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Effects of different resin sealing therapies on nanoleakage within artificial non-cavitated enamel lesions

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The aim of this study was to evaluate nanoleakage within the different lesion-sealing therapies applied to artificial non-cavitated enamel lesions. Thirty-two human anterior teeth were used. Artificial subsurface enamel lesions were produced on the labial surfaces of teeth. The specimens were then randomly divided into three groups (n=10): Group I- Clinpro Sealant application; Group II- ExciTE F adhesive resin application; and Group III- ICON resin infiltrant application. Each group was further divided into two subgroups: control and thermocycler. Nanoleakage was calculated by the digital image analysis software. In the control and thermocycled groups, there was no statistically significant difference between the Groups I, II, and III (p>0.05). The only significant leakage scores were obtained between the Group III control and thermocycler groups (p=0.027). ICON infiltrant can be used as an alternative to dental adhesives and fissure sealants in the sealing of initial non-cavitated enamel lesions. But the resin may become more affected by the water sorption than other resin materials over time. More studies are needed to evaluate long-term durability of resin infiltrants.

Keywords: Artificial enamel lesion, Nanoleakage, Resin infiltrant, Thermocycle

INTRODUCTION

The prevalence of enamel caries is still comparably high, despite a general decline of dental decay in the western industrialized nations in the past few decades. Enamel caries lesions are characterized by a loss of mineral beneath an apparently intact surface layer[1]. These lesions appear clinically as white spot lesions, which are the result of increased porosities in the enamel surface. This porous layer may permit the acid produced by bacteria to diffuse more freely along prisms and intercrystal spaces, affecting the enamel subsurface[2].

The common treatment strategies for caries lesions include either noninvasive (local fluoridation, oral hygiene education, and dietary education) or invasive (removing the carious tissue and placing a restoration) applications. Noninvasive treatments have usually been recommended if they have the potential of lowering the long-term caries risk by influencing the etiological factors of caries and promoting the natural repair process of teeth[3]. On the other hand, invasive treatment options, especially for proximal lesions, involve the destruction of considerable amounts of sound tissue to gain access to the caries[4].

Microinvasive approaches like fissure sealant or dental adhesives play a role of bridging the gap between noninvasive and minimally invasive treatment options. In pits and fissures, fissure sealing with light curing resins is an effective method to prevent caries formation and lesion progression[5]. Since the porosities of enamel caries act as diffusion pathways for acids and dissolved minerals, infiltration of these pores with resins occludes the pathways and thus hampers or arrests the lesions' progression in a demineralizing environment when using various resin materials[6,7]. In a previous study by Arslan *et al.*[8], it has been also confirmed that sealing of non-cavitated enamel lesions with the resin infiltrant ICON increased surface hardness and decreased bacterial adhesion.

The concept of sealing over non-cavitated lesions has been transferred to proximal surfaces as well. Using temporary tooth separation, proximal non-cavitated caries lesions have been sealed by using dental adhesives[9,10]. However, adhesives have been developed mainly with regard to adhesion, therefore these materials show inferior penetration capabilities with only superficial penetration into natural enamel lesions[6,11].

Nanoleakage is an important indicator to evaluate the ability of the restoration to be sealed by a resin material[12,13]. The term "nanoleakage" was introduced by Sano *et al.*[14] as an internal leakage describing the nanometer-sized spaces around the collagen fibrils within the hybrid layer. Leakage occurs laterally, through submicron porosities (estimated to be about 20 to 100 nm in width) at the base of the hybrid layer, which have not been filled with a resin or left poorly polymerized. Although there is still no clear evidence of the negative effects of nanoleakage on restoration durability, the existence of such pathways in gap-free cavity margins may have potential long-term consequences for adhesion quality[14]. For nanoleakage analysis, evaluation of silver

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uptake (*i.e.* nanoleakage evaluation) has provided good spatial resolution of submicron defects in resin infiltration or inadequate polymerization[15,16].

The effects of resin sealing therapies in caries progression have been investigated by several researchers in dental literature[6,11,17]. Although, the leakage in the interface of resin sealant and tooth surface leads to the degradation of bond between two surfaces, no clear information is available regarding nanoleakage of these materials. The aim of this current study was to evaluate a quantitative analysis of nanoleakage within three different lesion-sealing therapies applied to artificial non-cavitated enamel lesions with and without thermal aging. The null hypotheses tested were: 1) The test materials such as; resin infiltrant, fissure sealant and dental adhesive would show similar values of nanoleakage at the resin/enamel interfaces. 2) The thermal aging with thermocycling process would not affect the nanoleakage of tested materials.

MATERIALS AND METHODS

Teeth selection

Thirty-two extracted non-carious human anterior teeth were used in the study. The teeth surfaces were polished with paste (Qartz, Dharma Research, Miami, FL, USA) using a slow-speed hand piece with soft rubber polishing cups, rinsed thoroughly with water, and examined stereo microscopically for defects in the enamel after removal of the residual tissues using a scaling instrument. Until the time of the study, the teeth were stored in 1% chloramines T solution at 4°C.

The enamel samples (6×6×3 mm^3) were cut from the labial surface of the anterior teeth using a water-cooled cutting wheel (Struers, Birmensdorf, Switzerland). The samples were embedded into auto-polymerizing acrylic resin by leaving the outer enamel surfaces uncovered with resin (Fig. 1).

Formation of artificial enamel lesions

Artificial enamel lesions were created on each of the outer tooth surfaces by immersing the embedded teeth 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 and 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 (Fig. 1). This procedure was repeated every day for 14 days. Randomly selected two teeth were inspected under the scanning electron microscope (SEM) (LEO 440, Oxford, England) for lesion accuracy and depth (Fig. 2).

Test materials application

The tooth samples were randomly divided into three main study groups, as below (Fig. 1):

Group I: Clinpro Sealant (3M ESPE, Seefeld, Germany) was applied to this group

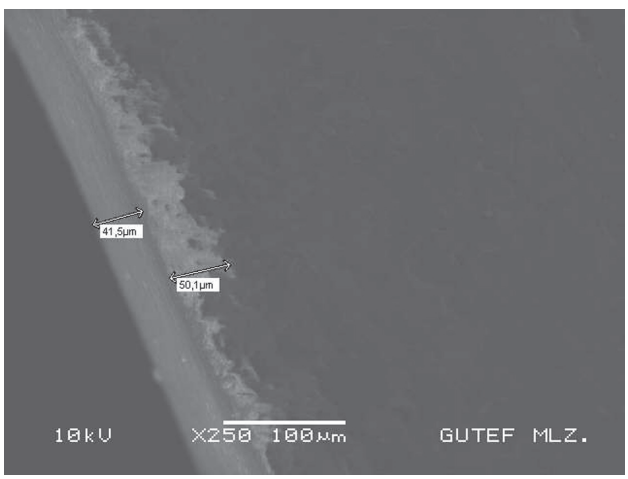

Fig. 2 A SEM image of an artificial enamel lesion before sealing therapies.

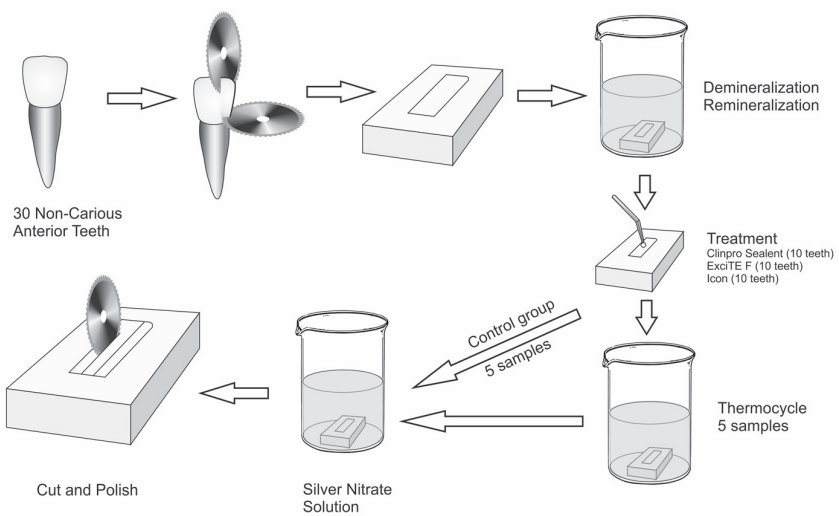

Fig. 1 Schematic drawing of the study method.

Table 1 Composition of tested materials

Materials	Composition	Manufacturer
Clinpro Sealant	Bis-GMA, TEGDMA, initiators, stabilizers, reinforced inorganic fillers	3M ESPE, Seefeld, Germany Batch No: 35763
ExciTE F	Phosphonic acid acrylate, HEMA, Bis-GMA, UDMA, glycerin-1.3-dimethacrylate, highly dispersed silicone dioxide, initiators, stabilizers, potassium fluoride	Ivoclar Vivadent, Schaan, Liechtenstein Batch No: B06150
ICON	ICON-Etch: hydrochloric acid, pyrogenic silicic acid, surface-active substance ICON-Dry: 99% ethanol ICON-Infiltrant: TEGDMA-based resin matrix, initiators.	DMG, Hamburg, Germany Batch No: 665992

(*n*=10, Table 1). Demineralized enamel surfaces of the samples were etched with 37% phosphoric acid for 30 s; thorough washing and air-drying followed the etching. The sealant was then applied to the etched surfaces and light-cured for 20 s (Valo/Standart Power Mode, Ultradent, UT, USA).

Group II: ExciTE F (IvoclarVivadent AG, Schaan, Liechtenstein) was used in this group (*n*=10, Table 1). Demineralized enamel surfaces of the samples were etched with 37% phosphoric acid for 30 s and followed by thorough washing and air-drying. ExciTE F was applied to the etched surfaces using a scrubbing motion for 10 s, gently air dried for 3 s, and were light activated for 10 s.

Group III: ICON (DMG, Hamburg, Germany) was applied to this group (*n*=10, Table 1). ICON-Etch was applied onto the demineralized enamel surfaces of the samples and let sit for 2 min, and then rinsed with water for at least 30 s. The enamel surfaces were then dried with oil-free and water-free air. An ample amount of the ICON-Dry was applied onto the etched enamel, let sit for 30 s, and then dried with oil-free and water-free air. An ample amount of ICON-Infiltrant was applied on the surface and let sit for 3 min. Excess material was removed with a cotton roll. The enamel surfaces were light-cured for 40 s. ICON-Infiltrant was applied for a second time and let sit for 1 min and then light-cured again for at least 40 s.

Thermocycling preparation

Five samples in each treatment group were exposed to a thermocycling regimen of 10,000 cycles between 5 and 55°C with a dwell time of 60 s and a transfer time of 3 s in each water tank (Fig. 1).

Nanoleakage preparation

For nanoleakage measurements, the specimens were immersed in a 50% (w/v) silver nitrate solution (pH=9.5) for 24 h. Silver nitrate solution was prepared from the dissolution of 25 g of silver nitrate crystals in 25 mL of distilled water. Concentrated (28%) ammonium hydroxide was used to titrate the black solution until it became clear, as ammonium ions complexed the silver into diamine silver ions ($[Ag(NH_3)_2]$). The solution was diluted to 50 mL with distilled water, yielding a 50% (w/v) solution (pH=9.5). The tooth samples were then washed with water for 5 min and cut perpendicularly (2 mm in thickness) to the surface using a low speed, diamond saw (Fig. 1). Both sides of the middle section of each sample were used for the leakage evaluation as this provided the best cross sectional interface for imaging. The cut surfaces of the tooth slabs were polished with silicon carbide papers (600, 800, and 1200 grits) under running water for 2 min then polished using diamond pastes in a descending order (6, 3 and 1 μm) followed by ultra-sonication in a water bath for 10 min. Samples were then dried and prepared for SEM (LEO 440).

Nanoleakage evaluation

To quantify silver nanoleakage along the enamel-treatment material interface, samples were analyzed with an SEM using the backscattered electron mode. In this mode, elements of higher atomic mass appear bright, making silver readily detectable in comparison to the rest of the calcium phosphate-based tooth structure. Once a bright spot was identified along the interface, such as the one as shown in Fig. 3A, they were analyzed using energy dispersive spectroscopy (EDS) to insure significant silver presence. EDS actually recorded the amount of silver grains (wt%) present in area at resin/enamel interface and demonstrated by Ag peaks (Figs. 3B–C). After white spots were found and identified on the sample as silver, representative images for leakage analysis were taken between 500 and 600 times magnification of portions where the enamel-treatment material interface showed the greatest nanoleakage or silver presence, such as in the Fig. 3A image. A representative image of the five samples in the control and thermocycling groups for each of the main groups,

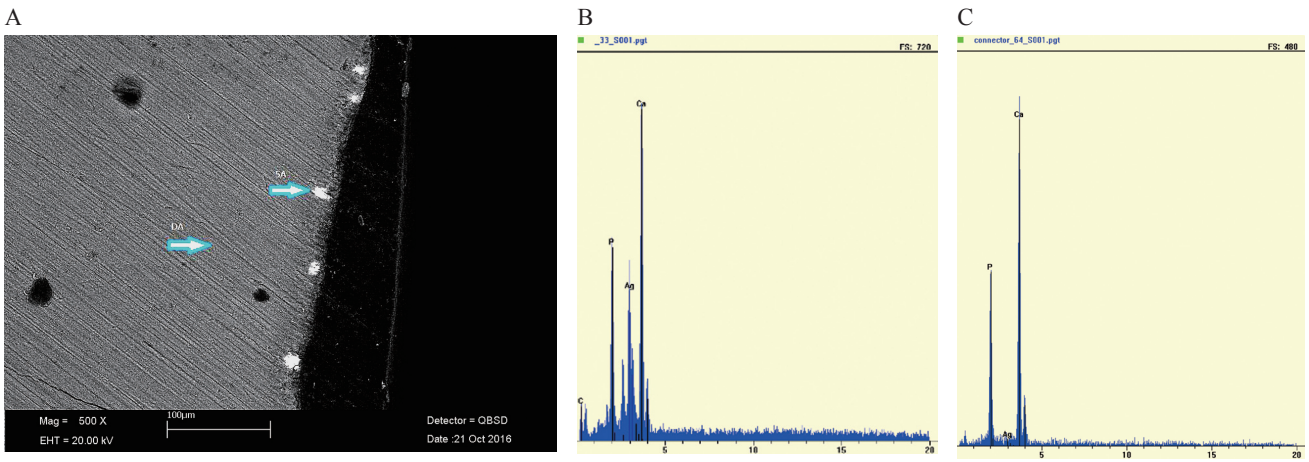

Fig. 3 A: SEM image of silver particles at the enamel-resin interface (SA: Silver area; DA: Dark area). B: EDS scan of bright spots with peak showing significant silver presence. C: EDS scan showing negligible silver presence in a neighboring dark section of bright spots.

Table 2 Nanoleakage values of the study groups

	Control GM (min–max)	Thermocycler GM (min–max)	*p*
Group I (Clinpro Sealant)	0.63 (0.23–3.045)	0.56 (0.24–2.08)	0.660
Group II (ExciTE F)	0.46 (0.17–2.14)	0.60 (0.33–0.82)	0.372
Group III (ICON)	0.3 (0.15–0.62)	0.55 (0.29–1.03)	0.027
p	0.052	0.945	

GM: Geometric mean; data were given by multiplying 100.

Clinpro Sealant, ExciTE F, and ICON was obtained on both sides of the sample slab, for a total of 60 images for nanoleakage analysis. This means that for each group 10 values were obtained.

Nanoleakage, or percentage of silver particle penetration at the enamel-treatment material interface, was calculated by using the digital image analysis software (Image J 1.42 q, National Institutes of Health, Bethesda, MD, USA), and Equation 1: N=[p/A](100)]. N is the degree of nanoleakage through the material of interest, and p is the total sum of the areas of white silver spots over the fixed area, A is the preparation wall length. Data was taken for all samples over a box of standard area 50 µm from the treatment material surface and 300 µm in height. This was done to be able to compare nanoleakage values over all samples.

Statistical analysis was performed using two-way ANOVA at $p<0.05$. Because some findings included extreme values, the statistics was performed on logarithmic data and then converted to normal range. Multiple comparisons were made using the *post-hoc* Holm Sidac test.

RESULTS

In the non-thermocycled control samples, no statistically significant differences were observed among the main study Groups I, II and III, even though Group III showed the least numerical value of nanoleakage. The same results were received with thermocycled samples, there were no significant differences among the Groups I, II, and III ($p>0.05$). There was an increase in the nanoleakage numerical values of Group II and III after thermal cycling but only statistically significant difference was observed between the Group III control and thermocycler groups ($p=0.027$). However, there were no statistically significant differences between leakage scores of the control and thermocycler groups of Group I and Group II ($p>0.05$, Table 2, Figs. 4, 5).

The SEM inspections of artificial enamel lesions confirmed that subsurface caries-like demineralization on the enamel surfaces of samples were created before application of resin sealing therapies. Artificial enamel lesion depth was also calculated on the images (Fig. 2).

DISCUSSION

Nanoleakage is one of important indicators to evaluate the sealing ability of resin adhesive materials. The aim of the present study was to optically quantify the efficacy of ICON, a resin infiltrant, by means of interfacial nanoleakage and compare the results with two other enamel sealing therapies in artificial non-cavitated enamel lesions. Our study findings showed that there

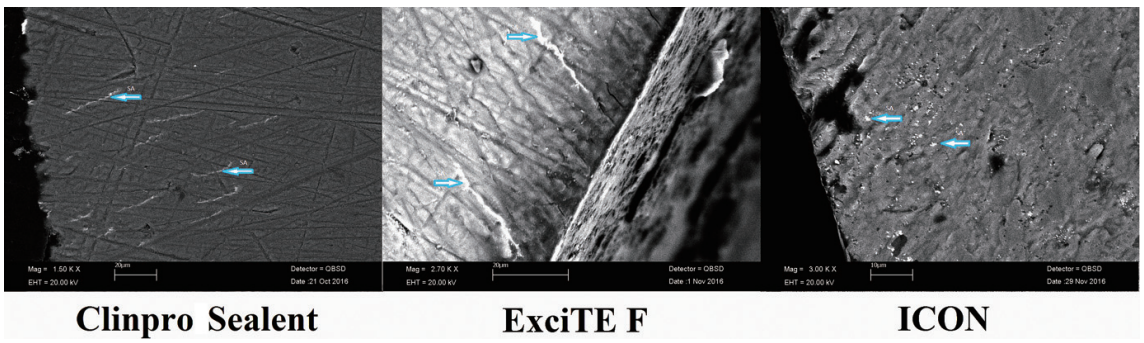

Fig. 4 SEM images showing silver infiltration in control samples.

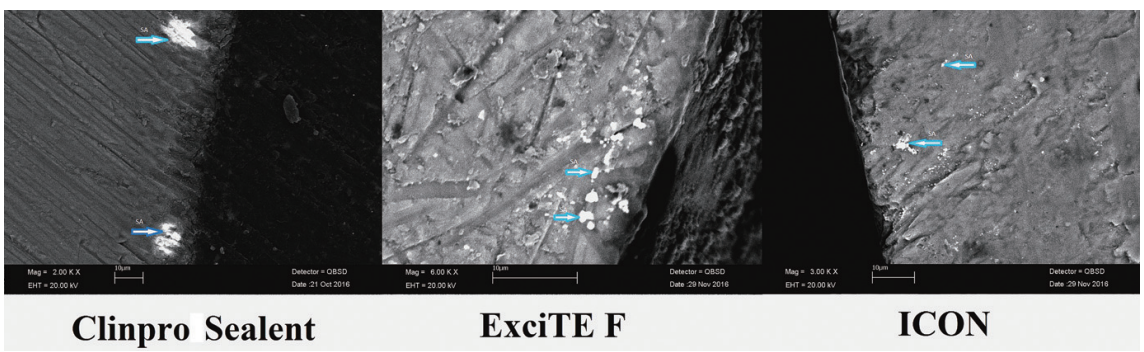

Fig. 5 SEM images showing silver deposition in thermocycled samples.

was no statistically significant difference in microleakage values of three sealing materials. However, and thermal aging increased silver deposition in the group III of ICON resin application. Therefore, the second null hypothesis of the study was rejected while the first null hypothesis of the study was accepted.

The majority of nanoleakage studies have focused on resin-dentin bonds, assuming that high-energy enamel surfaces created by acid etching were optimized for resin infiltration and created stable interface with low susceptibility to degradation over time. Nanoleakage expression in the resin/enamel interface has not been previously studied under different thermocycling regimens; there are some studies which provide information on the effects of aging on both nanoleakage and the bond strength of dentin, but in the case of enamel, reliable data are still scarce[17,18]. This study is the first to address how simulated oral environment with thermocycling can affect nanoleakage values of different resin sealing therapies applied to non-cavitated enamel lesions.

Visual assessment and scoring have been frequently used to evaluate the extent and characterize the patterns of nanoleakage expression at the interface. But, there is a lack of established quantitative techniques to evaluate the nanoleakage within the adhesive interface on both enamel and dentin. For this reason, the authors of current study preferred to use a quantitative analysis of nanoleakage expression within the resin interface on enamel.

In this current study, the bonded specimens were subjected to *in vitro* thermocycling process in order to evaluate the durability of the bondin a simulated oral environment. Thermocycling is applied to introduce an artifical aging parameter that would allow evaluation of the temperature dependent degradation of the materials[19]. It has been reported that hot water may accelerate hydrolysis of the interface components with subsequent water uptake and leaching of the breakdown products or poorly polymerized resin oligomers. In addition, thermal cycling may induce stress between the tooth substrate and the restorative material, which is generated by the different thermal conductivites and the coefficient of thermal expansion of the substrates and bonded materials[20]. These stresses may lead to craks that propagate along bonded interfaces, accelerating chemical degradation of the bonds.

Many leakage tests have used thermal stress to stimulate the clinical situation[21]. This phenomenon may cause the breakdown of the bond and the subsequent failure of the restoration[22]. When examining the effect of thermocycling on bond strength, Asaka *et al.*[20] found that 10,000 thermalcycles had no influence on bond durability, however thermal cycles up to 20,000 decreased the bond strength significantly. According to the study of Miyazaki *et al.*[23], the bond strength of dentin adhesive systems decreased after 30,000 cycles, especially in the self-etching systems. Harper *et al.*[24] showed that the rate

of temperature change beneath resin restorations was relatively low after taking cold or hot fluids, indicating that the linear coefficient of thermal expansion would not greatly influence the dimensional change of the material and therefore not affect leakage. It was also suggested that the bonding of the adhesive to the tooth structure is micro-mechanical in nature and would not be adversely affected by temperature cycling[25]. On the other hand, Holderegger *et al.*[26] confirmed that thermocycling affected the bonding performance of tested materials. Thermocycling of 10,000 cycles (5–55°C) was selected for this study to simulate one year of aging in an oral environment[27].

In the present study, while use of thermocycling did not significantly affect the degree of silver penetration at the fissure sealant and dental adhesive, ICON resin infitrant was significantly affected by thermocycling and nanoleakage increased more than other study materials after one-year *in vitro* thermal aging process. Therefore, we assume that these nanoleakage results may give us an important clue about the respective clinical outcomes of three sealing therapies used in this study.

Different effects of thermocycling on bond durability in previous and the present studies were related to differences in chemical structure of test materials used. Especially, the selection of the monomers in resin matrix strongly influences the reactivity, viscosity, and polymerization shrinkage of the materials, as well as the mechanical properties, water sorption, and swelling by water of the resin[28].

Oral environment plays an important role in the properties of dental restorative materials. Because water is always present in the mouths of healthy individuals, it is important to understand how the properties of resin adhesives are influenced by water. Water may promote a variety of chemical and physical processes that create biological concerns as well as produce deleterious effects on the structure and function of the polymer matrix itself[29]. Especially, water sorption has been found to affect physical and mechanical properties of materials[30-32]. Water sorption by resin materials is a diffusion-controlled process, and the water uptake occurs largely in the resin matrix. This absorption in the polymer matrix can cause filler-matrix debonding or even hydrolytic degradation of the fillers and may eventually affect mechanical properties of resin material[33,34].

The widely used monomer Bis-GMA exhibits a very viscos structure because of the hydrogen bond needs to be diluted with a less viscous and hydrophylic monomer like TEGDMA[35]. However, addition of TEGDMA increases water sorption and decreases general mechanical properties[36]. Some other high molecular weight monomers have been developed and introduced in commercial materials to overcome the limitations of Bis-GMA-based systems. Formulations based on UDMA have become increasingly common, due to the monomer's low viscosity and high flexibility in comparison to Bis-GMA. UDMA copolymers in general present higher flexural strength, elastic modulus, and hardness[37].

We can shortly summarize the findings of previous studies that TEGDMA creates the most dense polymer network, which is flexible and absorbs the highest amount of water. However, UDMA creates more rigid networks than TEGDMA, which absorbs less water. Bis-GMA leads to the formation of the most rigid netwok, which absorbs less water than TEGDMA but more than UDMA[37,38]. A study by Park *et al.*[29] indicated that water sorption and solubility of TEGDMA containing polymer were higher than Bis-GMA and HEMA. Their results confirm our findings that this monomer is highly hydrophilic and may undergo degradation in oral environment, reducing clinical performance. The results of another recent work is also in accordance with our findings[39]. The study showed that monomers' chemical characteristics influenced the behavior of experimental polymer blends. ICON resin infiltrant proved to be the most soluble material of all. Therefore we assume that increased nanoleakage exhibited by ICON infiltrant of this study could be due to the fact that resin network of the material is composed of a TEGDMA-based monomer.

Clinical studies remain the gold standard when measuring the performance of dental materials in oral environment. A very recent clinical study also supported our findings that a TEGDMA containing commercial fissure sealant and ICON infiltrant were both effective to prevent the caries progression in non-cavitated pit-and-fissures after 3 years. However, marginal integrity of the lesions was significantly reduced after 1 year for both materials[40].

Since the formation of nanoleakage within the adhesive-resin interface is an indicator for judging the material's sealing ability and the longevity of the restoration, the sealing capacity and long-term durability of resin infiltrants need to be evaluated further in different studies.

CONCLUSIONS

Within the limitations of this study, it can be concluded that ICON resin infiltrant can be used as an alternative to dental resin adhesives and fissure sealants in the sealing of initial non-cavitated enamel lesions. But TEGDMA containing resin infiltrants may become more affected by the water sorption than other resin materials over time.

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