Clin Oral Invest (2013) 17:2085–2089
DOI 10.1007/s00784-012-0909-8

ORIGINAL ARTICLE

Pretreatment of natural caries lesions affects penetration depth of infiltrants in vitro

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Received: 28 February 2012 / Accepted: 6 December 2012 / Published online: 23 December 2012

Abstract
Objectives Limited evidence exists about the usefulness of ethanol or acetone application to desiccate caries lesions before resin infiltration. Therefore, this in vitro study aimed to compare the penetration depths (PD) of an infiltrant (DMG, Germany) into natural caries lesions using various pretreatments.
Material and methods Extracted permanent human molars and premolars showing non-cavitated caries lesions were etched (90 s, 15 % HCl gel) and stored in pooled saliva (7 days). Lesions were etched again (30 s, 15 % HCl gel), washed (30 s), air-dried (30 s), and randomly allocated to one of the pretreatments: none (negative control), air-drying (incubator, 37 °C, 24 h; positive control), once ethanol (E1), twice ethanol (E2), once acetone (A1), and twice acetone (A2). Subsequently, the infiltrant was applied for 5 min and light-cured. Ground sections were prepared for analyses of lesion depths (LD) and PD using confocal microscopy.
Results Median LD (Q25/Q75) of all lesions (n=91) and lesions ≥500 μm (n=57) were 629 (395/798) and 731 (638/876) μm, respectively. When all lesions were analyzed, no significant differences between various pretreatments could be observed (p>0.05, Kruskal–Wallis). For lesions ≥500 μm, significantly deeper PP was observed in groups PC, E1, A1, and A2 compared with NC (p<0.05; Mann–Whitney), but not after adjustment for multiple comparison (p>0.05).
Conclusion Application of either ethanol or acetone, followed by air-drying, is suitable to prepare caries lesions for resin infiltration in vitro.
Clinical relevance This paper shows that proper drying is an important step prior to caries infiltration.

Keywords Caries · Infiltration · Microinvasive treatment · Caries infiltration

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Introduction

Non-cavitated proximal caries lesions are usually detected on bitewing radiographs taken for dental check-ups [1]. In recent years, a tendency to postpone operative treatment of proximal lesions has been supported by the principles of minimally invasive dentistry [2–4]. For non-cavitated lesions, noninvasive measures like plaque control, fluoridation, and dietary counseling are recommended to control caries progression [5, 6]. However, particularly in patients with medium to high caries risk, many proximal lesions progress and require restorative treatment [7].

Caries infiltration is an alternative treatment for proximal lesions and aims to arrest non-cavitated caries lesions by occluding the enamel porosities with low-viscosity light curing resins. Since the first experiments about resin penetration into caries lesions in the 1970s [8, 9], the technique could be significantly improved and especially the resin

penetration depth be increased. Dental adhesives, which nearly completely penetrated quite shallow artificial caries lesions [10, 11], but only superficially penetrated into deeper natural caries lesions [12], were replaced by special low-viscosity resins, optimized for caries infiltration. These so-called infiltrants, with high penetration coefficients, are able to infiltrate natural caries lesions almost completely after a relatively short application time [13, 14].

Not only the physical properties of the resins, but also the pretreatment of the enamel lesion prior to infiltration, influence the depth of resin penetration. Acid-etching with 15 % HCl gel for 2 min instead of the commonly used H_3PO_4 is required to erode the higher mineralized surface layer of natural caries lesions and to make the porosities in the subsurface accessible for the infiltrant [15]. Moreover, before resin application, drying of the enamel is required to remove water from the porosities and thus enable capillary action to soak the resin into the pores. Moreover, desiccation favors resin penetration by increasing the surface free energy [16, 17]. Shallow artificial caries lesions (80 to 205 μm) were significantly more deeply infiltrated after dehydration with ethanol than without [18]. For sound occlusal surfaces, dehydration of the enamel with acetone enhanced the penetration of a bonding agent into fissures [19], while ethanol or acetone used as drying agents did not improve the penetration ability of a fissure sealant [20]. In fact, only limited scientific evidence exists about appropriate pretreatment procedures to prepare natural caries lesions before resin penetration. Therefore, the aim of this in vitro study was to compare the penetration depth of an infiltrant into natural caries lesions after various drying pretreatments. We hypothesized that dehydration with ethanol or acetone before air-drying results in deeper resin penetration into enamel lesions compared with air-drying only.

Material and methods

One hundred and two extracted permanent molars and premolars with proximal active (chalky opacity, dull surface) non-cavitated white spot lesions scored as ICDAS 2 [21] were selected for the study. Lesions were etched with 15 % HCl gel for 90 s (DMG, Hamburg, Germany), shortly air-dried, and stained with 0.1 % ethanolic solution of tetramethylrhodamine isothiocyanate (Sigma-Aldrich, Steinheim, Germany) for 12 h for later confocal microscopic evaluation according to an introduced standard [22]. To simulate the natural contamination with organic saliva components, teeth were subsequently stored in pooled saliva also containing 0.1 % RITC. After 7 days, lesions were etched again with 15 % HCl gel for 30 s, washed with water spray for 30 s, and dried with compressed air for 30 s. Subsequently, the teeth were randomly allocated to one of the following pretreatment groups: no further pretreatment (negative control—NC), 24 h air-drying in incubator at 37 °C (positive control—PC), once application of 100 % ethanol (E1), twice application of 100 % ethanol (E2), once application of 100 % acetone (A1), and twice application of 100 % acetone (A2). Each application in E1, E2, A1, and A2 was followed by 30 s drying with compressed air. An infiltrant (Icon preproduct; DMG Hamburg, Germany) was applied onto the lesion surface using a microbrush. After 5 min, excessive material was removed from the tooth surface with a cotton roll and the infiltrant was light-cured for 60 s (400 mW/cm^2, Translux CL; Heraeus Kulzer, Hanau, Germany).

Three tooth sections with 1 mm thickness were prepared perpendicularly to the lesion surface (Exakt 300cl; Exakt Apparatebau, Norderstedt, Germany). Unbound red fluorophore was bleached by immersion in hydrogen peroxide (30 %) for 12 h at 37 °C. Subsequently specimens were washed with water, fixed on microscope slides, and polished plano parallel (Mikroschleifsystem 400 cs, Abrasive Paper 1200, 2400, 4000; Exakt Apparatebau, Norderstedt, Germany). To label porous structures, which were not infiltrated, specimens were immersed in a 50 % ethanol solution of 100 μM sodium fluorescein (NaFl; Sigma-Aldrich, Steinheim, Germany) for 3 min and subsequently washed with deionized water for 10 s.

Specimens were observed with a confocal laser scanning microscope (LSM 510; Carl Zeiss, Jena, Germany) using ×10 objective in dual fluorescence mode as described previously [22]. Confocal microscopic images were used to measure lesion and penetration depths. Infiltrated structures appeared in red (RITC) and porous structures (not infiltrated enamel or dentin) appeared in green (NaFl). Lesion depth (LD) was measured from the enamel surface to the deepest point of green fluorescence in enamel. Penetration depth (PD) of the infiltrant was measured from the enamel surface to the deepest point of red fluorescence along the same line where LD was measured.

SPSS (SPSS Inc., Chicago, IL, USA) was used to perform statistical analysis. Assumption of normal distribution of data was checked using Shapiro–Wilk test. Differences between group regarding lesion and penetration depths as well as percentage penetrations (PP=PD/LD×100) were analyzed using Kruskal–Wallis and Mann–Whitney tests (adjusted for multiple comparison). To consider the limiting influence of lesion depth on the penetration depth, subgroup analyses were performed for lesions with LD ≥500 μm. The level of significance was set at 5 %.

Results

From the 102 specimens, 11 broke during the preparation for analysis resulting in a final sample of 91 specimens.

Table 1 Median maximum lesion depths (LD) and median penetration depths (PD) of the infiltrant according to the various pretreatments (dehydration/drying) for all lesions and for lesions with LD ≥500 μm

Groups	All lesions			Lesion with LD ≥500 μm		
	n	LD	PD	*n*	$LD_{\geq 500}$	$PD_{\geq 500}$
NC	15	711 (352; 887)	295 (146; 510)	9	876 (746; 1,138)	290 (146; 809)
PC	14	687 (327; 832)	647 (327; 732)	10	783 (680; 1,107)	687 (641; 807)
Ethanol 1	16	658 (449; 875)	522 (394; 839)	9	807 (727; 955)	807 (727; 955)
Ethanol 2	14	630 (395; 731)	422 (303; 638)	10	679 (629; 788)	628 (406; 719)
Acetone 1	17	608 (444; 699)	484 (371; 699)	10	698 (618; 798)	698 (585; 798)
Acetone 2	15	629 (305; 729)	499 (264; 669)	9	695 (659; 759)	659 (591; 695)
p		$p>0.05$	$p>0.05$		$p>0.05$	$p>0.05$

25th percentile and 75th percentile are given in parenthesis. Kruskal–Wallis test was used for statistical comparisons between groups

NC negative control, *PC* positive control, *LD* lesion depth, *PD* penetration depth, *n* sample size per group

Both ethanol and acetone application to dehydrate and dry the enamel lesions prior to infiltration resulted in deep resin penetration of almost all lesions, whereas superficial infiltration was observed in some lesions in the negative control group. Median LD (25th percentile; 75th percentile) of all lesions ($n=91$) and of lesions being deeper than 500 μm ($n=57$) were 629 (395; 798) μm and 731 (638; 876) μm, respectively, and did not differ significantly between groups (Table 1; $p>0.05$; Kruskal–Wallis test).

In groups PC, E1, E2, A1, and A2, higher median PDs were observed than for NC, the differences between groups were not significant (Table 1; $p>0.05$; Kruskal–Wallis test). Median percentage penetration (PP) was below 100 % for the NC. The difference of PP between NC and the other groups was not statistically significant when all lesions were considered in the analysis ($p>0.05$). In the subanalysis with lesion depths of more than 500 μm, PPs differed significantly between groups ($p<0.05$; Kruskal–Wallis test), in particular for PC, E1, A1, and A2 compared with NC ($p<0.05$; Mann–Whitney test). No significant differences between certain groups could be observed after correction for multiple testing ($p>0.05$; adjusted Mann–Whitney test) (Fig. 1).

Discussion

The results of this in vitro study showed that the pretreatment in terms of dehydration of enamel caries lesions with ethanol or acetone prior to caries infiltration considerably enhanced resin penetration into deeper enamel lesions. This confirms previous studies reporting that ethanol and acetone application enhanced adhesive penetration into artificial caries lesions [18] and fissures [19]. This effect is attributed to the fact that organic solvents, as ethanol or acetone, lead to increased displacement of water from the enamel porosities which is a prerequisite for resin penetration [17–19, 23].

Regarding the selection of the caries lesions for the present study, only teeth showing active (chalky opacity, dull surface) non-cavitated proximal lesions scored as ICDAS 2 were included in the experiment. Despite of presenting similar clinical aspect, caries lesions varied histologically from lesions in the outer half of enamel to lesions reaching into the dentin. However, the median lesion depth did not differ significantly between groups. Nonetheless, as shallower lesions might be more easily completely infiltrated than deeper lesions, the lesion depth was taken into consideration by subgroup analysis and the main comparison between groups was based on the percentage penetration.

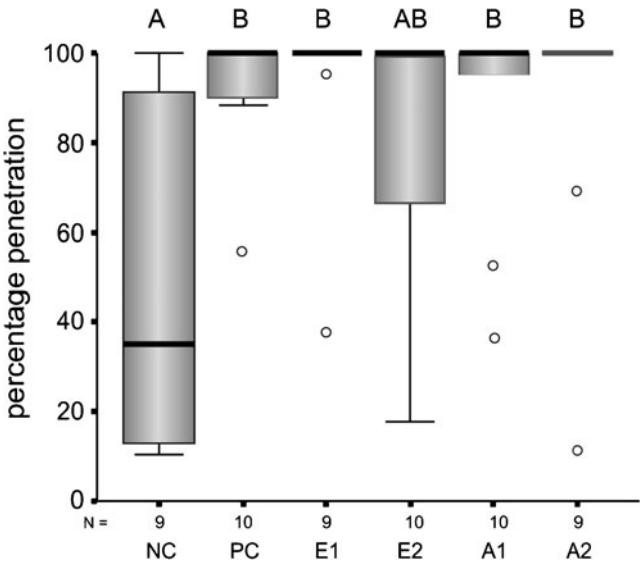

Fig. 1 Box plots of percentage penetration (PP) of the infiltrant after four dehydration/drying methods as well as of positive and negative control groups with LD ≥500 μm ($p<0.05$; Kruskal–Wallis test). *Different letters above the bars* indicate significant differences between the various groups (Mann–Whitney test; not adjusted for multiple comparisons). *n*=number of teeth

Air blowing with compressed air is the method most commonly used in clinical practice to dry the dental enamel prior to any resin application. As the water may be partially removed by air-drying [16], this might explain why in the present study some of the lesions in the negative control group (particularly the shallower ones) were deeply infiltrated after air-drying only. Besides, it is worth noting that 30 s air blowing used in the present study was rather a long time comparing with 5 to 15 s used in previous studies with adhesives or fissure sealants [18, 20], which might have somehow favored the results for the negative control.

Bonding agents penetrate into etched and dried enamel between 20 and 50 μm and this process is driven by capillary action [24–27]. However, much deeper penetration is observed when infiltrating natural caries lesions with infiltrant. In the present study, for example, the median penetration depth of the infiltrant reached 731 μm among the deeper enamel lesions. A prerequisite for capillary action is that the capillaries or pores to be penetrated are empty or filled with air. Therefore, proper drying of the lesions is necessary before caries infiltration. Unlike from dentin, where over-drying is deleterious, the enamel structure is not damaged by desiccation [28].

Surfaces with high free energy favor the wettability of a liquid. The lower the contact angle between a liquid and a solid surface, the more easily the liquid penetrates into a porous solid. However, the presence of a film of water or organic material on a solid surface decreases the free energy significantly and the contact angle between the solid and a liquid increases [16]. This explains why moisture hampers resin penetration into dental enamel and affects bonding strength between enamel and sealants or adhesives [17, 29].

It might have been advantageous to use natural caries lesions immediately after extraction, but this is technically almost impossible. If the pretreatment with ethanol or acetone in the present study also affected organic contaminations within the lesion is not clear. Acid-etching with 15 % HCl for 120 s is necessary to erode the surface layer of natural caries lesions and enable resin penetration into the lesion body in the subsurface [15]. It has been shown that etching with 37 % H_3PO_4 is not suitable to remove the highly mineralized surface layer, resulting in superficial penetration of the resin [15, 30]. In the present study, lesions were etched for 90 s with HCl before teeth were stored in pooled saliva. This was done to allow contamination of the lesion pores with organic saliva components and thus simulate natural conditions as closely as possible. Shortly before the various drying procedures and infiltration was performed, lesions were etched again for the "remaining" 30 s to simulate the clinical procedure.

Percentage penetration after twice application of ethanol did not differ significantly from those of the negative control group. One might speculate if excessive ethanol application might have resulted in incomplete evaporation of the ethanol and hampered infiltration in some lesions. However, percentage penetration was mainly lowered due to very shallow penetration in only three of the specimens of the E2 group. Thus, other aspects that also influence resin penetration into enamel such as the enamel porous size and surface layer thickness in some particular lesions might be the reason for the overall result and not the pretreatment itself.

Resin penetration into enamel caries lesions is driven by several factors and proper drying of the enamel plays a relevant role in the protocol for caries infiltration. In the present study, application of either ethanol or acetone, followed by air blowing, was shown to be suitable as a pretreatment in preparation for caries infiltration in vitro.

Acknowledgments This study was supported by the Deutsche Forschungsgemeinschaft (PA 1508/1-1). We are indebted to PD Dr. Dr. Werner Hopfenmüller for his statistical advice.

Conflict of interest HML and SP are appointed as inventors of US and European patents for an infiltration technique for dental caries lesions, held by Charité-Universitätsmedizin Berlin, and receive royalties from DMG.

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