Journal of Dental Sciences (2021) 16, 296—303

Available online at www.sciencedirect.com

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Journal of Dental Sciences

Original Article

Effect of resin infiltration application on early proximal caries lesions in vitro

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Received 23 February 2020; Final revision received 14 April 2020
Available online 1 May 2020

KEYWORDS
Resin infiltration;
Dental caries;
Carious lesions;
Caries infiltration;
Icon

Abstract *Background/purpose:* Resin infiltration (RI) material ICON is used in treating early proximal caries lesions, as it depends on a micro—invasive infiltration technology. This in vitro study aimed to evaluate the effect of ICON resin infiltration (RI) on early proximal caries by comparing surface roughness and hardness before and after treatment with RI using atomic force microscopy (AFM), automated microhardness testing system (AMHTS), and scanning electron microscopy (SEM) in vitro.

Materials and methods: Twenty seven premolars extracted for orthodontic reasons were sectioned buccolingually, creating 54 specimens. Each specimen was immersed in demineralizing solution to induce caries. Only teeth with "International Caries Detection and Assessment System" codes 1 and 2 were selected. The specimens were divided randomly into either AFM, AMHTS or SEM groups, which examined demineralized enamel before and after treatment with ICON.

Results: The mean average surface roughness and root mean square roughness values of demineralized enamel treated with ICON were significantly higher than untreated lesions ($P < 0.001$). The mean Vickers hardness values for demineralized enamel treated with ICON was significantly higher than untreated lesions ($P < 0.001$). SEM showed irregular, pitted and rough demineralized enamel surface with destruction of enamel rods and dissolution of enamel crystals. After ICON application, the surface showed complete blockage of enamel rods with RI and irregular, rough uneven topography.

Conclusion: RI application on proximal incipient caries increased surface roughness and hardness significantly.

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https://doi.org/10.1016/j.jds.2020.04.005

Introduction

Dental decay is a microbial, infectious and multifactorial disease and is still a highly prevalent pathology worldwide.[1] The prevalence of caries in the city of Jeddah ranged from 70 to 76 percent in children aged 6 years.[2]

Early proximal carious lesions have been given special attention, with full preservation of tooth structure.[3] This is mainly due to the fact that restorative therapy for interproximal lesions involves a significant amount of sound tissue to be removed and this brings the tooth into a circle of treatment and retreatment.[4] Therefore, the need for invasive treatment in the future will be reduced by early detection and minimally–invasive treatment of such lesions.

Incipient decay can now be managed with many conservative methods,[5–7] such as resin infiltration (RI) which was developed in Germany to treat early enamel proximal caries. Its low viscosity that penetrates the porosities of the lesions, makes it superior to the former technique that depends on sealing of the occlusal lesions by sealing materials.[8,9]

Resin infiltration has several benefits, like absence of tooth structure loss, allowing stability for white spot lesions, preventing caries progress, plugging of the micropore forms in the body of the lesion, delaying the necessity for a restoration, decreasing recurrent decay, absence of inflammation of the pulp and postoperative sensitivity, lowering the possibility of periodontitis and gingivitis and better aesthetic outcomes in covering demineralized enamel.[10,11]

Several researches investigated the characteristics of the ICON. However, few studies evaluated the material at a nanoscale using atomic force microscopy (AFM) on smooth surface lesions. This study aimed to evaluate the effect of the ICON RI on induced early proximal caries–like lesions in vitro by comparing the surface roughness and microhardness before and after treatment with the resin infiltration using AFM, automated microhardness testing system (AMHTS) (Vickers hardness) and scanning electron microscope (SEM) in vitro.

Materials and methods

Study design

This is an experimental in vitro study in which the specimens were examined using AFM (BRUKER, Innova® Atomic Force Microscope, Tokyo, Japan), AMHTS (BUEHLER, MicroMet 6040, An ITW Company, Lake Bluff, IL, USA) and SEM (FETEM: JEOL, JEM–2100F, Tokyo, Japan).

Ethical approval

Ethical approval was acquired from the Research Ethics Committee, Faculty of Dentistry, King Abdulaziz University (KAU), Jeddah, Saudi Arabia (Approval no. 063–16).

Specimen preparation

The study sample included twenty seven human sound premolars, collected from the orthodontic clinics, Faculty of Dentistry, KAU. The selected teeth were extracted for orthodontic reasons. Collected human premolars were stored in saline. For each tooth, the root was sectioned at the cementoenamel junction (CEJ) and then the crown was sectioned buccolingually into 2 halves (mesial and distal) using Isomet low–speed saw (TechCut 4™, 100–240, Allied, Compton, CA, USA) creating 54 specimens in total. Then, all teeth surfaces were covered with acid–resistant varnish, except for the proximal surfaces, which were exposed to the assigned treatment.

Each specimen was immersed individually for a period of 1–2 weeks in a separate glass that contained 4 ml of the demineralizing solution to inducing caries–like lesion.[12] The demineralizing solution used was composed of 2.2 mM/L calcium chloride ($CaCl_2$), 2.2 mM/L potassium phosphate (KH_2PO_4) and 50 mM/L acetic acid with pH equal to 4.5.[12] These containers were numbered from 1 to 50 and labelled with yellow stickers. After that, the teeth were taken out of the demineralizing solution and washed with deionized water, dried and placed back in their respective clean containers filled with deionized water.

The teeth were evaluated visually using the "International Caries Detection and Assessment System" "ICDAS" codes (Table 1).[13] Only the teeth with codes 1 and 2 (early–stage decay) were included. Afterward, each specimen was randomly allocated into either AFM (n = 25), AMHTS (n = 25) or SEM (n = 4).

ICON infiltration application

First, caries–like lesion was etched with slight excess for 2 min with ICON–etch "15% hydrochloric acid (HCl), pyrogenic silicic acid and surface–active substances". Second, the surfaces were washed with water for 30 s and dried. Then the ICON–Dry "99% ethanol" was applied to the lesion site with a slight excess and allowed to react for 30 s. Finally, the ICON infiltrant

Table 1 International caries detection and assessment system (ICDAS).

0	Sound tooth surface
1	First visual change in enamel
2	Distinct visual change in enamel
3	Localized enamel breakdown due to caries with no visible dentin
4	Underlying dark shadow from dentin (with or without enamel breakdown)
5	Distinct cavity with visible dentin
6	Extensive distinct cavity with visible dentin

"Methacrylate—based resin matrix, initiators, and additives" was applied to the lesion site with a slight excess and let to infiltrate for 3 min. The excess material was wiped off using a cotton roll before polymerization. The ICON infiltrant was light—cured from all sides for 40 s in total. The material was applied for a second time and allowed to react for 1 min. The surplus material was cleaned with a cotton roll before polymerization, then light—cured for 40 s from all sides. The light intensity in the curing unit was measured regularly to make sure that it remained at least 600 mW/cm^2.

Atomic force microscopy

The AFM (BRUKER, Innova® Atomic Force Microscope) was utilized to assess the surface roughness of the demineralized enamel before and after ICON application. Twenty—five specimens were observed with the AFM. The AFM images were recorded in contact mode, using AFM probes (CONT20A—CP, MPP—31123—10, BRUKER, Tokyo, Japan) mounted in cantilevers (3um) with a spring constant of 0.9 N/m. Three—dimensional images of (30×30 μm) with a resolution of 256×256 pixels were taken. The data were processed by BRUKER Innova AFM software. The mean average surface roughness (Ra) (arithmetic average) and root mean square roughness (Rq) (geometric average) values were the topographical parameters used in this study.[14] The same specimens were examined before and after treatment with the ICON.

Automated microhardness testing systems (Vickers hardness)

Twenty—five specimens were tested by transversal Vickers hardness with a force (F) of 0.891 N for indentation time 10 s and 50 μm distance to the enamel surface and between indents. Automated microhardness testing system (BUEHLER, MicroMet 6040, An ITW Company) evaluated the indentation by calculating transverse and longitudinal axis of indent (d_1d_2 (μm). Vickers hardness number (VHN) was calculated using the equation "VHN (N/μm^2) = 0.102x2xsin (136/2) x (F/d^2), with $d = (d_{1+}d_2)/2$". The same specimens were examined before and after treatment with the ICON.

Scanning electron microscope

Four specimens were divided into two groups, group 1 which served as an experimental group (ICON) and group 2 which served as a control group (no treatment). The specimens were stored in 4 ml of deionized water and sent for examination with SEM (FETEM: JEOL, JEM—2100F).

Statistical analysis

Data analysis was conducted utilizing the "Statistical Package for Social Sciences (SPSS) version 20.0" (SPSS Inc., Chicago, IL). Since the sample size was 25 in each group (less than 30), and the measurements of the same group were compared before and after ICON application, Wilcoxon—Signed Rank test was used. $P < 0.05$ was considered statistically significant.

Results

Surface roughness

A total of 25 samples of demineralized enamel were examined before application of ICON using AFM. Each of the examined samples showed the same appearance (Fig. 1). After application of the ICON, samples were also evaluated by the AFM analysis. In each sample, the ICON material displayed a non—homogenous appearance and a rough surface (Fig. 2). Following statistical analysis, the Ra and Rq values were evaluated and it was determined that the mean Ra and Rq values after ICON were significantly higher than the demineralized enamel ($P < 0.001$) (Tables 2 and 3).

Microhardness

Statistical analysis of mean microhardness values revealed a statistically significant difference between the values of the demineralized enamel before and after treatment with ICON ($P < 0.001$) (Table 4). The mean microhardness values after treatment with ICON group were significantly higher than the demineralized enamel ($P < 0.001$).

Surface topography

Sound enamel surface shows a smooth homogenous surface. In the control group, enamel surface after demineralization showed irregular pitted rough surface with multiple craters of variables depths with the destruction of enamel rods and dissolution of the enamel crystals (Fig. 3). After ICON application, the enamel surface showed complete blockage of the enamel rods with resin infiltration, irregular rough uneven topography and the presence of a mineralized layer that completely covered the enamel surface (Fig. 4).

Discussion

This research is an in vitro experimental study evaluating the surface roughness and hardness before and after

a-

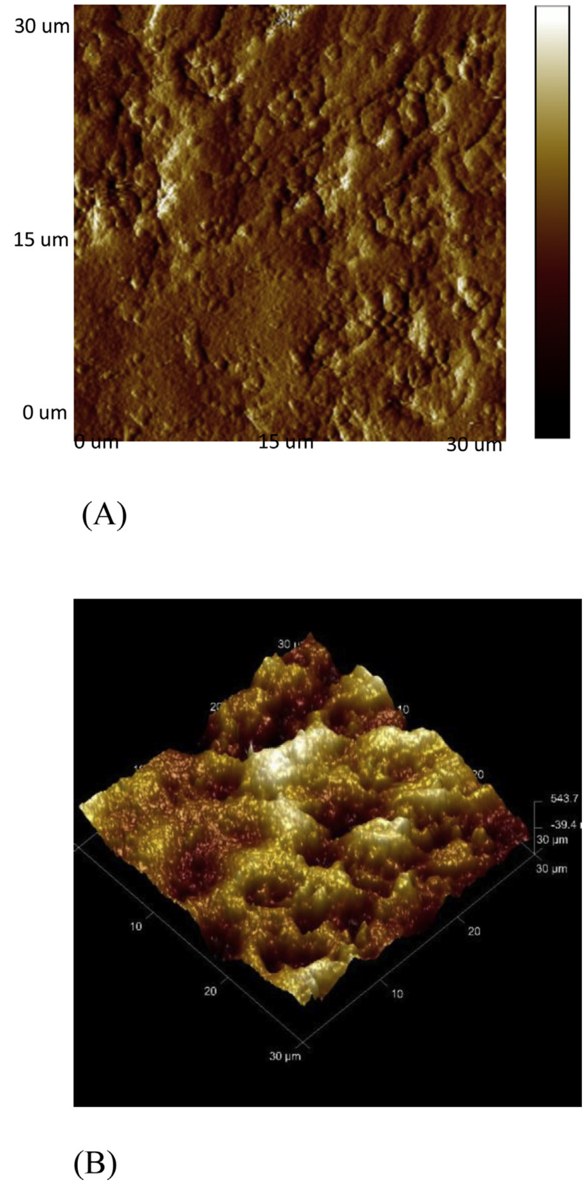

Figure 1 (A) Two— dimensional (30um X 30um) AF images of demineralized enamel showing rough irregular surface and (B) Three—dimensional AF images of demineralized enamel showing different profile heights of surface topography of the caries—like lesion on the enamel surface.

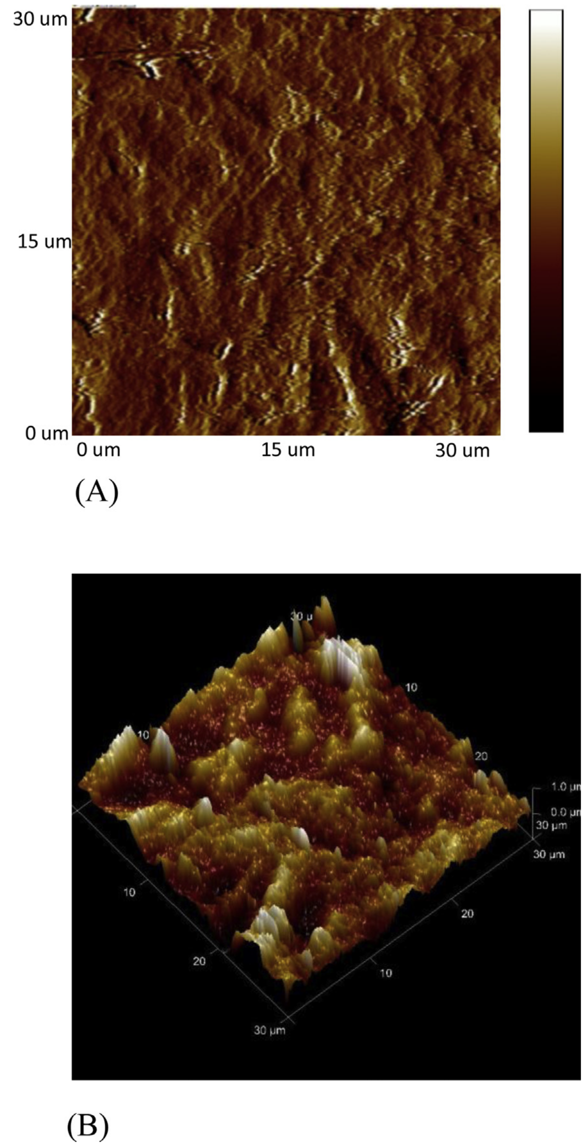

Figure 2 (A) Two—dimensional (30um x 30um) AFM images after treatment with ICON showing rough irregular surface and (B) Three—dimensional AFM images after treatment with ICON showing different profile heights of the caries—like lesion on the enamel surface with the presence of multiple cavities with variable depths displaying rough pitted irregular enamel surface of the examined areas.

pplication of ICON RI, on induced early proximal caries lesions at a nanoscale level using AFM, AMHTS and SEM.

In the current study, when we compared treated and untreated demineralized enamel, there was a significant increase in the surface roughness and hardness in the ICON group compared to the control group. This suggests that the ICON material tends to increase plaque accumulation in proximal surfaces but also penetrates the porosity of artificial white spot lesions and increases the surface hardness and caries resistance, thus perform well clinically.

In a study done by Taher et al.[15] on human premolars with healthy enamel (Teeth cleaned using slurry pumice and a prophylaxis brush in a contra-angle handpiece to create a smooth buccal surface), there was no significant

Table 2 Comparison of the means of the average surface roughness values of the demineralized enamel before and after treatment with ICON.

Ra	N	Mean $\pm$ SD	P
Before (demineralized enamel)	25	251.95 ± 64.11	$< 0.001^*$
After (ICON)	25	458.36 ± 72.32	

Ra: Average surface roughness values, N: Number of teeth in each group, SD: Standard deviation, ICON: Resin infiltration material.
*Statistically significant at $P < 0.05$ level.

Table 3 Comparison of the means of the root mean square roughness values of the demineralized enamel before and after treatment with ICON.

Rq	N	Mean ± SD	P
Before (demineralized enamel)	25	331.3 ± 110.4	< 0.001*
After (ICON)	25	524.4 ± 128.8	

Rq: Root mean square roughness values, N: Number of teeth in each group, SD: Standard deviation, ICON: Resin infiltration material.
*Statistically significant at P < 0.05 level.

Table 4 Comparison between both mean microhardness values of the demineralized enamel before and after treatment with ICON.

VHN	N	Mean ± SD	P
Before (demineralized enamel)	25	60.37 ± 6.26	< 0.001*
After (ICON)	25	181.25 ± 34.01	

VHN: Vickers hardness number, N: Number of teeth in each group, SD: Standard deviation, ICON: Resin infiltration material.
*Statistically significant at P < 0.05 level.

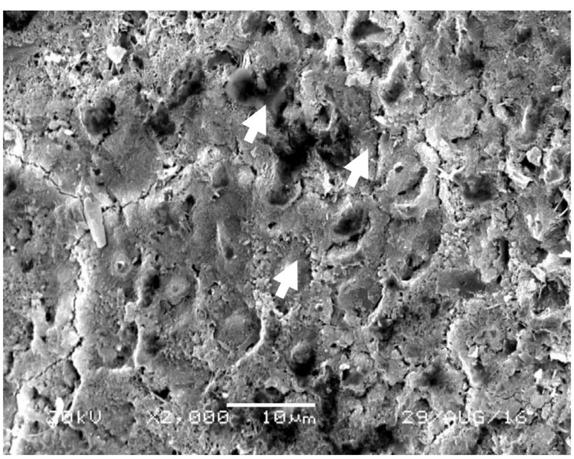

Figure 3 Different pattern of enamel demineralization showing cobblestone like appearance with areas of irregular pitted enamel surface (x 2000).

difference in the surface roughness before and after application of ICON material to enamel using a profilometer. In contrast to our study, there was a significant increase in the surface roughness (proximal surface) after application of the ICON using AFM. Taher in 2013[16] used the AFM in another research, and 3D surface images were obtained from ICON, showing a non—homogenous layer with groups of small enamel grains scattered on the surface.

In the present study, the surface roughness results coincided with the results of Gurdogan et al.,[17] where the ICON had a significantly rougher surface than the control group. In Gurdogan et al.[17] study, they used sound bovine incisors (smooth buccal surface) in contrast to our study which used human premolars (proximal surface). Artificial

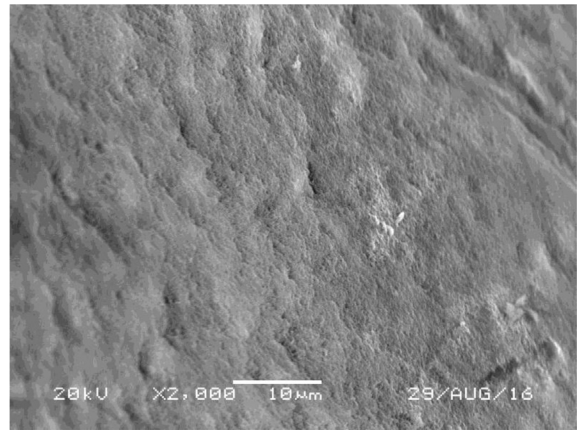

Figure 4 Scanning electron micrograph of enamel surface following ICON application. The surface showing complete blockage of the enamel rods with resin infiltration, irregular rough uneven topography. Note the presence of a mineralized layer that completely covered the enamel surface (x 2000).

lesions in bovine teeth may vary from natural human teeth, with higher resin penetration due to lower lesion depth.[18]

In a study conducted by Ulrich et al.[19] on human premolars (proximal surface), ICON was applied to natural lesions to examine the surface roughness parameter (Ra) using a 3D scanning microscope. Similar to the present research, they found that the surface roughness quality of the ICON specimens was higher than that of enamel.

In 2018 Yazkan and Ermis[20] studied the surface roughness of demineralized bovine teeth before and after application of ICON RI on the labial surfaces (Specimen surfaces were ground flat with sequential aluminium oxide abrasive papers) using a stylus profilometer, they found that the roughness values after application of ICON were significantly lower than demineralized enamel. In contrast to our study, there was a significant increase in the surface roughness (proximal surface) after application of the ICON compared to the demineralized enamel.

Paris et al.[21] advised to wipe off the excess infiltrant material before polymerization because the residual thin resin layer may lead to plaque collection and thus cause decay. In the present investigation, according to Paris et al.[21] findings and the manufacturer instructions, the surplus material was cleaned with a cotton roll prior to polymerization.

Earlier investigations indicated that if a surface has a roughness value of less than 200 nm (0.2 lm), this roughness does not contribute to plaque collection, but if the value is higher, plaque collection is inevitable.[19,22,23] In our study, all of the samples revealed a higher value when compared to the 0.2 lm value and it was concluded that ICON can lead to plaque collection.

In a study carried out by Taher et al.,[15] the infiltrant group VHN values are notably higher than the fissure sealant group values. ICON infiltrant is a methacrylate—based resin matrix that contains Bis—GMA and TEGDMA, while Seal—Rite contains 34.4% UDMA. This compositional variation may explain the different measurements of surface hardness between the materials. Also, in our study, the VHN values were significantly increased after ICON application on the demineralized enamel surface compared to untreated demineralized enamel.

Gurdogan et al.[17] performed a study on bovine incisors (smooth buccal surface), they showed significantly lower values for demineralized enamel microhardness than the values of the sound enamel and the demineralized enamel treated by ICON, while there was no significant difference between sound enamel and demineralized enamel treated with ICON. Taher et al.[15] also found no significant difference between premolar (smooth buccal surface) microhardness values infiltrated with ICON and sound enamel.

El—zankalouny et al.[24] evaluated the penetration depth and enamel microhardness of incipient enamel lesions treated with ICON and casein phosphopeptide—amorphous calcium phosphate (CPP—ACP). ICON group revealed the highest penetration depth followed by CPP—ACP. Also, RI significantly increased microhardness of demineralized enamel in comparison to CPP—ACP. Therefore, a resin—infiltrated layer strengthens the demineralized enamel structure and prevents further wear and cavitation. However, in our study, the microhardness values of the premolars (proximal surface) were significantly higher in the demineralized enamel treated by ICON compared to the untreated demineralized enamel.

Paris et al.[18] used transversal microhardness test and found that the microhardness values of the infiltrated enamel were significantly higher than the demineralized enamel of bovine teeth. The VHN value was 93 ± 23 for demineralized enamel, after a single layer application of ICON and 146 ± 29 after twice application of RI. Based on this research, lesions infiltrated are much more likely to resist forces and stop enamel caries than untreated lesions.

Also, Paris et al.[18] observed no difference in mineral loss or microhardness between lesions infiltrated with different types of resins (Bisphenol A—glycidyl methacrylate (Bis—GMA), Triethylene glycoldimethacrylate (TEGDMA)). In addition, repeated application of resin improved both microhardness and lesion resistance to demineralization.[18] The commercially available infiltrant ICON mixtures containing large amounts of TEGDMA, and ethanol are associated with high coefficients of penetration and adequate hardening; thus, they could be promising resources for quick caries penetration.[25] The Bis—GMA based infiltrants intended to increase the microhardness of infiltrated lesions because of their aromatic backbone, Bis—GMA has a more rigid molecular structure. Bis—GMA resins exhibited higher flexural strength and elastic modulus compared to TEGDMA, thus increasing polymer hardness.[26,27]

Infiltration with Bis—GMA—containing resins did not lead to increased microhardness or resistance to demineralization relative to resins containing neat TEGDMA.[18] This may be due to decreased penetration depth, since Bis—GMA has a higher viscosity, thus decreasing the resin's penetration coefficient.[28] Nevertheless, as the artificial lesions were shallow in their sample compared to natural lesions, complete infiltration was expected to occur for all resins. In addition, a decrease of hardness is due to reduced conversion related to Bis—GMA that may have outweighed the higher polymer rigidity.[26]

Microhardness measurements are not necessarily equal to fracture or wear resistance of teeth and their surface, and were shown to vary between dentition, teeth and even tooth or resin restoration surface.[18] Hardness was shown to be inversely related with elasticity, and high elasticity may be beneficial to resist polymerization shrinkage.[29] This elasticity however warrants caution when interpreting measured hardness values, as elastic deformation reverses after indentation, as shown in comparison between micro— and nanoindentation procedures.[29] This effect may have artificially increased the obtained microhardness values. Additionally, TEGDMA—based resins, like infiltrants, are even more elastic, thus increasing systematic measurement bias.[26] However, infiltrated proximal lesions do not need to resist masticatory forces, but reinstate proximal surfaces, thus resisting proximal abrasion or attrition whilst preventing cavitation of lesions.

Using the HCl gel with ICON erodes the surface layer more effectively than 37% phosphoric acid (H_3PO_4) and the longer the use of the HCl conditioning for 2 min with the ICON could have resulted in deeper resin penetration than etching with H_3PO_4 gel.[30] Also, the use of ICON—dry which contains 99% ethanol for 30 s before application of the infiltrant increases the penetration coefficient by reducing the viscosity and contact angle and improves infiltration depth.[25] However, using ethanol containing resins did not result in improved hardness or resistance to demineralization of infiltrated lesions. This may be due to porosities in the resin, as solvent evaporation is considered more unlikely for infiltrated resins.[28] In addition, higher ethanol content can inhibit polymerization of resin and thus reduce infiltration success.[28] In conclusion, solvent—free resins tend to be more appropriate for infiltration of caries. Twice application of RI was done in our study, which caused increase in microhardness of the lesions, compensated to polymerization shrinkage and filled porosities and crevices in the infiltrated lesion body, thereby increasing hardness and decreasing mineral loss when exposed to demineralization again. This agrees with Paris et al.[18] and could explain why the drawbacks of the ethanol dissolved resins were compensated when the infiltrants were applied twice.[4] Using higher initiator concentrations within the resin might increase the conversion, and increase hardness. If the results of penetration and conversion were examined, it would be of more clinical application.[18]

In a study performed by Taher et al.[15] on human premolars (smooth buccal surface), the SEM outcomes showed that the application of ICON to the enamel surface had sealed the enamel porosities and the resulting product appeared smooth, while the sealant appeared to be protruding from the sealed surface. In the current study after ICON application, the surface showed complete blockage of the enamel rods with resin infiltration, irregular rough uneven topography and the presence of a hypermineralized layer that completely covered the enamel surface.

According to Belli et al.[31] bovine teeth macroscopic pores can accelerate the decay process and it is difficult to penetrate and occlude these large pores with resin and therefore demineralized areas with poorer mechanical properties can remain after the technique of infiltration. Nonetheless, you can also see areas with high mineral content and perfect mechanical characteristics after resin infiltration.

ICON material is technique sensitive, the working and setting times of ICON RI is acceptable. ICON needs a completely dry field and the material can fail due to any moisture on the tooth surface. The tooth surface, was dried using dry, moisture—free air, further procedures should be performed to dry the surface when treating the lesion area with ethanol. During its use, other problems were found with the ICON material, including the packaging of the ICON material, which involves a syringe from which the substance is dispensed. As the syringe plunger is depressed, the quantity of material that is dole out is much greater than necessary and the excess material must be wiped away with a cotton swab. Laboratory studies similar to our study have some characteristic limitations. They are carried in artificial and very controlled settings, so it is not possible to generalize the study findings (external validity). Laboratory—based research are artificial by kind, and susceptible to human bias and mistakes, making it difficult to replicate the outcomes.

In this in—vitro study, extracted human premolars were used and the procedure and time needed for the formation of lesions, the application of the treatment material and how the material works on the lesions may be different from what happens in—vivo. The lesions in each tooth may also be different, i.e. the amount of demineralization in each tooth may vary depending on the amount of fluoride to which they were exposed before extraction. Other limitations unique to our research are that the pH cycling cannot fully replicate the oral environment. In about one to two weeks of exposure to demineralization acids, the lesions were formed, while oral cavity lesions may develop over a period of months or years. Furthermore, mouth lesions develop when there has been both demineralization and remineralization.

ICON material performed well in increasing the hardness and the enamel stability. The ICON material is used as an infiltrant and to prevent further demineralization by formation of resin tags (where the material penetrates into the enamel superficially) on the surface.

Resin infiltration application on proximal incipient caries lesions increased the surface roughness and hardness significantly.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia under grant no. G: 116—165—1440. The authors, therefore, acknowledge with thanks DSR for technical and financial support.

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