©Operative Dentistry, 2014, **39-5,** 481-488

Laboratory Research

In Vitro Progression of Artificial White Spot Lesions Sealed With an Infiltrant Resin

R Gelani • AF Zandona • F Lippert
MM Kamocka • G Eckert

Clinical Relevance

Infiltration of initial *in vitro* enamel lesions by resin seems to reduce or even stop the progression of carious lesions.

SUMMARY

This study assessed the ability of an infiltrant resin (Icon, DMG Chemisch-Pharmazeutische Fabrik GmbH, Hamburg, Germany) to prevent artificial lesion progression *in vitro* when used to impregnate white spot lesions and also assessed the effect of saliva contamination on resin infiltration. Enamel specimens (n=252) were prepared and covered with nail varnish, leaving a window of sound enamel. After demineralization (pH 5.0; four weeks), specimens were divided into six groups (n=42 per group): group 1, 2% fluoride gel (positive control); group 2, resin infiltrant; group 3, resin infiltrant + fluoride gel; group 4, no treatment (negative control); group 5, resin infiltrant application after saliva contamination; and group 6, resin infiltrant + fluoride gel after saliva contamination. Specimens from each group were cut perpendicular to the surface, and one-half of each specimen was exposed to a demineralizing solution for another four weeks. The other half was set aside as a record of initial lesion depth and was used later in the determination of lesion progression. Lesion progression and infiltrant penetration were measured using confocal laser scanning microscopy (CLSM) and transverse microradiography (TMR). For lesion depth, based on CLSM, groups 2 and 3 showed the least changes when submitted to demineralization challenge, followed by group 1, then groups 5 and 6, and finally group 4. There were no significant differences between groups 2

*Rakhi Gelani, BDS, MSD Preventive Dentistry student, Indiana University School of Dentistry, Indianapolis IN, USA

Andrea Ferreira Zandona, DDS, MSD, PhD, associate professor/director, MSD Preventive Dentistry, Department of Preventive and Community Dentistry, Indiana University School of Dentistry, Indianapolis IN, USA

Frank Lippert, MSc, PhD, assistant research professor/director, OHRI Remineralization Research Program, Department of Preventive and Community Dentistry, Indiana University School of Dentistry, Indianapolis IN, USA

Malgorzata Maria Kamocka, PhD, assistant research professor of medicine, Department of Medicine/Division of Nephrology, Research Institute, Indiana University School of Medicine, Indianapolis IN, USA

George Eckert, biostatistician supervisor, Department of Biostatistics, Indiana University School of Medicine, Indianapolis IN, USA

*Corresponding author: 1121 W. Michigan St., Indianapolis IN 46202; e-mail: rakhigelani@gmail.com

DOI: 10.2341/13-202-L

and 3 or groups 5 and 6 in their ability to inhibit further lesion progression ($p<0.05$). Based on TMR, groups 2 and 3 also showed the fewest changes when submitted to demineralization challenge, followed by group 5, then groups 1 and 6, and finally group 4. In terms of mineral loss as measured by TMR, all groups that contained fluoride (groups 1, 3, and 6) show less percentage change in mineral loss than the groups that did not contain fluoride (groups 2, 4, and 5). It can be concluded that infiltrant penetration into early enamel lesions inhibited further demineralization *in vitro*, especially in the presence of fluoride. Saliva contamination decreased the ability of the infiltrant to prevent further demineralization, but the presence of fluoride seemed to counteract this effect.

INTRODUCTION

Dental caries is one of the most prevalent chronic diseases globally, with individual susceptibility lasting a lifetime. Early recognition of the disease process (before cavitation) is important to implement intervention in an attempt to stop and even reverse the disease process (remineralization of the noncavitated lesion).[1] Within the modern concept of dental caries management, prevention and hard-tissue preservation are the primary goals, and dentists are encouraged to prefer a more conservative and biological rather than a surgical approach.[1] Common nonoperative treatment for enamel caries includes fluoride application, sealants, and behavioral modification. For pits and fissures, mainly on the occlusal surfaces of permanent molars, sealing with light-curing resins has been shown to be an effective preventive measure.[2,3] A promising alternative therapy to arrest caries lesions on proximal surfaces might be the infiltration of subsurface lesions with low-viscosity, light-curing resins. Early white spots have increased enamel porosity. Since porosities of enamel caries lesions act as diffusion pathways for acids and dissolved minerals, infiltration of these pores with low-viscosity resins might occlude the pathways and thus hamper or arrest caries progression.[4] These same porosities can also be the ideal loci for infiltration of adhesives.[5]

Reduction in pore volume after the sealing of artificial initial enamel lesions has been reported in several studies[5-14] either by dental adhesives[15-17] or by fissure sealants. However, dental sealants and adhesives are not optimized for high penetrability and have therefore shown only superficial penetration into natural enamel lesions.[18] Special resins, optimized for rapid capillary penetration (so-called infiltrants), penetrate significantly deeper.[19] In laboratory experiments, resin-infiltrated enamel lesions without a covering resin coat showed a significantly reduced lesion progression in a demineralizing environment compared to untreated lesions.[14,15] The aim of caries infiltration is to saturate the porous lesion body with a low-viscosity resin (infiltrant) that is subsequently hardened with blue light.[4,18,19] Thereby, diffusion pathways for cariogenic acids are blocked and lesions sealed. However, in contrast to conventional caries sealing,[20,21] with this technique, the diffusion barrier is created inside the lesion and not on the surface, facilitating clinical application, especially in the interproximal space.[22] Recently, a resin based on this concept was introduced to the market (Icon, DMG Chemisch-Pharmazeutische Fabrik GmbH, Hamburg, Germany). This material, composed of triethylene-glycol-dimethacrylate–based resin, bisphenol A glycerolate dimethacrylate, camphorquinone, and ethyl 4-(dimethylamino) benzoate and ethanol, has an extremely high penetration coefficient that facilitates deeper penetration. However, little is known about the performance of the new infiltrant resin on the progression of white spot lesions[23] or the effect that salivary contamination might have on the penetration of the infiltrant. Studies show that one second of contact between saliva and etched enamel is enough to noticeably modify enamel topography.[24] Furthermore, Taskonak and Sertgoz reported that etched enamel absorbs salivary components, decreasing surface energy and impairing potential adhesion.[25] Therefore, it can be assumed that infiltrant, as it is based on the same principle, would likely suffer the same consequences of saliva contamination.

As a noninvasive treatment, the use of topical fluoride associated with plaque removal is indicated to promote lesion remineralization.[26] Remineralization is the natural repair process for noncavitated lesions and relies on calcium and phosphate ions, assisted by fluoride, to rebuild a new surface on existing crystal remnants in subsurface lesions remaining after demineralization. Fluoride ions incorporate into remineralizing enamel/dentin, changing carbonated apatite to a fluoroapatite-like form that is more acid tolerant and makes the hard tissues more acid resistant.[26] To date, there are no data comparing the preventive effect of resin infiltrant, fluoride application, or a combination of the two on lesion progression. Therefore, the purpose of this study was to assess the in vitro ability of an

infiltrant resin to impregnate artificial white spot lesions to prevent lesion progression when done alone and also in conjunction with fluoride treatment. Additionally, as a secondary objective, this study assessed the effect of saliva contamination on resin infiltration. The null hypotheses tested were 1) that caries progression was not altered by the different treatments tested 2) that there was no effect of saliva contamination on resin infiltration.

METHODS AND MATERIALS

Sample Preparation

Enamel slabs (approximately 5×5 mm^2) were prepared from the facial aspect of bovine incisors stored in an aqueous 0.1% thymol solution. The slabs were cut from the middle third of the labial coronal surfaces. Initially, the pulpal surface of the slabs was flattened in a grinding machine (Roto-Pol31/Roto-Force4 polishing unit, Struers, Westlake, OH, USA). Next, the external/experimental surface was sequentially flattened with 500-, 1200- and 4000-grit silicon carbide grinding papers (MD-Fuga, Struers) and then polished (1-μm diamond suspension; Struers). Specimens with white spots, cracks, or any other defect were discarded. The remaining specimens were mounted on an acrylic rod with sticky wax, and their baseline surface microhardness (SMH) was determined by the mean length (Lb) of five indentations placed 100 μm apart from each other in the center of the specimen using a Knoop diamond indenter with a load of 50 g and a dwell time of 15 seconds (2100 HT, Wilson Instruments, Norwood, MA, USA).[27] Only specimens with baseline SMH between 300 and 350 KHN were selected for the study (n=252).

Preparation of Caries-Like Lesions

The enamel surface of each specimen was partly covered with acid-resistant nail varnish, leaving an experimental window of sound enamel of about 5×3 mm. Specimens were then demineralized using a hydroxyethylcellulose (HEC) acid gel. The gel was prepared by adding HEC (Sigma 54290, Cellosize QP-40, 80-125 cP, at a ratio of 140 g/L) to a pH 5.0–adjusted solution containing 0.05 M lactic acid. The solution was continuously stirred until the HEC was partially hydrolyzed (about 30 minutes). The gel was then poured into a container and placed into an incubator set at 37°C for about 24 hours. Specimens were placed into a second, sealable container, and fully hydrolyzed (ie, clear) HEC gel was poured over them. The specimens were demineralized at 37°C for four weeks. The SMH of the enamel specimens was measured again (SMH1). Indentations were spaced 100 μm from each other and from the baseline indentation sites.

Treatment of White Spot–Like Lesions

All specimens were balanced according to microhardness values (SMH1) and divided into six groups (n=42 per group; Figure 1). In group 1 (positive control), enamel specimens were treated with a fluoride gel (2% NaF, neutral ssodium fluoride) for 4 minutes, which was removed by rinsing the specimens under running distilled water for 2 minutes. For all groups that received the infiltrant (groups 2, 3, 5, and 6), the indirect staining technique[28] was used prior to application of the infiltrant: the surface was dried with compressed air for five seconds and etched for five seconds with 37% phosphoric acid, and specimens were stored in an ethanolic solution of 0.1% Rhodamine B isothiocyanate (RITC) for 12 hours to dehydrate and label all accessible porosities with the red fluorophore. Because etching with phosphoric acid removes the surface layer in bovine teeth,[9] HCl was not used. Group 2 specimens were dried using compressed air for 10 seconds, and pure infiltrant (ICON preproduct) was applied onto the lesion surface. In order to ensure that inhibition of lesion progression was achieved only by infiltration of the lesion body and not by a superficial resin layer, after five minutes resin surplus was removed from the surface using a cotton roll, and the material was then light cured for 60 seconds (530 mW/cm^2; Astralis 5; Ivoclar Vivadent,Schaan Liechtenstein). To bleach all red fluorophore that had not been enclosed by infiltrant, specimens were stored in 30% hydrogen peroxide solution for 12 hours at 37°C. Subsequently, specimens were washed with water for 60 seconds. Group 3 specimens were first treated the same way as group 2, and then fluoride gel (2% NaF, neutral sodium fluoride) was applied for 4 minutes. Subsequently, the fluoride gel was removed by rinsing in distilled water for two minutes. Group 4 specimens served as the negative control and received no treatment. Group 5 specimens were treated the same way as group 2 (dry, acid etch, and label with red fluorophore) and then exposed to saliva before placing the resin infiltrant (after the acid-etching step). Stimulated, frozen pooled whole human saliva collected under Indiana University Institutional Review Board (IRB) approval (IRB no. 1105005588) was microbrushed onto the selected specimens, left undisturbed for five seconds, and gently air blown for three to five seconds. Subsequently, resin

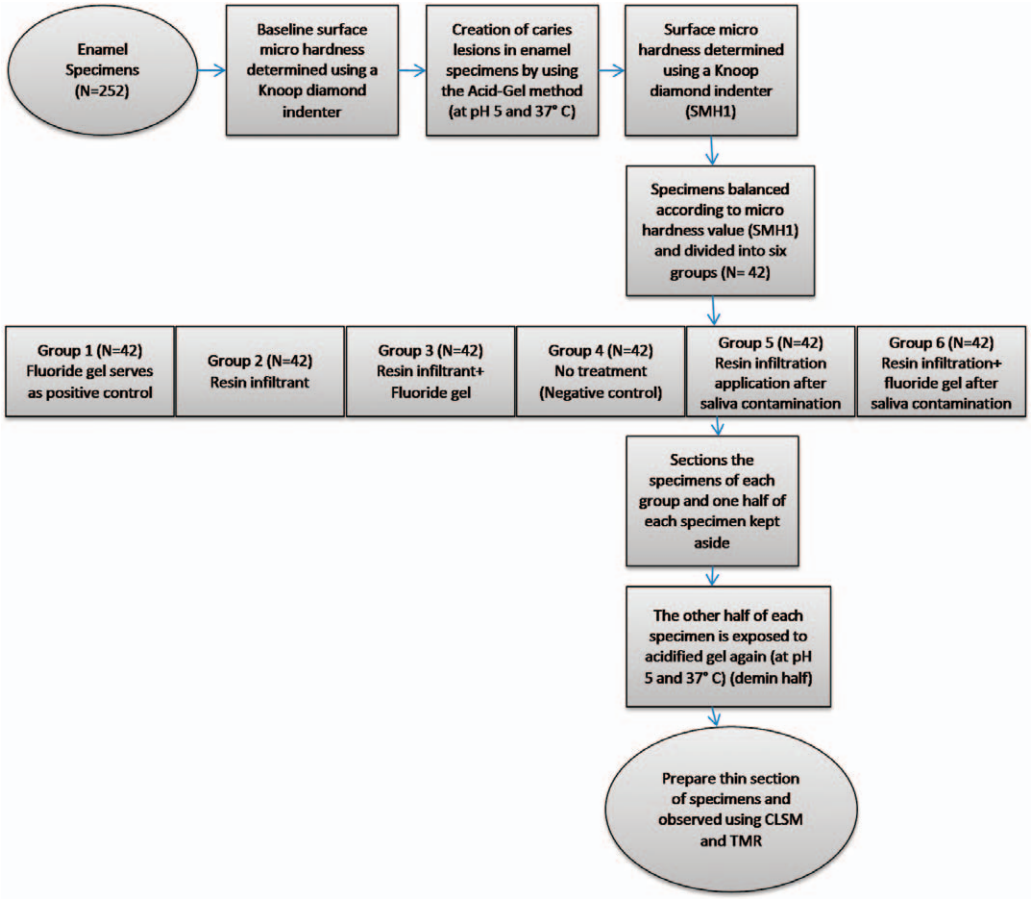

Figure 1. *Schematic representation of specimen preparation.*

infiltrant application and storing specimens in 30% hydrogen peroxide took place as previously described.

In group 6, specimens were dried, acid etched, labeled with red fluorophore, and then exposed to saliva. The resin infiltrant application and storing in 30% hydrogen peroxide took place after the saliva exposure. This was followed by a four-minute application of fluoride gel (2% NaF, sodium fluoride gel), which then was removed by rinsing in distilled water for 2 minutes.

In order to evaluate the progression of sealed lesions in a demineralizing environment, specimens from each group were cut perpendicular to their surface using a hard-tissue microtome yielding two halves for each lesion. One-half of each specimen (initial half) was set aside to be used later as a record of initial lesion depth when determining lesion progression. For the remaining specimen halves (demineralization half), the cut surface of each specimen was covered with nail varnish and treated as per above, and the specimens exposed to the hydroxyethylcellulose gel for another four weeks to simulate a cariogenic environment.

Confocal Laser Scanning Microscopy and Transverse Microradiography

The nail varnish covering the cut surface of each of the demineralization specimen halves was removed with acetone. For examination by confocal laser scanning microscopy and transverse microradiography (TMR), thin sections (100 μm) of both initial and demineralization halves of each specimen were prepared. First, specimens were observed using a confocal laser scanning microscope (Olympus Fluoview FV1000 MPE, Olympus, Center Valley, PA, USA) at the Indiana Center for Biological Microscopy (Indianapolis, IN, USA). To visualize the pore structures of the uninfiltrated portion of the lesions, both halves of each specimen were immersed in 50% ethanol solution containing 100 μM sodium fluorescein (Sigma Aldrich, Saint Louis, MO, USA) for 10 minutes. Subsequently, specimens were thoroughly washed in deionized water for three minutes and

Table 1: *Change in Confocal Lesion Depth After Demineralization Challenge*

Group	n	Confocal Lesion Depth (in μm)		
		Initial Mean (SE)	After-Challenge Mean (SE)	% Change[a] Mean (SE)
1. Fluoride (F)	42	110.1 (2.7)	146.4 (4.1)	33.9 (3.1) A
2. Icon	42	116.2 (3.3)	121.0 (3.2)	4.3 (0.6) B
3. Icon + (F)	42	106.9 (3.1)	111.0 (3.1)	4.0 (0.6) B
4. No treatment	42	109.7 (2.2)	172.3 (3.7)	58.3 (3.3) C
5. Saliva contamination + Icon	42	116.1 (3.7)	165.0 (5.3)	42.3 (2.2) D
6. Saliva contamination + Icon + (F)	42	108.4 (3.2)	155.1 (4.8)	43.4 (2.7) D

[a] *Percentage change after challenge within group significantly different at* $p<0.0001$.
Statistical significance between groups indicated by different letters ($p\leq0.008$).

visualized with a confocal laser scanning microscope; a 488-nm excitation light and 500-545-nm emission filter band was used to visualize fluorescein isothiocyanate, and a 559-nm excitation laser and 570-650-nm emission filter band was used for RITC. Images were recorded with a lateral resolution of 1024 × 1024 pixels (635 × 635 μm) and analyzed using Metamorph imaging software (Molecular Devices, Downingtown, PA, USA).

At three defined points per image (depending on the lesion depth indicated by a 50-μm grid), both initial lesion depth and resin penetration depth were measured and their mean values calculated. The lesion depth was defined as the distance from the surface of the specimen to the point where the prism cores were no longer fluorescent.[14] Lesion depth was measured for each specimen individually. In the case of complete infiltration, the initial lesion depth was the same as the resin penetration depth. If the lesion depth could not be exactly determined in "demin" halves due to complete infiltration, it was assumed that the lesion had not progressed. Subsequently, thin sections were examined using TMR.[29] Lesion depths as well as integrated mineral loss for both lesion halves were measured using TMR software (TMR for Windows, version 2.0.27.2, Inspektor Research System, Amsterdam, The Netherlands). Lesion progression was then evaluated by subtracting lesion depths of paired "initial" halves from "demin" halves.[15] The outcomes measure was depth of penetration of the resin infiltrant for both halves of the specimens and lesion depth and mineral loss on both halves of the specimens.

Statistical Analysis

For each treatment group, the significance of the lesion depth and mineral loss changes between the treatment and demineralization periods was tested using Wilcoxon signed rank tests. The treatment groups were compared for differences in percentage change in lesion depth and percentage change in mineral loss using Wilcoxon rank sum tests. Resin penetration depth was calculated as the average penetration from the two halves of each specimen. Penetration depth was also compared between groups using Wilcoxon rank sum tests.

RESULTS

With the new indirect staining technique, the confocal microscopic images obtained in dual fluorescence mode showed red-infiltrated structures (RITC), whereas porous structures (noninfiltrated part of the lesion) appeared green due to staining with NaFl. Nonporous structures, like sound enamel, showed no fluorescence and were displayed dark.

A total of 10 specimens were damaged during preparation for TMR. Thus, averages of 39-40 specimens from each group were analyzed by TMR. Based on confocal lesion depth, group 2 (resin infiltrant) and group 3 (resin infiltrant + fluoride) showed the fewest changes when submitted to the demineralization challenge, followed by group 1 (fluoride), group 5 (saliva contamination + resin infiltrant), and group 6 (saliva contamination + resin infiltrant + fluoride), then group 4 (negative control, no treatment; Table 1). There were no significant differences between groups 2 and 3 or groups 5 and 6 in their ability to inhibit further lesion progression.

When analyzing lesion depth using TMR, group 2 (resin infiltrant) and group 3 (resin infiltrant + fluoride) showed the fewest changes when submitted to the demineralization challenge, followed by group 5 (saliva contamination + resin infiltrant), then group 6 (saliva contamination + resin infiltrant + fluoride) and group 1 (fluoride), then group 4 (negative control, no treatment; Table 2).

In terms of mineral loss as measured by TMR, all groups that included fluoride (groups 1, 3, and 6)

Table 2: *Change in Transverse Microradiography Lesion Depth and Mineral Loss Measurements After Demineralization Challenge*

Group	n	TMR Lesion Depth (in μm)			TMR Mineral Loss (in vol% × μm)		
		Initial Mean (SE)	After-Challenge Mean (SE)	% Change[a] Mean (SE)	Initial Mean (SE)	After-Challenge Mean (SE)	% Change[a] Mean (SE)
1. Fluoride (F)	41	155.1 (1.8)	197.1 (3.0)	27.6 (2.2) A	2267 (49)	3076 (111)	37.2 (5.1) A
2. Icon	40	187.4 (3.3)	190.9 (3.4)	1.9 (0.3) B	2546 (70)	3910 (109)	57.1 (5.5) B
3. Icon + F	42	195.1 (3.6)	197.3 (3.5)	1.2 (0.2) B	2852 (87)	3605 (87)	31.4 (5.0) A
4. No treatment	39	155.2 (2.4)	221.8 (4.1)	43.8 (3.0) C	2467 (61)	3991 (133)	63.8 (5.8) B
5. Saliva contamination + Icon	40	177.5 (3.0)	207.5 (3.2)	17.8 (2.2) D	2477 (63)	3811 (101)	57.1 (5.5) B
6. Saliva contamination + Icon + F	40	151.9 (3.4)	187.8 (3.1)	24.9 (2.2) A	2428 (82)	3259 (70)	39.7 (5.3) A

[a] *Percentage change after challenge within group significantly different at* $p<0.0001$.
Statistical significance ($p\leq0.03$) *between groups indicated by different letters.*

Table 3: *Measurements of Infiltrant Resin Penetration by Confocal Microscope*[a]

Group	n	Lesion Depth	Infiltrant Resin Penetration[a]
Icon	42	116.2 (3.3)	112.8 (2.9) A
Icon + F	42	106.9 (3.1)	103.0 (2.9) B
Saliva contamination + Icon	42	116.1 (3.7)	110.1 (3.3) AB
Saliva contamination + Icon + F	42	108.4 (3.2)	106.0 (2.5) AB

[a] *Statistical significance* ($p=0.004$) *between groups indicated by different letters.*

showed less percentage change in integrated mineral loss than the groups that did not include fluoride (groups 2, 4, and 5; Table 2). Resin penetrated significantly deeper in group 2 (resin infiltrant; $p=0.0041$) than in group 3 (resin infiltrant + fluoride), but there were no other statistically significant differences in penetration depth among other groups (Table 3). Although saliva did not interfere with the penetration of resin, the resin layer was not homogeneous, as seen in Figure 2, and thus there was greater lesion progression in saliva-contaminated groups (Tables 1 through 3).

DISCUSSION

Resin infiltration of enamel lesions aims to reduce or even stop the progression of white spot lesions based on the available clinical and laboratory stud-

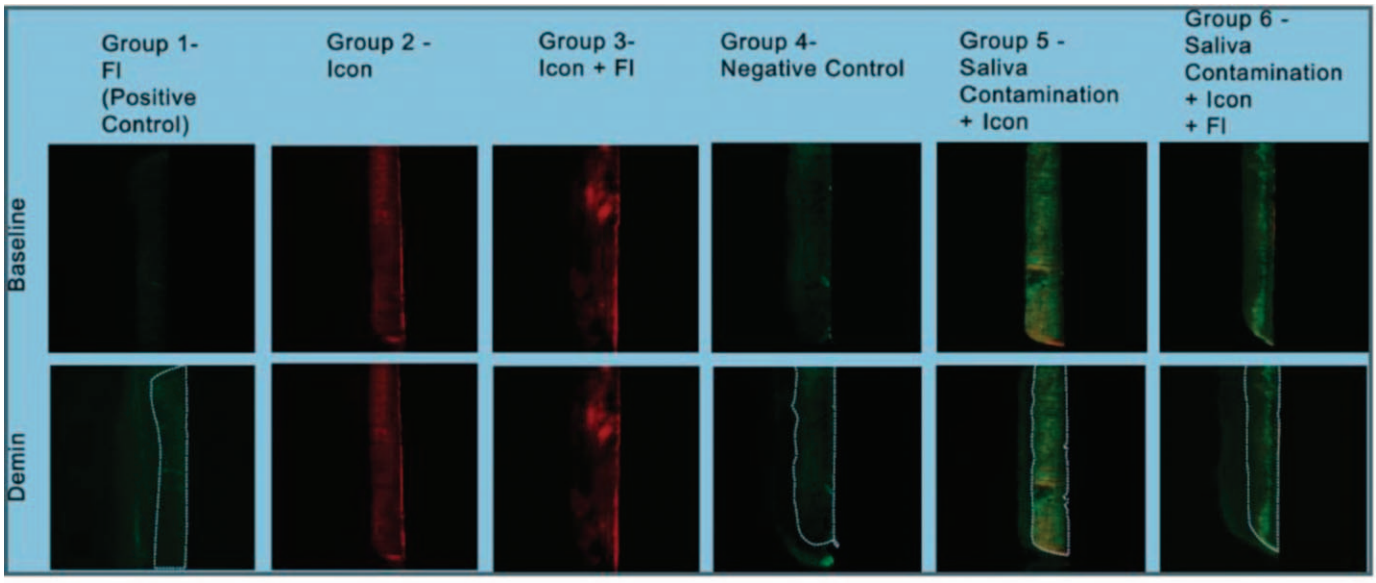

Figure 2. *Representative confocal laser scanning microscopy (CLSM) images of corresponding lesion halves of all groups. On the images of the "demin" half. the extent of the corresponding baseline lesion is indicated by a dotted line. Group 1 shows slight progression of lesion, and groups 2 and 3 infiltrated the complete lesion body with resin. Here, no progression of lesion depth could be observed using CLSM. Group 4, the untreated control in the "demin" half, progressed significantly compared to the baseline. In groups 5 and 6, the lesion is not homogeneously infiltrated, and progression of lesion depth could be observed. Notice the inhomogeneity of the resin layer in groups 5 and 6; the inhomogeneous layer of resin tags appears as a mix of red and green (ie, yellow) or more green.*

ies.[11,15,19,23,30,31] This novel technique might bridge the gap between noninvasive and minimally invasive treatment of initial dental caries, postponing as long as possible the need for a restoration.[23] Fluoride's ability to inhibit or even reverse the initiation and progression of dental caries is well documented.[32] As a prophylactic, the frequent use of fluorides for the noninvasive treatment of initial enamel lesions is generally recommended, and remineralization of the lesion may be obtained by improving the patient's oral hygiene and the use of fluoride toothpaste. The aim of the current study was to evaluate the synergistic effect of treatment with the resin infiltration technique and remineralization of enamel caries with fluoride gel on the progression of initial dental caries. According to the results of this study, all the treatments tested hampered lesion progression although at different levels. The first null hypothesis of the study was rejected, as caries progression differed among the different treatments (fluoride, resin infiltration + fluoride, or resin infiltration). After the new acid challenge, lesion depth values for the groups treated with resin infiltrant and resin infiltrant + fluoride were similar and exhibited less change than specimens in the group treated with fluoride only (Tables 1 and 2). This is in agreement with other studies indicating that resin infiltration can assist in hampering the progression of dental caries.[19,28,30] However, in relation to mineral loss, the resin infiltrant + fluoride group showed less change in mineral loss values compared with the resin infiltrant group alone (Table 2).

To date, no study has shown the effect of saliva contamination on resin infiltration. In this investigation, when the lesions were contaminated with saliva prior to the application of the resin infiltrant, there was no effect on the penetration of the infiltrant. However, due to saliva contamination, the lesion was not homogeneously infiltrated, and the lesions were not protected from further progression (Tables 1 and 2). Thus, in agreement with a previous study, this indicates that not only a deep infiltration but also a homogeneous resin layer within the lesion body is essential for a leakproof seal.[14] In terms of mineral loss, group 6 (saliva contamination + resin infiltration + fluoride) showed less change in mineral loss than group 5 (saliva contamination + resin infiltration), indicating that in the presence of fluoride, saliva contamination did not have a strong negative effect. Although short-term contamination with saliva did not alter the penetration of the infiltrant, it reduced the ability of resin infiltration to hamper the progression of early enamel lesions, and thus proper isolation should be performed during application of resin infiltrant. In case of inadvertent saliva contamination, an application of topical fluoride is recommended. It should be taken into consideration that an artificial bovine enamel lesion model was used and that the mean lesion depths of the initial lesions were on average 110 μm. This limits the validity of the study because, under clinical situations, the enamel lesions to be resin infiltrated are usually deeper (500-900 μm).[19] More studies are needed to confirm the efficacy of resin infiltration in conjunction with fluoride treatment in clinical conditions.

CONCLUSION

Resin infiltrant, especially in combination with fluoride, has great potential for inhibiting further progression of small white spot lesions. Saliva contamination can negatively affect lesion progression, but fluoride can counteract this effect.

Acknowledgements

This research project was done as part of the requirements for the MSD in preventive dentistry at the Indiana University School of Dentistry. The author thanks the committee members (Drs Norman Cook, Armando Soto, Masatoshi Ando, and Jeffrey Platt) and Drs Paris and Meyer-Lueckel for their valuable advice. Dr Kamocka is thanked for her contributions with the confocal measurement and George Eckert for statistical analysis. This study was partially funded by Delta Dental Foundation. DMG supplied the infiltrant (Icon).

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 August 2013)

REFERENCES

1. Steinberg S (2002) A paradigm shift in the treatment of caries *General Dentistry* **50(4)** 333-338.
2. Mejare I, Lingstrom P, Petersson LG, Holm AK, Twetman S, Kallestal C, Nordenram G, Lagerlof F, Soder B, Norlund A, Axelsson S, Dahlgren H (2003) Caries-preventive effect of fissure sealants: A systematic review *Acta Odontologica Scandinavica* **61(6)** 321-330.
3. Ahovuo-Saloranta A, Hiiri A, Nordblad A, Makela M, & Worthington HV (2008) Pit and fissure sealants for preventing dental decay in the permanent teeth of children and adolescents *Cochrane Database of Systematic Reviews* **(4)** CD001830.
4. Paris S, Meyer-Lueckel H, Colfen H, & Kielbassa AM (2007) Resin infiltration of artificial enamel caries lesions

with experimental light curing resins *Dental Materials Journal* 26(4) 582-588.

5. Davila JM, Buonocore MG, Greeley CB, & Provenza DV (1975) Adhesive penetration in human artificial and natural white spots *Journal of Dental Research* **54(5)** 999-1008.
6. 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.
7. Garcia-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.
8. 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.
9. Gray GB, & Shellis P (2002) Infiltration of resin into white spot caries-like lesions of enamel: An in vitro study *European Journal of Prosthodontics and Restorative Dentistry* **10(1)** 27-32.
10. 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.
11. Meyer-Lueckel H, Paris S, Mueller J, Colfen 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 Journal* **22(1)** 22-28.
12. Rodda JC (1983) Impregnation of caries-like lesions with dental resins *New Zealand Dental Journal* **79(358)** 114-117.
13. Donly KJ, & Ruiz M (1992) In vitro demineralization inhibition of enamel caries utilizing an unfilled resin *Clinical Preventive Dentistry Journal* **14(6)** 22-24.
14. 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.
15. Meyer-Lueckel H, & Paris S (2008) Progression of artificial enamel caries lesions after infiltration with experimental light curing resins *Caries Research* **42(2)** 117-124.
16. Buonocore M (1970) Adhesive sealing of pits and fissures for caries prevention, with use of ultraviolet light *Journal of the American Dental Association* **80(2)** 324-330.
17. Buonocore MG (1971) Caries prevention in pits and fissures sealed with an adhesive resin polymerized by ultraviolet light: A two-year study of a single adhesive application *Journal of the American Dental Association* **82(5)** 1090-1093.
18. Paris S, Meyer-Lueckel H, & Kielbassa AM (2007) Resin infiltration of natural caries lesions *Journal of Dental Research* **86(7)** 662-666.
19. Meyer-Lueckel H, & Paris S (2008) Improved resin infiltration of natural caries lesions *Journal of Dental Research* **87(12)** 1112-1116.
20. Martignon S, Ekstrand KR, & Ellwood R (2006) Efficacy of sealing proximal early active lesions: An 18-month clinical study evaluated by conventional and subtraction radiography *Caries Research* **40(5)** 382-388.
21. Griffin SO, Oong E, Kohn W, Vidakovic B, Gooch BF, Bader J, CDC Dental Sealant Systematic Review Work Group (2008) The effectiveness of sealants in managing caries lesions *Journal of Dental Research* **87(2)** 169-174.
22. 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.
23. Paris S, & Meyer-Lueckel H (2010) Inhibition of caries progression by resin infiltration in situ *Caries Research* **44(1)** 47-54.
24. Silverstone LM, Hicks MJ, & Featherstone MJ (1985) Oral fluid contamination of etched enamel surfaces: An SEM study *Journal of the American Dental Association* **110(3)** 329-332.
25. Taskonak B, & Sertgoz A (2002) Shear bond strengths of saliva contaminated "one-bottle" adhesives *Journal of Oral Rehabilitation* **29(6)** 559-564.
26. Torres CR, Rosa PC, Ferreira NS, & Borges AB (2012) Effect of caries infiltration technique and fluoride therapy on microhardness of enamel carious lesions *Operative Dentistry* **37(4)** 363-369.
27. Hara AT, Kelly SA, Gonzalez-Cabezas C, Eckert GJ, Barlow AP, Mason SC, Zero DT (2009) Influence of fluoride availability of dentifrices on eroded enamel remineralization in situ *Caries Research* **43(1)** 57-63.
28. Paris S, Bitter K, Renz H, Hopfenmuller W, & Meyer-Lueckel H (2009) Validation of two dual fluorescence techniques for confocal microscopic visualization of resin penetration into enamel caries lesions *Microscopy Research and Technique* **72(7)** 489-494.
29. Lippert F, Lynch RJ, Eckert GJ, Kelly SA, Hara AT, & Zero DT (2011) In situ fluoride response of caries lesions with different mineral distributions at baseline *Caries Research* **45(1)** 47-55.
30. Ekstrand KR, Bakhshandeh A, & Martignon S (2010) Treatment of proximal superficial caries lesions on primary molar teeth with resin infiltration and fluoride varnish versus fluoride varnish only: Efficacy after 1 year *Caries Research* **44(1)** 41-46.
31. Meyer-Lueckel H, Bitter K, & Paris S (2012) Randomized controlled clinical trial on proximal caries infiltration: Three-year follow-up *Caries Research* **46(6)** 544-548.
32. Hicks J, Garcia-Godoy F, & Flaitz C (2004) Biological factors in dental caries: Role of remineralization and fluoride in the dynamic process of demineralization and remineralization (part 3) *Journal of Clinical Pediatric Dentistry* **28(3)** 203-214.