©Operative Dentistry, 2006, **31- 3,** 338-345

Inhibition of Lesion Progression by the Penetration of Resins *In Vitro*: Influence of the Application Procedure

J Mueller • H Meyer-Lueckel
S Paris • W Hopfenmuller • AM Kielbassa

Clinical Relevance

The penetration of adhesives into initial enamel demineralization seems to be a promising approach to providing a non-operative treatment regimen for carious lesions.

SUMMARY

This study compared the progression of sealed initial enamel lesions penetrated with a fissure sealant (Helioseal, Vivadent) or various adhesives (Heliobond, Excite, Vivadent; Resulcin, Merz; Solobond M, Voco; Prompt L-Pop, 3M-ESPE) after exposure to a demineralizing solution, *in vitro*. From 27 bovine teeth, 54 enamel specimens were prepared and covered with nail varnish (control), thus obtaining three windows for treatment. After demineralization (pH 5.0; 14 days), two of the windows (A, B) were etched with phosphoric acid (20%; 5 seconds); whereas, the third area served as the control (C). The specimens were divided randomly into six groups (n=9), and the material was applied (90 seconds) either once (A) or twice (B). Light-curing followed each application. Half of the area of each specimen window was then covered with nail varnish, and the samples were again stored in the demineralizing solution (pH 5.0; 14 days). The specimens were cut perpendicular to the surface, and both enamel slabs were studied after infiltration using a fluorescent, low viscous resin (VIRIN) and confocal microscopy (CLSM). Lesion depths were calculated (ImageJ) from the surface to that point in the lesion where the grey values clearly changed to a darker grey. After demineralization, mean lesion depths (SD) (14 days) were measured at 105 (21) µm. The second demineralization led to a mean progression of the lesion depths of 52 (31)%. Adper Prompt L-

Jan Mueller, DDS, postdoctorate, Department of Operative Dentistry and Periodontology, School of Dental Medicine, Campus Benjamin Franklin, Charité–Universitätsmedizin, Berlin, Germany

*Hendrik Meyer-Lueckel, DDS, assistant professor, Department of Operative Dentistry and Periodontology, School of Dental Medicine, Campus Benjamin Franklin, Charité–Universitätsmedizin, Berlin, Germany

Sebastian Paris, DDS, postdoctorate, Department of Operative Dentistry and Periodontology, School of Dental Medicine, Campus Benjamin Franklin, Charité–Universitätsmedizin, Berlin, Germany

Werner Hopfenmuller, DMD, PhD, assistant professor, Department of Medical Informatics, Biometry and Epidemiology, Campus Benjamin Franklin, Charité–Universitätsmedizin, Berlin, Germany

Andrej M Kielbassa, DDS, PhD, professor and head, Department of Operative Dentistry and Periodontology, School of Dental Medicine, Campus Benjamin Franklin, Charité–Universitätsmedizin, Berlin, Germany

*Reprint request: Aßmannshauser Straße 4-6, 14197, Berlin, Germany; e-mail: hendrik.meyer-lueckel@charite.de

DOI: 10.2341/05-39

Pop and Solobond M could not significantly prevent lesion progression after a single application ($p>0.05$; *t*-test); however, the second application of Solobond M significantly decreased lesion progression ($p<0.05$; *t*-test). Helioseal, Heliobond, Resulcin Monobond and Excite showed significantly better inhibition of the demineralization compared to the other materials ($p<0.05$; Bonferroni). It can be concluded that the penetration of adhesives into initial lesions inhibited a further demineralization *in vitro*.

INTRODUCTION

As a prophylactic, the frequent use of fluorides for the non-invasive treatment of initial enamel lesions on smooth or proximal surfaces is generally recommended. White spot lesions on smooth surfaces are only supposed to remineralize if patients have good oral hygiene and are frequently exposed to fluorides. Backer-Dirks (1966) showed that early white spot lesions on smooth surfaces can, indeed, disappear with time. Nevertheless, proximal dental plaque can only be completely removed by few patients who achieve adequate remineralizing conditions. Therefore, in patients with inadequate oral hygiene and low exposure to fluoride compounds, white spot lesions will most likely progress to caries.

On the other hand, the restoration of enamel and initial dentinal lesions results in an unfavorable damage-benefit relation. Moreover, restorations possess a limited life span. Therefore, the sealing of initial enamel lesions with resins might be a promising approach, as it can be deduced from the fissure sealing technique (Simonsen, 1991) where a physical barrier between the lesion and the source of acid production is established. However, this treatment relies upon maintenance of an intact margin between the sealant and tooth.

In 1976, Robinson and others were the first to describe the infiltration of carious lesions with organic resins and demonstrate a reduction in pore volume following the application of resorcinol-formaldehyde resin. However, this resin was unsuitable for clinical use because of its toxic nature. Follow-up studies also reported a significant reduction in pore volume after the sealing of artificial initial enamel lesions (García-Godoy, Summit & Donly, 1997; Robinson & others, 2001; Gray & Shellis, 2002; Schmidlin & others, 2004; Meyer-Lueckel & others, 2006).

The superficial sealing of initial natural lesions seems to be successful in inhibiting lesion progression after exposure to a carious challenge (Goepferd & Olberding, 1989). Caries did not progress due to resin coatings that were applied onto the surface (García-Godoy & others, 1997; Robinson & others, 2001; Schmidlin & others, 2004). However, this is not considered to be a favorable method for preventing secondary caries and periodontitis in proximal sites. The original convex proximal enamel surface is affected negatively by resin coats on top of the lesion surfaces and it should be difficult to clean these surfaces. Nevertheless, a clinical study revealed no lesion progression for 77% of the sealed lesions after two years (Ekstrand & Martignon, 2004). Thus, this non-operative approach might be a promising alternative in preventive dentistry.

Currently, no investigations exist in the accessible literature that compare the inhibition of lesion progression of different materials without resin coatings above the surface. Thus, the aim of this study was to investigate the inhibition of lesion progression in artificial bovine enamel lesions after penetration by five adhesives or one fissure sealant, respectively, when applied either once or twice. A recently developed procedure for the visualization of porous microstructures (VIRIN) was used in this investigation (Meyer-Lueckel & others, 2006).

METHODS AND MATERIALS

Sample Preparation

From 27 bovine incisors stored in 0.9% sodium chloride solution, 54 enamel slabs (approximately 5x4x3 mm^3) were prepared (Band Saw Exakt 300cl; Exakt Apparatebau, Norderstedt, Germany). The specimens were embedded in epoxy resin (Technovit 4071; Heraeus Kulzer, Hanau, Germany), and the surfaces were ground flat and polished up to 4000 grit (Phoenix Alpha; Buehler, Düsseldorf, Germany; Abrasive Paper 600, 1200, 2400, 4000; Exakt Apparatebau). The specimen surfaces were partly covered with acid resistant nail varnish (Betrix, Frankfurt/Main, Germany), leaving three uncoated windows of enamel.

Subsequently, the specimens were stored in a demineralizing solution (Buskes, Christoffersen & Arends, 1985) at pH 5 (37°C) for 14 days. After demineralization, each specimen showed three initial enamel lesions (A, B, C) separated by two lines of nail varnish. One of the lesions (C) served as the untreated control; whereas, the other two lesions (A, B) were etched for 5 seconds with 20% phosphoric acid gel (Gluma Etch 20 Gel; Heraeus Kulzer). The etching gel was washed for 30 seconds using a dental sprayer. The lesions were then dried with oil-free pressured air for another 30 seconds. The specimens were randomly divided into six groups and the adhesives Heliobond (Ivoclar Vivadent, Schaan, Liechtenstein), Resulcin Monobond (Merz, Lütjenburg, Germany), Excite (Ivoclar Vivadent), Solobond M (Voco, Cuxhaven, Germany), and Adper Prompt L-Pop (3M-ESPE, Seefeld, Germany) and fissure sealant Helioseal (Ivoclar Vivadent) were care fully applied to the lesions using a micro brush (Ivoclar Vivadent). After 90 seconds of penetration time (A and

B), the resins were light cured for 20 seconds (Translux CL; Heraeus Kulzer). On lesion part B, each material was applied for an additional 90 seconds and light cured. Before each photopolymerization, the overlying liquid material was wiped away manually after each application using a rubber cup (Brasseler, Lemgo, Germany). Half of the area of each specimen's window was then covered with nail varnish, while the other half served as the control. Some varnish on the specimen's sound surface applied prior to the demineralization was removed (scalpel; Aesculap, Tuttlingen, Germany) in order to document the effect of the second demineralization period (Figure 1). The samples were then stored in the demineralizing solution for an additional 14 days (pH 5).

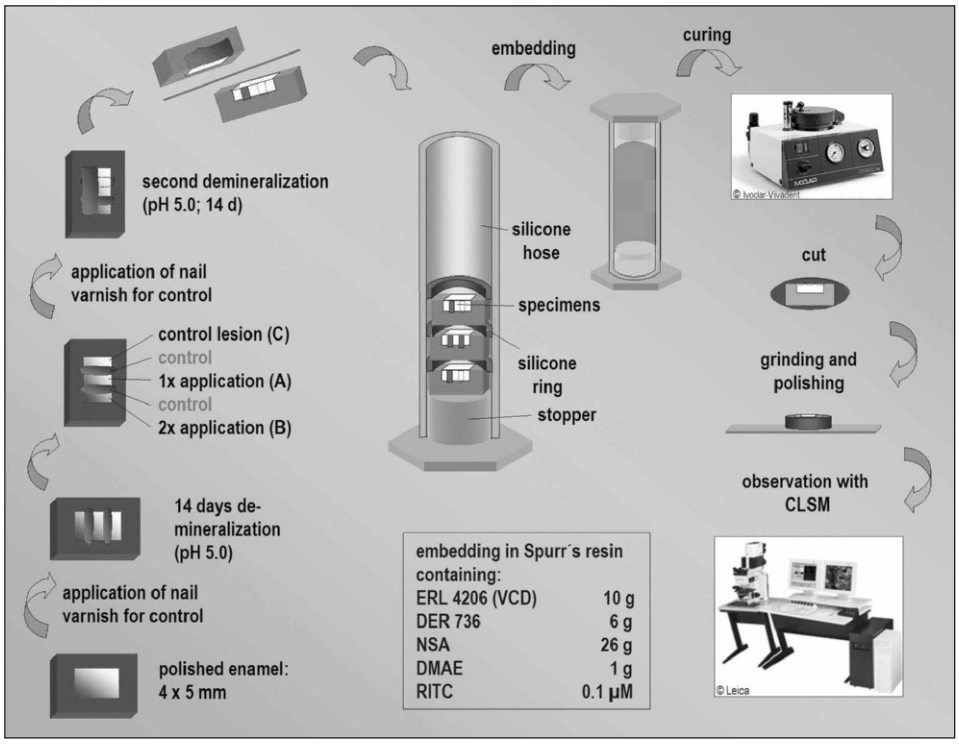

Figure 1. *Experimental setup showing the specimen preparation used for CLSM observations of the enamel lesions (modified from Meyer-Lueckel & others, 2005a).*

Subsequently, the specimens were cut perpendicular to their surfaces. The specimen halves were air dried (5 minutes) and put into a silicone hose, where one end was closed with a stopper. Spurr's resin (Spurr, 1969), which was labeled with 0.1 mmol of the fluorescent dye Rhodamine B Isothiocyanate (RITC), was doused over the specimens and the hose was closed with another stopper. The resin was cured in an autoclave (Ivomat IP3; Ivoclar Vivadent, Schaan, Liechtenstein) at 0.8 MPa and 70°C for 3 hours. After curing, the specimens were again cut perpendicular to their surfaces, fixed on object holders (diaplus, Oststeinbeck, Germany) and parallelized. Thereby, approximately 100 µm of the surface was reduced to minimize artifacts and the surfaces were polished up to 4000 grit (Exakt Mikroschleifsystem; Exakt Apparatebau).

CLSM Imaging

The specimens were studied using a confocal laser scanning microscope (CLSM) (Leica TCS NT; Leica, Heidelberg, Germany). The excitation light was generated with an Ar/Kr-Laser and had a maximum wavelength at 568 nm. The images were recorded in fluorescent mode. The emitted light passed through a 590 nm long pass filter to ensure that only fluorescent light was detected and reflected light was suppressed. The specimens were observed approximately 10 µm below the surface using a 10x objective.

The images (1000x1000 µm) were taken with a resolution of 1024x1024 pixels, using 256 pseudo color steps (red/black). Laser beam intensity and Photo Multiplier Amplification (PMT) were kept constant during the investigation. Only ranges of the sample that contained the RITC-colored Spurr's resin appeared red, while all other areas were black. The concentration of the dye was selected, likewise. Thus, the self-fluorescence of the enamel could be neglected. Three images of each lesion were made with different detector sensitivities (PMT-adjusted in three steps: 300, 500 and 700). The first image was taken with small detector sensitivity (PMT 300), thus depicting only the beginning of the lesion in good quality. The so-called pseudointact surface layer contained lower dye amounts than the lesion body and, therefore, appeared to be a thin, dark line beneath the surface. The middle PMT (500) was used to configure the lesion body. The third adjustment (PMT-700) showed the progressive demineralization underneath the lesion body (Figure 2). The three images were combined (ImageJ, v1.29x, National Institute of Health, Bethesda, MD, USA) (AVERAGE mode), resulting in one computed picture.

Analysis of the Images

The lesion depths could be differentiated due to the pseudo colors of the different grey values. The distance from the surface to the depth of the lesion, where a relatively high fluorescence could be measured, was defined as the depth of the lesion body (I). The darker zone below the body of the lesion was seen as the pro-

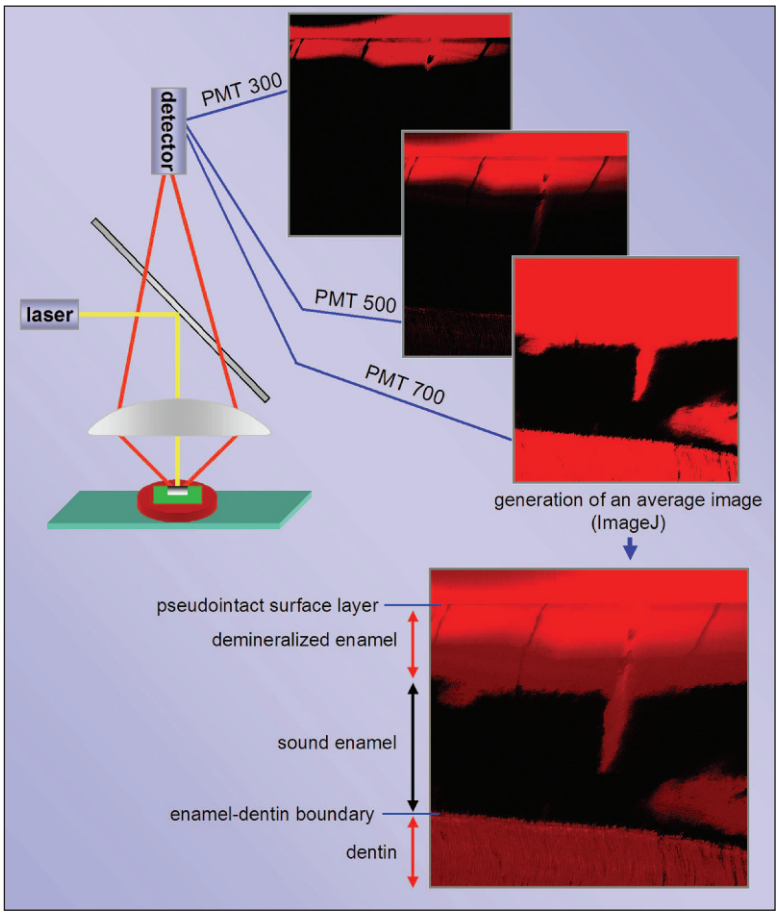

Figure 2. *Function mode of the CLSM. Three images of each lesion were taken with different detector sensitivities. The three images were combined, resulting in one computed image.*

gressing lesion front, and the maximum depth from the original surface to the depth, where fluorescence could no longer be observed, was defined as the maximum lesion depth (II) (Figure 3). A vertical grid was placed over each picture, and the mean lesion depths were calculated from the values of three neighboring lines. If it was not possible to measure the depths in area A or B, because some adhesives had penetrated the lesion completely, the values of the unfilled (control) lesion of the respective sample were used. For quantitative evaluation of the inhibition of lesion progression by the various materials, lesion depths prior to and after the second demineralization were related.

Statistical Analysis

The values were tested for normal distribution using the Kolmogorov-Smirnov-Test. Differences in lesion progression between the materials were analyzed using ANOVA and the Bonferroni post hoc test. A comparison of lesion depths after one or two applications was performed using *t*-test. All analyses were performed using the SPSS program at the 5% level of significance (SPSS 11.5 for windows, Munich, Germany).

RESULTS

Helioseal, Heliobond, Resulcin Monobond, Solobond M and Excite formed homogeneous black layers; whereas, Adper Prompt L-Pop infiltrated the lesions rather inhomogeneously. Figure 4 shows the qualitative differences in the resin layers between Helioseal and Adper Prompt

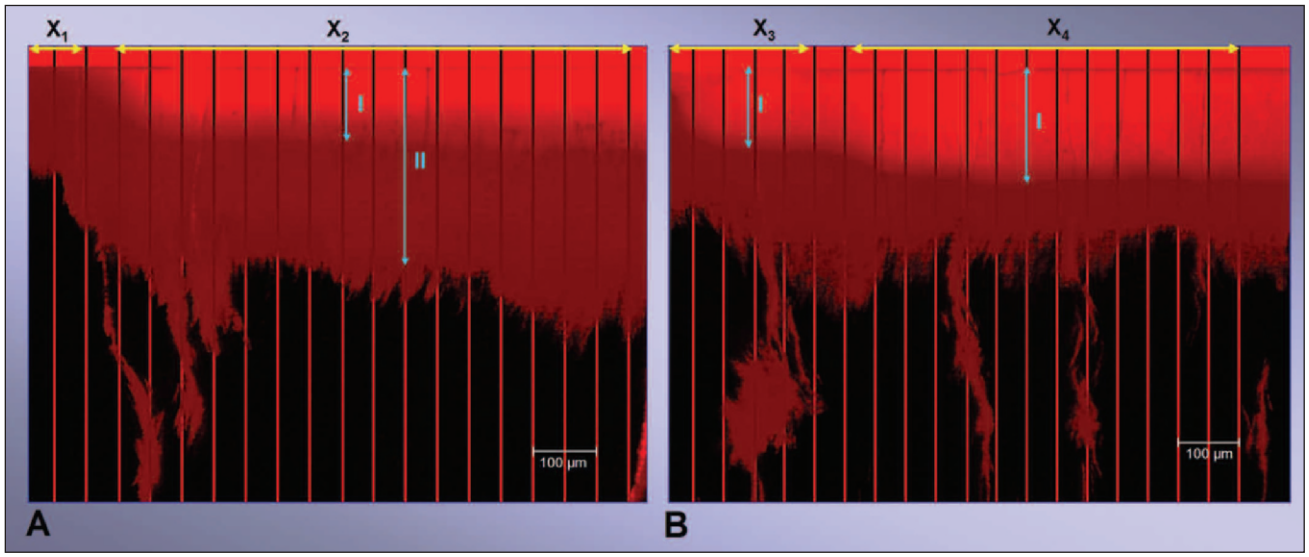

Figure 3. *A: Control lesion (14 days) (X_2) and a control zone beneath a layer of nail varnish (X_1). B: Control lesion (exposed group) depicting an area after the first (X_3) and the second demineralization (28 days) (X_4). Label I defines the lesion body x. The darker zone below the body of the lesion was seen as the progressing lesion front. The distance from the original surface to the depth, where fluorescence could no longer be observed, was defined the maximum lesion depth (label II) (control lesion, 10x).*

L-Pop before (unexposed) and after the second demineralization (exposed). None of the applied materials hardened completely at the lesion surfaces, so that the superficial enamel appeared red in the CLSM image due to the Spurr's resin infiltration. The layers of Helioseal, Heliobond, Resulcin Monobond, Solobond M and Excite were invariably homogeneous, and Adper Prompt L-Pop showed unchanged resin layers with large porous areas after the second demineralization.

The mean (SD) lesion depths I and II of the control lesions (C) were measured at 105 (21) µm and 237 (53) µm, respectively, after the first demineralizing period. After the first demineralization, the lesion depths of the various groups did not differ significantly from one another ($p>0.05$; ANOVA, Bonferroni).

After the second demineralization, the untreated lesions showed a mean progression of 52 (31)%. Helioseal, Heliobond, Resulcin Monobond and Excite were able to inhibit lesion progression completely ($p<0.05$; t-test). There were no differences between lesion depths after the first or second application ($p<0.05$; t-test) (Figure 5). In contrast, Adper Prompt L-Pop and Solobond M were not able to inhibit further lesion progression when applied once ($p>0.05$; t-test). After two applications of Solobond M, a significantly decreased lesion progression ($p<0.05$; t-test) could be observed. The other materials proved to be significantly better in hampering further lesion progression ($p<0.05$; ANOVA, Bonferroni).

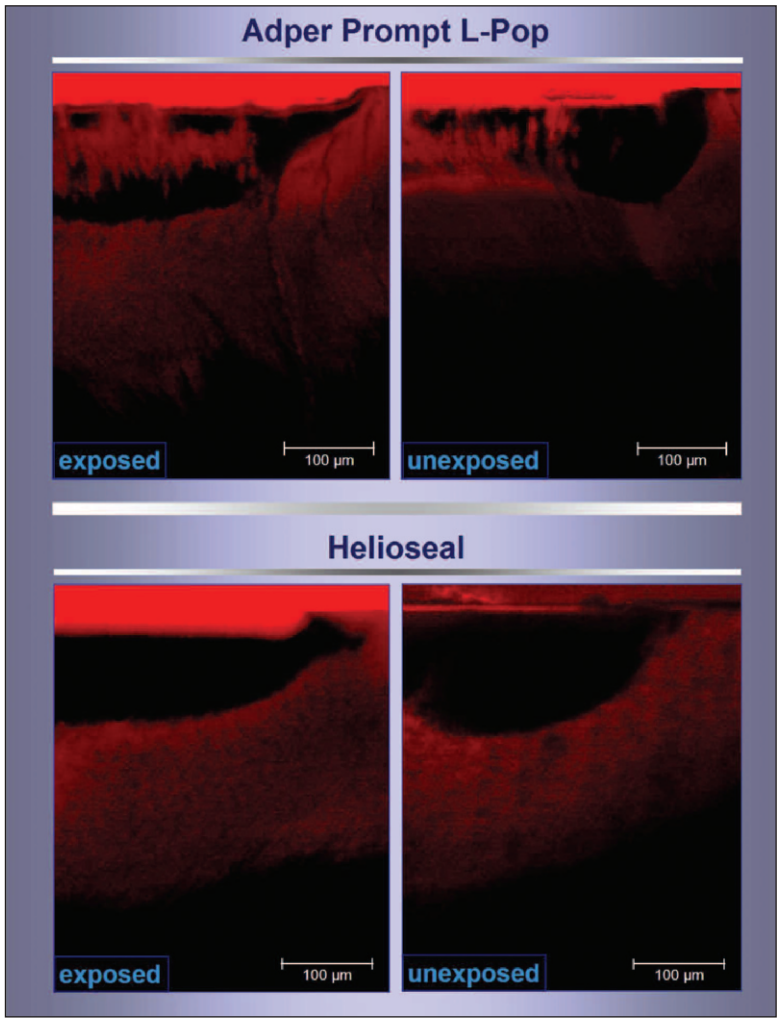

Figure 4. *Qualitative comparison of the resin layers of Adper Prompt L-Pop and Helioseal. Helioseal showed a homogenous resin layer with no qualitative differences before (unexposed) and after (exposed) second demineralization (area of 2x application, 10x). Adper Prompt L-Pop penetrated irregularly deep and showed partially polymerized resin layer in both cases (area of 2x application, 10x).*

DISCUSSION

The introduction of Confocal Laser Scanning Microscopy (CLSM) in combination with fluorescent dyes has provided a valuable new technique for the visualization of porous structures in dental hard tissues. The advantages of confocal microscopy include non-destructive examination, since the layer visualized can be situated up to 100 µm below the surface. Moreover, drying of the samples, which is required for conventional Scanning Electron Microscopy (SEM) or Transmission Electron Microscopy (TEM), is not necessary with CLSM, thus, leading to a decreased risk of shrinkage or other artifacts (Pioch, D'Souza & Staehle, 1996). Procedures based on aqueous, fluorescent solutions penetrating into the pores of enamel lesions have been described for the fluorescence-microscopic visualizing of porous structures in dental hard tissues (Fontana & others, 1996; Gonzalez-Cabezas & others, 1998). This method seems to be unfavorable, since the colorants are not bound firmly to the dental hard tissue and might be easily washed out during sample processing.

This problem is avoided with the help of a special high-pressure method (Uchtmann & Wilkie, 1997). Infiltration of the dental hard tissues with a colored resin under high pressure (200 MPa) allows for an even filling of the tiny pores (<0.1 µm). Moreover, an increased resistance of fractile structures during preparation can be likewise achieved. However, due to the high pressure, this method requires a sophisticated apparatus (technical expenditure). Finally, since the resin has a rather high viscosity, the weakened demineralized enamel structures might be destroyed.

In this study, Spurr's resin (Spurr, 1969) was used for visualization; this resin is commonly used for embedded samples studied with electron microscopy. Due to very low viscosity and excellent wetting abilities, penetration of the tiny pores (<0.1 µm in diameter) can be achieved at a relatively low pressure of 0.8 Mpa. The

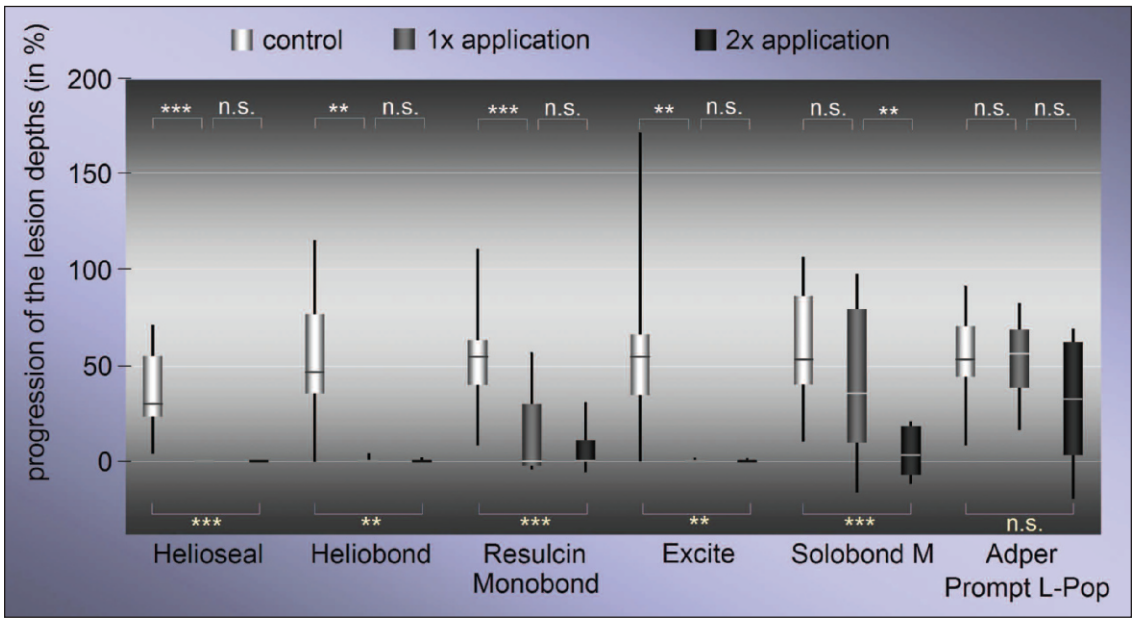

Figure 5. *Box-and-whisker plot depicting the progression of lesion depths (in %) of the tested materials. After the second demineralization, the untreated lesions showed a mean progression of 52 (31)%. Differences between the groups are indicated with asterisks (*p*>0.05 ns; *p*<0.01**; *p*<0.001***; t-test).*

lesion body of enamel lesions is not affected by the penetration of Spurr's resin and even areas beyond the lesion body can also be labeled (Meyer-Lueckel & others, 2005, 2006).

The surface zone of an initial caries lesion possesses 10 to 50 times more pores than sound enamel (Silverstone, 1973), although this pore volume amounts to only 1% (Gray & Shellis, 2002). Nevertheless, in unetched lesions, the surface layer almost completely inhibits the penetration of adhesives. To improve access into lesions etched with 37% phosphoric acid, 5 seconds seems sufficient for artificial subsurface lesions of human origin. Longer etching time has resulted in increased enamel loss and a partial breakdown of the lesion surface *in vitro*, but no increase in penetration depths could be observed (Gray & Shellis, 2002). In bovine enamel, using a lower concentrated phosphoric acid gel (20%) and applying it to a wet enamel surface (dilution effect by wet etching) resulted in less destruction of the surface and the lesion body and was therefore chosen in this study.

Adper Prompt L-Pop shows self-etching capabilities because of polyalcenic acid (pH<1). Nevertheless, preliminary tests showed that the etching effect is too small to degrade the surface layer in order to allow for penetration into the lesions. Moreover, by using the same etching regimen for all materials, this material could be better compared to the other materials.

The relatively short demineralization period of 14 days is long enough to create an initial lesion in bovine enamel or to deepen (up to 50%) an existing lesion as seen in this study. However, it is assumed that after longer demineralizing periods, partially filled lesions might progress. Nevertheless, the chosen demineralizing model seems suitable for comparing the initial inhibition of lesions by adhesives.

Operative intervention should not be a treatment option for the non-cavitated initial lesion. An early report revealed that more than half of initial lesions may disappear with time (Backer-Dirks, 1966). Early enamel caries might remineralize, and it has been suggested that progress of the disease is very slow. Nevertheless, patients with less compliance could be treated by resin infiltration to arrest the initial enamel lesions. The results of this paper show that the pores of the lesion body and the deeper lesion areas (front of demineralization) can be infiltrated successfully with an adhesive, thus protecting the lesions against further demineralizing influences, although this still has to be proven for natural lesions.

Previous studies pertaining to the sealing of subsurface demineralizations left a coat of resin on top of the lesions (García-Godoy & others, 1997; Robinson & others, 2001; Gray & Shellis, 2002; Schmidlin & others, 2004). Unfortunately, from these studies, it remains unclear whether the seal was accomplished by obturation of the pores in the lesion body or by the covering resin coat. In this investigation, overlying resin was wiped away before light curing the sealants. Therefore, it can be assumed that only the infiltrated material influenced the progression of demineralization. Cleaning of the overlying material from the surface of the infiltrated lesions prior to polymerization is advantageous, since the ideal form of the surface can be retained. After light curing, the overlying resin cannot be that easily polished (for example with abrasive stripes) without damaging the lesion surface or adjacent sound enamel. So-called "adhesive patches" for proximal sealing were suggested as a proximal sealant (Schmidlin & others, 2005). Nevertheless, application of these patches seems to be rather difficult and might result in an unfavorable hygienic situation.

A preliminary investigation showed that Helioseal and Resulcin Monobond almost completely penetrated the lesion body (Meyer-Lueckel & others, 2005). Heliobond and Excite even penetrated into less demineralized areas beneath the lesion body (progressing front of demineralization). The results of this paper showed that lesion progression can be significantly reduced even by incomplete penetration (Solobond M) of the lesion body. Thus, it does not seem necessary to completely fill the lesion in order to prevent progression of a lesion. This is supported by a previous study where superficially sealed lesions did not progress (Goepferd & Olberding, 1989). After etching the pseudo intact surface of initial natural lesions, only an etched surface texture was produced. The adhesive also penetrated only up to 50 µm into the superficial pores of the surface layer, but not into the lesion body. Moreover, a thick layer of adhesive was also left on the lesion surface (Goepferd & Olberding, 1989). However, even superficial sealing inhibited progression of the lesion. Thus, it is not surprising that even partially filled lesion bodies were capable of preventing further demineralization under the experimental conditions of this study. However, with Solobond M, the progression of lesion formation (4%) was measured; whereas, Helioseal, Heliobond and Excite were able to inhibit further demineralization with complete penetration, as seen in this study. In a previous study, it was shown that, with these materials, partially filled lesion bodies almost completely inhibited lesion progression (Paris & others, 2006). Nevertheless, it can be assumed that, after longer demineralizing periods, partially filled lesions might progress. Therefore, complete infiltration of a sealant should be the goal.

When applied once, Excite showed a very thick oxygen inhibition layer compared to the other materials, although the established adhesive layer was thick enough to prevent further demineralization. Adper Prompt L-Pop showed a very inhomogenous, and only partly polymerized resin layer even after the second application due to the solvent (water), and it could not protect the lesions from further acidic influences (Meyer-Lueckel & others, 2006). Monobond, Helioseal and Heliobond contained no solvents and showed no pores within the adhesive layers, thus preventing a successful lesion progression. Therefore, solvent-free materials seem preferable for the infiltration of enamel lesions.

The aspect of the clinical feasibility of sealing proximal lesions should also be considered. A slight separation of the teeth can be ascertained by orthodontic elastomeric separating rings or wires. An interdental gap of 0.5 to 1 mm is readily produced after some days (Seddon, 1989), a procedure that might be even easier in posterior teeth. Since etching with syringes and application of the resin with a micro brush seems to be unfeasible, application tools based on stripes are being developed.

CONCLUSIONS

Five of the six materials were capable of protecting the initial lesions from further demineralization. A resin layer on top of the lesion is not required to accomplish this goal, if the lesion body is homogeneously infiltrated with a resin. Future studies using natural lesions should be implemented to prove this procedure *in vitro* before its clinical application.

Acknowledgements

The authors are indebted to Dr M Hummel (Institute of Pathology, CBF) for the possibility of using the CLSM.

(Received 12 March 2005)

References

Backer-Dirks O (1966) Posteruptive changes in dental enamel *Journal of Dental Research* **45(3)** 503-511.

Buskes JA, Christoffersen J & Arends J (1985) Lesion formation and lesion remineralization in enamel under constant composition conditions. A new technique with applications *Caries Research* **19(6)** 490-496.

Ekstrand KR & Martignon S (2004) Managing approximal carious lesions: A new non-operative approach *Caries Research* **38(4)** 357-412 Abstract #12.

Fontana M, Li Y, Dunipace AJ, Noblitt TW, Fischer G, Katz BP & Stookey GK (1996) Measurement of enamel demineralization using microradiography and confocal microscopy. A correlation study *Caries Research* **30(5)** 317-325.

García-Godoy F, Summitt JB & Donly KJ (1997) Caries progression of white spot lesions sealed with an unfilled resin *Journal of Clinical Pediatric Dentistry* **21(2)** 141-143.

Goepferd SJ & Olberding P (1989) The effect of sealing white spot lesions on lesion progression *in vitro Pediatric Dentistry* 11(1) 14-16.

Gonzalez-Cabezas C, Fontana M, Dunipace AJ, Li Y, Fischer GM, Proskin HM & Stookey GK (1998) Measurement of enamel remineralization using microradiography and confocal microscopy. A correlational study *Caries Research* **32(5)** 385-392.

Gray GB & Shellis P (2002) Infiltration of resin into white spot caries-like lesions of enamel: An *in vitro* study *European Journal of Prosthodontic Restorative Dentistry* **10(1)** 27-32.

Meyer-Lueckel H, Paris S, Mueller J, Coelfen H & Kielbassa AM (2006) Influence of the application time on the penetration of different dental adhesives and a fissure sealant into artificial subsurface lesions in bovine enamel *Dental Materials* **22(1)** 22-28.

Meyer-Lueckel H, Mueller J, Paris S, Hummel M & Kielbassa AM (2005) [The penetration of various adhesives into early enamel lesions *in vitro*] *Schweizer Monatsschrift für Zahnmedizin* **115(4)** 316-323.

Paris S, Meyer-Lueckel H, Mueller J, Hummel M & Kielbassa AM (2006) Progression of sealed initial bovine enamel lesions under demineralizing conditions *in vitro Caries Research* **40(2)** 124-129.

Pioch T, D'Souza PD & Staehle HJ (1996) Resin-dentin interface studied by SEM & CLSM *Microscopy and Analysis* **42(1)** 15-16.

Robinson C, Brookes SJ, Kirkham J, Wood SR & Shore RC (2001) *In vitro* studies of the penetration of adhesive resins into artificial caries-like lesions *Caries Research* **35(2)** 136-141.

Robinson C, Hallsworth AS, Weatherell JA & Kunzel W (1976) Arrest and control of carious lesions: A study based on preliminary experiments with resorcinol-formaldehyde resin *Journal of Dental Research* **55(5)** 812-818.

Schmidlin PR, Zehnder M, Zimmermann MA, Zimmermann J, Roos M & Roulet JF (2005) Sealing smooth enamel surfaces with a newly devised adhesive patch: A radiochemical *in vitro* analysis *Dental Materials* **21(6)** 545-550.

Schmidlin PR, Zehnder M, Pasqualetti T, Imfeld T & Besek MJ (2004) Penetration of a bonding agent into de- and remineralized enamel *in vitro Journal of Adhesive Dentistry* **6(2)** 111-115.

Seddon RP (1989) The detection of cavitation in carious approximal surfaces *in vivo* by tooth separation, impression and scanning electron microscopy *Journal of Dentistry* **17(3)** 117-120.

Silverstone LM (1973) The susceptibility to dissolution of fissure-sealed enamel surfaces artificially abraded *in vitro Helvetica Odontolgica Acta* **17(3)** 64-67.

Simonsen, RJ (1991) Retention and effectiveness of dental sealant after 15 years *Journal of the American Dental Association* **122(10)** 34-42.

Spurr AR (1969) A low-viscosity epoxy resin embedding medium for electron microscopy *Journal of Ultrastructural Research* **26(1)** 31-43.

Uchtmann H & Wilkie D (1997) High-pressure replica technique for *in vitro* imaging of pore morphologies in teeth *Advances in Dental Research* **11(4)** 467-471.