheim, Germany). To create slices of approximately 1,200 µm thickness, teeth were cut across perpendicularly to the lesion surface (Band Saw 300cl; Exact Apparatebau). The specimens were fixed on a microscope slide (Locite 493; Henkel, Munich, Germany) and polished to 1,000 µm thickness (±20 µm; Mikroschleifsystem 400cs; Abrasive Paper 1,200, 2,400, 4,000, Exact Apparatebau). Unbound red fluorophore RITC was bleached by immersion of the specimens in

30% hydrogen peroxide for 12 h. Then, the non-infiltrated porous structures of the lesion were counterstained with 50% ethanol solution of 100 µM sodium fluorescein (Sigma Aldrich) for 3 min. Finally, the specimens were rinsed with deionized water for 10 s.

Image Analysis

Specimens were observed using dual fluorescence and confocal laser scanning microscopy (Carl Zeiss, Jena, Germany; 5× objective) [Paris et al., 2009]. Multiple images of 1,024 × 1,024 µm were combined with the help of graphic software (Photoshop Elements 6; Adobe Systems GmbH, Munich, Germany).

Measurement

Lesion depths and penetration depths were measured from the enamel surface to the deepest point of green or red fluorescence, respectively. Lesion and penetration depth were both measured at 18 defined points (9-point measurement on each side of the fissure) along the enamel prisms (Axiovision; Carl Zeiss) [Paris et al., 2014].

Statistical Analysis

Data were checked for normal distribution (Shapiro-Wilk test). We analysed the lesion depth (LD_{max}) and penetration depth (PD_{max}) at the deepest lesion side as well as the average lesion depth (LD_{av}) and penetration depth (PD_{av}) for each specimen (Kruskal-Wallis test).

Percentage penetration (PP_{max}) was calculated as $PP_{max} = PD_{max}/LD_{max} \times 100$ and compared between the various treatments by Mann-Whitney tests. To minimize the limiting influence of (low) lesion and penetration depth, subset analyses of PD_{max500}

Table 2. Medians (μm ; interquartile ranges in parentheses) of the mean lesion (LD_{av}) and penetration depths (PD_{av} ; each 18 measurements) as well as the maximum lesion depths (LD_{max}) and penetration depths at LD_{max} (PD_{max}) of the 7 treatments for all lesions and for lesions with $\text{LD}_{\text{max}} > 500 \mu\text{m}$

Groups	All lesions					Lesions with $\text{LD}_{\text{max}} > 500 \mu\text{m}$		
	n	LD_{av}	PD_{av}	LD_{max}	PD_{max}	n	$\text{LD}_{\text{max}500}$	$\text{PD}_{\text{max}500}$
Etch + Inf	17	723 (405–876)	146 (118–338) ^{a, b}	1,248 (916–1,472)	234 (116–491) ^{a, b}	16	1,254 (974–1,493)	261 (147–622) ^{a, b}
Etch _{NB} + Inf	18	746 (573–977)	123 (80–235) ^a	1,392 (1,168–1,690)	176 (75–371) ^a	17	1,470 (1,184–1,690)	174 (75–371) ^a
Etch _{OB} + Inf	15	619 (418–919)	322 (189–544) ^b	1,185 (959–1,558)	459 (272–891) ^b	13	1,214 (978–1,558)	503 (272–891) ^b
Etch _{OB} + Inf _{OB}	17	547 (264–823)	181 (137–289) ^{a, b}	1,288 (700–1,496)	320 (190–633) ^{a, b}	15	1,304 (828–1,515)	304 (159–667) ^{a, b}
Etch _{FB} + Inf	19	780 (458–929)	255 (167–359) ^b	1,205 (1,025–1,510)	339 (170–502) ^{a, b}	18	1,242 (1,033–1,510)	295 (170–502) ^{a, b}
Etch _{MB120} + Inf	17	569 (407–775)	429 (288–697) ^c	1,103 (736–1,386)	844 (598–1,208) ^c	17	1,103 (736–1,386)	844 (598–1,208) ^c
Etch _{MB30} + Inf	20	667 (527–842)	500 (345–704) ^c	1,244 (1,004–1,556)	963 (758–1,324) ^c	20	1,244 (1,004–1,556)	963 (758–1,324) ^c

Different superscript letters indicate statistically significant differences ($p < 0.05$; Mann-Whitney test) between the treatments; for results without any superscript letters, no significant differences were observed ($p > 0.05$). n = Sample size per group.

and $\text{PP}_{\text{max}500}$ values for lesions having a maximum lesion depth $> 500 \mu\text{m}$ ($\text{LD}_{\text{max}500}$), were performed (Mann-Whitney test). The level of significance was set at 5% for all tests (SPSS version 20.0; Munich, Germany).

Results

The confocal laser scanning microscopy images showed the penetration of the infiltrant (red fluorescence) into the porous lesion body (green fluorescence; fig. 1d) that was most pronounced after abrading the surface layer using the respective brush. No cavitation of the enamel surface could be observed for any of the treatments.

Overall, 17 lesions were judged as being too shallow ($\text{LD}_{\text{max}} < 200 \mu\text{m}$) or destroyed during confocal laser scanning microscopy preparation and therefore excluded. The median LD_{max} ($n = 123$) and $\text{LD}_{\text{max}500}$ ($n = 116$) were $1,232 \mu\text{m}$ (interquartile range 882–1,513) and $1,270 \mu\text{m}$ (971–1,530), respectively. No significant differences in lesion depth between the various groups were observed ($p > 0.05$; Kruskal-Wallis test; table 2).

Micro-abrasive treatments (Etch_{MB120} + Inf, Etch_{MB30} + Inf) resulted in the highest PD_{max} compared with all other treatments being significantly different compared with the control group (Etch + Inf; $p < 0.05$). Similar results were evaluated for $\text{PD}_{\text{max}500}$ (table 2).

Analysis of PD_{max} (1-point measurement) and PD_{av} (multiple measurements) revealed similar results (table 2).

$\text{PP}_{\text{max}500}$ values of the various groups are shown in figure 2. An almost complete penetration was observed for

Etch_{MB120} + Inf and Etch_{MB30} + Inf, not being significantly different from each other ($p > 0.05$; Mann-Whitney test), but both being significantly higher compared with the other 5 treatments ($p < 0.05$).

Discussion

In the present study, the infiltrant was capable to penetrate several hundred micrometres into fissure caries lesions. This corroborates findings for proximal lesions [Meyer-Lueckel and Paris, 2010; Soviero et al., 2012]. The study hypothesis that modifications of the pretreatment would lead to a significantly enhanced and almost complete infiltration into occlusal lesions could only partially be confirmed, since a modified HCl gel (Etch_{NB} + Inf) alone could not improve the penetration abilities of the infiltrant. Oscillation of the HCl gel or the infiltrant (Etch_{OB} + Inf, Etch_{OB} + Inf_{OB}) or micro-abrasion with a normal flat brush (Etch_{FB} + Inf) enhanced penetration. But only with micro-abrasive pretreatments using a modified brush (Etch_{MB120} + Inf, Etch_{MB30} + Inf) fissure caries lesions were infiltrated almost completely.

Previous studies argued that the measurement of the infiltrated area reflects penetration abilities of an infiltrant better compared with a 1-point measurement [Neuhaus et al., 2012; Soviero et al., 2012]. In the present study penetration was evaluated by both a 1-point measurement at the deepest lesion site as well as by mean values of a multiple-point measurement. As with area measurements, mean values include shallow parts of the lesion ($< 500 \mu\text{m}$) as well. Since these parts should be penetrated

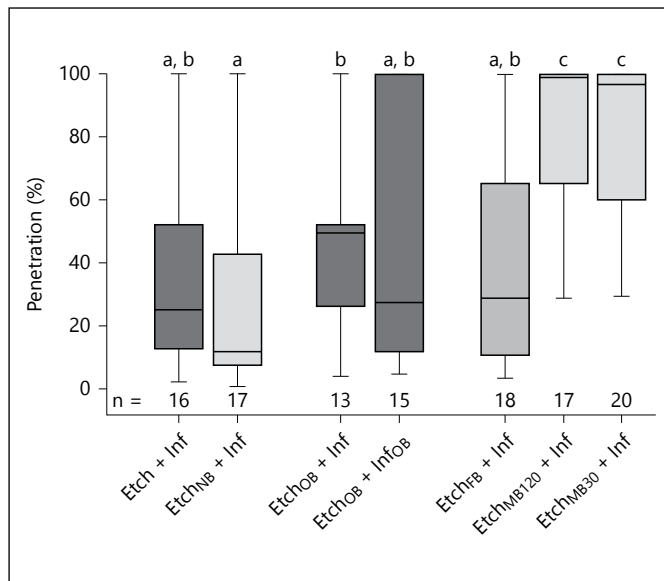


Fig. 2. Percentage penetration (PP_{max500} ; box and thick line = 25th and 75th percentile and median, error bars = minima and maxima) of the infiltrant (Inf) into lesions with $LD_{max} > 500 \mu m$ for the various treatments (Etch = etchant, Inf = infiltrant, Etch_{NB} + Inf = no brush, Etch_{OB} + Inf, Etch_{OB} + Inf_{OB} = oscillating brush, Etch_{FB} + Inf = flat brush, Etch_{MB120} + Inf = modified brush for 120 s, Etch_{MB30} + Inf = modified brush for 30 s). Use of abrasives and a special brush (Etch_{MB120} + Inf, Etch_{MB30} + Inf) led to almost complete penetration. Statistically significant differences between various treatment groups are indicated by different letters ($p < 0.05$; Mann-Whitney test). n = Number of teeth.

rather completely [Meyer-Lueckel and Paris, 2010], the 1-point measurement seems to be more eligible to describe the maximum penetration abilities of an infiltrant. However, in the present investigation both measurements revealed similar outcomes.

It has been shown that HCl gel is capable of eroding the surface layer of proximal caries lesions [Meyer-Lueckel et al., 2007]. However, due to its rather high viscosity, conventional HCl gel might not have reached occlusal lesions situated within deeper parts of the fissure resulting in a rather shallow lesion infiltration [Paris et al., 2014]. Furthermore, it was assumed that even if HCl gel reaches deeper areas within fissures, enamel surfaces might only be insufficiently wetted due to rather high surface tension of the HCl gel. Consequently, we reduced the viscosity and surface tension of the HCl gel; nonetheless, these modifications could not improve infiltration properties considerably.

Possibly, air bubbles within the fissure hampered the HCl gel (and also the infiltrant) to reach the lesion areas

particularly in deep fissures [Asmussen, 1977; Paris et al., 2014]. To remove these air bubbles, the etchant as well as the infiltrant were applied with the help of an oscillating brush. This procedure led to enhanced but compared with proximal lesions still relatively low penetration depths [Paris et al., 2011]. Probably, air bubbles were destroyed, but the oscillation was not capable of eroding the surface layer sufficiently in order to improve resin infiltration considerably.

Contrary to the other treatments, abrasion of the enamel surface by using a modified brush and abrasive etching gel allowed an almost complete infiltration. Studies on micro-abrasion techniques showed enhanced erosion when an HCl gel pumice slurry was applied with a rotating rubber cup ($360 \pm 130 \mu m$) compared with HCl gel application alone ($100 \pm 47 \mu m$) [Tong et al., 1993] although this effect also depends on the force used. The erosion of the enamel surface layer was not measured directly in the present study but as a result after a rather abrasive pretreatment the penetration depths were significantly higher. It seems that the hard bristles of the modified brush (bristles differing in lengths) combined with HCl gel pumice slurry removed air bubbles as well as calculus remnants and therefore eroded the highly mineralized surface layer sufficiently.

Nonetheless, micro-abrasion techniques also involve the risk of cavity formation of the enamel surface that would change a lesion from ICDAS-2 to ICDAS-3 (cavitated in enamel). Fortunately, no cavitation could be observed in the present investigation for the various pretreatments.

Resin penetration seems to depend on the mode of etching [Neuhaus et al., 2012; Paris et al., 2014] and shorter etching times should result in less erosion of the surface layer. However, for the treatment of fissure caries the combination of abrasives with HCl gel allowed an almost complete penetration of the infiltrant, even if the time of etching was reduced to 30 s.

In clinical practice a careful handling with HCl gel should be recommended because even short exposures to HCl gel (≥ 30 s) might evoke ulcerations of the gingiva [Croll et al., 1990]. However, if a rubber dam is applied to avoid contact of acid with soft tissues, the use of HCl gel seems to be safe. It is still unclear how much of the surface layer has to be eroded for a sufficient infiltration to hamper further caries progression. Nonetheless, for clinical practice short application of a small amount of HCl gel is advantageous, especially for the treatment of children.

In conclusion, the use of the conventional infiltration technique for proximal and smooth surface lesions seems

to result in lower penetration depths when used for fissure caries lesions. A more liquid HCl gel as well as oscillation of the etchant could not considerably enhance resin penetration. Micro-abrasion of the enamel surface by a modified brush and abrasive HCl gel slurry resulted in a nearly complete infiltration and therefore appears to be most suitable for pretreatment of occlusal caries lesions situated in fissures.

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Author Contributions

H.M.-L. and S.P. designed and J.L. planned the study; J.L. and T.S. prepared the samples; J.L. performed the measurements and statistical analysis; J.L. wrote the manuscript. H.M.-L. and S.P. commented on, and all authors revised the manuscript. The results of this paper have been also published in form of the doctoral thesis of J.L. at RWTH Aachen University.

Disclosure Statement

The Charité Universitätsmedizin Berlin holds patents for an infiltration technique for dental caries lesions in which two of the authors (S.P. and H.M.-L.) are appointed as inventors. These patents have been licensed by the DMG, Hamburg, Germany. S.P. and H.M.-L. receive royalties from this license as well as a research grant from the DMG. J.L. receives a research grant from the DMG.