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Effect of resin infiltration on enamel surface properties and *Streptococcus mutans* adhesion to artificial enamel lesions

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The aim of this study was to evaluate and compare the effects of resin infiltration and sealant type on enamel surface properties and *Streptococcus mutans* adhesion to artificial enamel lesions. Artificial enamel lesions were produced on the surfaces of 120 enamel specimens, which were divided into two groups: Group A and Group B (n=60 per group). Each group was further divided into four subgroups (n=15 per subgroup) according to sealant type: Group I–Demineralized enamel (control); Group II–Enamel Pro Varnish; Group III–ExciTE F; and Group IV–Icon. In Group A, hardness and surface roughness were evaluated; in Group B, bacterial adhesion was evaluated. Icon application resulted in significantly lower surface roughness and higher hardness than the other subgroups in Group A. In Group B, Enamel Pro Varnish resulted in lowest bacterial adhesion, followed by Icon. This study showed that resin infiltration of enamel lesions could arrest lesion progress.

Keywords: Bacterial attachment, Microhardness, Resin infiltration, Roughness

INTRODUCTION

Enamel caries lesions are characterized by mineral loss beneath an apparently intact surface layer. Increased porosity within the lesion body causes the characteristic whitish appearance of these lesions. Thus, these lesions are often called white spots[1)]. Enamel caries lesions are a common side effect of orthodontic treatment with fixed appliances. Some predisposing factors associated with these smooth-surface white spots include extremely poor oral hygiene and salivary hypofunction[2)].

Initial white spot lesions have an intact surface and are reversible. The most effective way of managing early carious lesions is to practice adequate oral hygiene and remove dental plaque[3,4)]. Therefore, the first line of lesion arrestment is to promote lesion remineralization.

Different methods are available for the arrestment of white spot lesions: resin infiltration[4)], conventional resin bonding[5)], remineralization of enamel subsurface lesions with the use of topical agents such as fluorides[6)] and amorphous calcium phosphates (ACP)[7)], microabrasion[8)], and various types of veneers[9)]. The use of topically applied fluoride on enamel surfaces, shown to be both safe and effective, has played a major role in markedly reducing caries incidence and prevalence. In the United States, the use of fluoride varnishes has become a common modality for in-office topical fluoride treatments[10)]. Ease of application, efficacy, and minimal risk of fluorosis also make the use of fluoride varnishes on young children a logical choice[9)].

New fluoride varnishes contain ACP and release more fluoride[7)]. ACP, generally viewed to be a precursor to hydroxyapatite formation, has shown anti-cariogenic properties with remineralization potential and the ability to promote higher fluoride uptake into tooth structure[7)]. ACP-containing bioactive materials stimulate mineral growth by increasing calcium and phosphate concentrations within the lesion, especially in the acidic oral environment, to levels that exceed those existing in ambient oral fluids. This shifts the thermodynamic driving force toward apatite formation[11,12)]. ACP can also sustain these supersaturation conditions, which are favorable for apatite formation, over extended periods of time[11,12)].

Tiny pores within the enamel lesion body act as diffusion pathways for acids and dissolved minerals[1)]. An alternative approach to arrest carious lesions is to infiltrate these pores with light-curing resins[4)]. This approach not only seals the microporosities and blocks the access of acids to any remaining pores, it also significantly increases surface hardness and provides significant mechanical support to tooth tissue[1,4)]. From biomedical engineering standpoint, surface hardness is an important property because resistance to wear by either friction or erosion increases with hardness. Several studies have confirmed that resin infiltration significantly increased the microhardness of initial enamel carious lesions[6,13,14)].

When the micropores of infiltrated lesions are filled with resin, cariogenic acids are blocked from entering these carious lesions, causing caries progression to slow down or even be arrested[1)]. Thus, the resin infiltration technique bears several advantages: mechanical stabilization of demineralized enamel, preservation of sound hard substance, permanent occlusion of superficial micropores and cavities, obturation of porous

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and deeply demineralized areas, arrest of lesion progress by increasing resistance to demineralization, minimized risk of secondary caries development, and high patient acceptance[4,13,15].

Attachment of certain microorganisms to specific surfaces in the human oral cavity and the resulting formation of dental plaque on teeth and dental materials are primary causes for oral diseases such as gingival inflammation and secondary caries[16]. The quantity and quality of bacterial accumulation on specific substrata are determined by variable surface characteristics[17]. For instance, high surface roughness and high surface free energy significantly promote bacterial adhesion[16].

The resin infiltration technique is a novel approach. Therefore, it needs to be investigated in detail. A survey of published literature revealed that no researches have been conducted to evaluate and discuss these different aspects of resin infiltration technique within a single study — namely, surface roughness and microhardness of sealed white spot lesions and bacterial adhesion thereupon. The aim of this study was to evaluate and compare the effects of resin infiltration and carious lesion sealant type, such as dental adhesive and ACP-containing fluoride varnish, on the surface roughness and microhardness of artificial enamel carious lesions. Also for the first time, the influence of sealant type on *Streptococcus mutans* (*S. mutans*) adhesion was investigated in this study.

The null hypotheses tested were: (1) Resin infiltration and the two different sealant types tested in this study would not alter the surface roughness or microhardness of artificial enamel carious lesions; and (2) Tested sealant types would not have any influence on *S. mutans* adhesion.

MATERIALS AND METHODS

Tooth specimen preparation

This study was approved by the Ethics Committee of Erciyes University, Kayseri, Turkey. All patients gave written informed consent. One hundred and twenty extracted non-carious human anterior central incisors were used in this study. Residual tissues were removed using a scaling instrument. Teeth were polished with a paste (Prophylaxis Paste, Sultan Topex, Englewood, NJ, USA), thoroughly rinsed with water, and examined under a stereomicroscope (Olympus SZ61, Olympus Optical Co., Tokyo, Japan) for enamel defects. All teeth were stored in 1% chloramine T solution until use.

From the labial surfaces of 120 anterior incisor teeth, enamel specimens (approximately 6×6×3 mm) were obtained by cutting using a water-cooled cut-off wheel (Struers, Birmensdorf, Switzerland). Specimens were randomly divided into two main study groups: Group A and Group B (n=60 per group). Each group was further divided into four subgroups (n=15 per subgroup). Specimens in Group A were embedded in squares molds (7×7×4 mm) filled with an epoxy resin (Technovit 4004, Heraeus Kulzer, Hanau, Germany). Specimens in Group B were left unembedded for bacterial adhesion test.

Artificial enamel lesions were created on tooth surfaces by immersion in a demineralizing solution ($CaCl_2$: 12 mM; KH_2PO_4: 10 mM; lactic acid: 50 mM; NaCl: 100 mM; pH=4.5) at 37°C for 6 h, followed by immersion in a remineralizing solution ($CaCl_2$: 1.5 mM; KH_2PO_4: 5 mM; Acetic acid: 100 mM; NaCl: 100 mM; pH=6.5) at 37°C for 18 h. This procedure was repeated every day for 14 days[18].

Specimen preparation for roughness and microhardness testing

Specimens in Group A were equally divided into four subgroups of 15 specimens each. Table 1 presents the materials applied on Group A specimens, and their chemical compositions and manufacturers. The test groups are as follows:

Group I: Demineralized enamel surfaces were not applied with any material and used as the control.

Group II: Enamel Pro Varnish (Premier Dental Products Company, PA, USA) was applied as a thin layer to the enamel surfaces. Treated specimens were allowed to dry for 10 s.

Group III: Specimens were etched with 37% phosphoric acid for 15 s. After specimens were rinsed of the etchant and thoroughly air-dried, ExciTE F adhesive bonding agent (Ivoclar Vivadent AG, Schaan, Liechtenstein) was applied to the enamel surfaces using a scrubbing motion for 10 s. This

Table 1 Compositions and the manufacturers of the study materials

Materials	Composition	Manufacturer Batch number
Enamel ProVarnish	Amorphous calcium phosphate, 5% NaF	Premier Dental Products, PA, USA. Batch no: 35763
ExciTE F	Phosphonic acid acrylate, HEMA, dimethacrylate, highly dispersed silicone dioxide, initiators, stabilizers, potassium fluoride	Ivoclar Vivadent AG, Schaan, Liechtenstein Batch no: B06150
Icon	Icon-Etch: hydrochloric acid, pyrogenic silicic acid, and surface-active substance Icon-Dry: 99% ethanol Icon-Infiltrant: TEGDMA-based resin matrix, initiators.	DMG, Hamburg, Germany Batch no: 665992

was followed by gentle air-drying for 3 s and light activation for 20 s.

Group IV: Icon-Etch (DMG, Hamburg, Germany) was applied to the lesion site and allowed to sit for 2 min. Etchant was rinsed off with water for at least 30 s, and specimens were dried using an oil-free, water-free air stream. Icon-Dry (DMG, Hamburg, Germany) was applied to the lesion site and left for 30 s, followed by drying with an oil-free, water-free air stream. An ample amount of Icon-Infiltrant (DMG, Hamburg, Germany) was applied to the etched surfaces and allowed to sit for 3 min, then light-cured for 40 s using a light emitting diode (LED) light curing unit (Elipar S10, 3M ESPE, Seefeld, Germany). Icon-Infiltrant was applied for a second time and allowed to sit for 1 min. After excess material was removed by hand using a rubber cup[19], light curing was performed for 40 s.

All specimens were kept in artificial saliva for 24 h before roughness and microhardness testing. Artificial saliva was prepared by adding 7.69 g of K_2HPO_4, 2.46 g of KH_2PO_4, 5.3 g of NaCl, and 9.3 g of KCl to 1,000 mL of distilled water (pH=6.5)[20].

Surface roughness test

Surface roughness was analyzed using a contact-type profilometer with a stylus (P-16+, KLA-Tencor Confidential, San Jose, CA, USA). Specimens (n=60) were placed on a flat table, and the needle of the roughness tester was placed on the tooth surface. The machine then recorded the surface roughness values of the specimens, which were digitally displayed on the screen of the roughness tester. Surface roughness was evaluated using the arithmetic mean of the sum of roughness profile values (Ra).

Microhardness test

After surface roughness test, enamel surface microhardness of each specimen was determined using a Vickers hardness tester (HMV-700 Microhardness Tester, Shimadzu, Kyoto, Japan). At a minimum of three widely separated locations on the surface of each specimen, indentations were made using a Vickers diamond indenter with a 2-N load and for a 15-s dwell time. Recorded values were averaged to produce the mean hardness value for each specimen.

Scanning electron microscopy analysis

Before surface roughness and microhardness tests, one specimen randomly selected from each subgroup was used to investigate the enamel surface sealing efficacy of the tested materials. Specimens were sputter-coated with gold and observed under a scanning electron microscope (SEM; LEO 440, LEO Electron Microscopy, Oxford, England) in different magnifications.

Bacterial adhesion test

Specimens in Group B were first sterilized using ethylene oxide gas and then randomly divided into four subgroups (n=15 per subgroup). Decalcified enamel surfaces were sealed in the same way as Group A specimens.

Adhesion of *S. mutans* UA159 (ATCC 700610) to enamel was investigated using a protocol adapted from a previous study[21]. Starting inoculum for the test was 10^8 CFU/mL. A quantity of 100 L of this inoculum was spread over the surface of blood agar. After enamel specimens were placed on this agar surface, agar plates were incubated overnight at 37°C. After enamel specimens were rinsed with 15 mL of a sterile saline solution, each specimen was suspended in 1.0 mL of sterile saline solution and sonicated three times with 10-s pulses at 5-s intervals at 30 W. This suspension was diluted in sterile saline at ratios of 1/10, 1/100, and 1/1000. A quantity of 100 L of all dilutions was spread over the surface of blood agar and incubated for 18–24 h at 37°C to determine the total microorganism count. Results were expressed as CFU/mL.

Statistical analysis

Statistical analysis was performed using ANOVA and Kruskal-Wallis test at p<0.05. Multiple comparisons were made using the Tukey test.

RESULTS

Tables 2 and 3 respectively present the surface roughness and microhardness values of enamel surfaces in Groups I to IV. Group IV (Icon) showed significantly lower surface roughness and higher hardness than the other groups (p<0.05). Group II (Enamel Pro Varnish) showed significantly lower surface roughness and higher hardness than Groups I (control) and III (ExciTE F) (p<0.05). There were no significant differences in both surface roughness and microhardness between Groups

Table 2 Surface roughness values of the study groups

Groups	Median (μm)	25%	75%
Group I (Demineralized Enamel)	1.668 [a]	0.233	3.510
Group II (Enamel ProVarnish)	0.361 [b]	0.262	7.326
Group III (ExciTE F)	3.361 [a]	1.571	5.761
Group IV (Icon)	0.306 [c]	0.174	0.758

Different letters (a, b, c) reveal statistical differences.

Table 3 Surface microhardness values of the study groups

Groups	Mean (VHN)	Standard Deviation
Group I (Demineralized Enamel)	30.646 [a]	25.826
Group II (Enamel ProVarnish)	109.577 [b]	47.409
Group III (ExciTE F)	36.607 [a]	23.654
Group IV (Icon)	318.240 [c]	43.919

Different letters (a, b, c) reveal statistical differences.

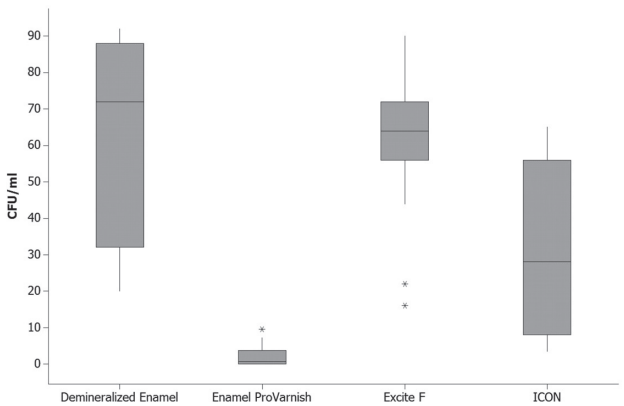


Fig. 1 Concentrations of *S. mutans* on enamel surfaces (median; 25%/75%).

I and III.

Figure 1 shows the concentrations of *S. mutans* on the enamel surfaces in Groups I–IV. Group II exhibited the lowest *S. mutans* adhesion ($p<0.05$), followed by Group IV. There was no significant difference between Groups I and III ($p>0.05$).

Figure 2 shows the SEM micrographs of Groups I–IV. Group I exhibited a honeycomb-shaped demineralized enamel surface. In Group IV, resin infiltrant application to the demineralized enamel surface sealed the enamel microporosities. In Groups II and III, their demineralized enamel surfaces were improperly sealed with the sealants used.

DISCUSSION

Resin infiltrant (Icon)-applied enamel surfaces in Group IV exhibited the lowest surface roughness and highest microhardness. Enamel Pro Varnish-applied enamel surfaces showed higher surface roughness than sealing with ExciTE F adhesive bonding agent. In addition, Enamel Pro Varnish-applied group presented the lowest *S. mutans* adhesion. Results of this *in vitro* study indicated that resin infiltration and sealant type affected the amount of bacterial adhesion and enamel surface properties. Therefore, the first and second null hypotheses of this study were rejected.

Penetration into and arrest of artificial enamel lesions by dental adhesives have been investigated in several laboratory studies. Although a complete infiltration of shallow lesions could be accomplished using these products, penetration into natural, deeper lesions could be achieved, at most, superficially. Since adhesives have been developed mainly for the purpose of adhesion, it was not surprising that these materials showed inferior penetration capability[22-24]. In the present study, sealed enamel lesions were observed using SEM. Although the penetration depths of the tested materials were not evaluated, the dental adhesive ExciTE F did not efficiently seal the enamel microporosities (Fig. 2).

To ensure good long-term prognosis of a treated tooth, a high-quality treated surface is an essential requirement. The term "high-quality surface" refers to a restoration surface with low roughness and wear in a solid state, and which can withstand the chemical and mechanical challenges of the oral cavity. Hardness, which could be defined as the resistance of a material or surface against indentation or penetration, is an important mechanical property from a biomedical engineering standpoint. This is because resistance to wear by either friction or erosion with water or other substances generally increases with hardness[25]. It has been demonstrated that high mineral-containing enamel exhibits relatively low wear rates when compared with dentin (higher organic content)[26].

In the present study, the surface microhardness of resin-infiltrated enamel was expectedly higher than the treated enamel of other groups. This result indicated the ability of low-viscosity resin to fill the spaces between the remaining crystals of porous lesions and create a diffusion barrier not only at the surface, but also within the enamel lesion body. Therefore, a resin-infiltrated layer should be able to strengthen the demineralized enamel structure and prevent further wear and cavitation. Some clinical studies have reported that micro-invasive caries treatment with resin infiltration was an effective and safe approach to arrest initial caries lesions and preserve demineralized enamel[27-29].

Findings of this study agreed with those reported by Torres *et al.*[6] and Paris *et al.*[13], in that the microhardness of carious lesions was significantly improved with resin infiltration. Taher *et al.*[14] also indicated that enamel surfaces treated with an infiltrant showed significantly higher surface hardness than

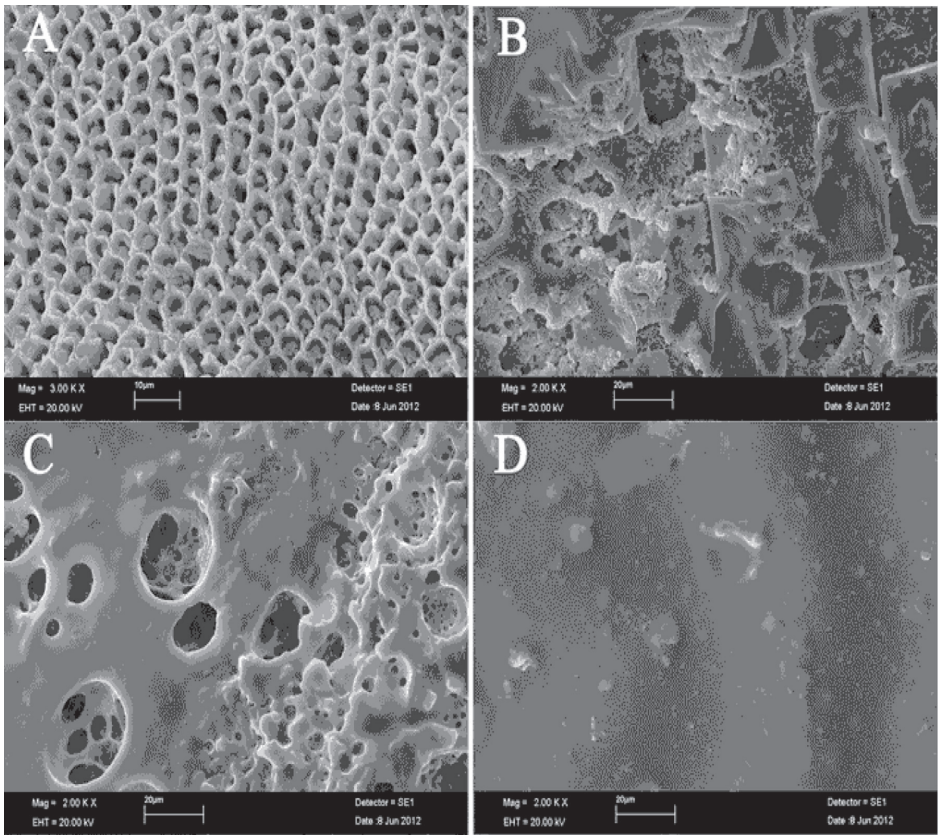


Fig. 2 SEM micrographs of the test groups.
A: Demineralized enamel (×3,000); B: Enamel Pro Varnish application (×2,000); C: ExciTE F application (×2,000); D: Icon application (×2,000).

treatment with a fissure sealant.

In this study, a highly sensitive contact-type surface profilometer with a stylus was used for surface roughness measurements. It delivered automated step height analysis, surface contour, waviness, and roughness measurements with detailed two-dimensional (2D) or three-dimensional (3D) analysis of the topography for a variety of surfaces and materials.

For the resin infiltration technique, the resin must be applied twice to compensate for polymerization shrinkage and occlude microporosities that may persist within the infiltrated lesion body[30]. With the microporosities of demineralized enamel successfully obturated *via* the resin infiltration technique, enamel surface roughness thereafter is expected to be reduced[4]. Impact of dental treatments on surface roughness is material-dependent. The use of some dental materials results in a very smooth surface, while others make the surface rather rough. A rough surface enhances bacterial adhesion and provides the site for rapid development of cariogenic plaque, hence increasing the risk of demineralization in enamel[31]. In the present study, enamel surfaces in Group IV which were applied with the resin infiltrant Icon had significantly lower surface roughness ($p<0.05$) than the other groups.

Fluoride reduces dental caries incidence by promoting enamel remineralization and reducing the rate of demineralization. Fluoride concentrations in plaque can reach the millimolar range, and thus able to exert inhibitory effects on oral microflora growth[32]. *In vitro* experiments have demonstrated that the acid production of *S. mutans* and *lactobacilli* was reduced in layers overlying fluoridated enamel[33]. On the contrary, as the fluoride concentration decrease, the viable bacterial count increase[32].

Enamel Pro Varnish was a 5% sodium fluoride varnish which also contained ACP. Previous studies have shown that Enamel Pro Varnish had the highest fluoride release in the first 8 h or one week[7,34], delivering up to four times more fluoride than other fluoride varnishes[7]. It has also been shown that ACP could facilitate remineralization and improve fluoride uptake[35,36]. In this study, lowest bacterial adhesion was found in Enamel Pro Varnish-applied group, followed by the group treated with the resin infiltrant Icon. Therefore, the high fluoride concentration and fast fluoride release action of Enamel Pro Varnish in the initial hours hindered the bacterial adhesion of *S. mutans* to enamel surfaces. As for Icon-applied enamel surfaces, their reduced *S. mutans* adhesion could be

attributed to the lower surface roughness obtained after resin infiltration.

CONCLUSION

Within the limitations of this *in vitro* study, an increase in microhardness and a decrease in roughness of demineralized enamel surfaces, coupled with low bacterial adhesion, showed that the resin infiltration technique was capable of arresting initial enamel carious lesions. Nonetheless, further clinical studies are needed to confirm the effectiveness of resin infiltrants in caries prevention.

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