Comparison of Resin Infiltration Technique with Conventional Preventive Applications on Occlusal Fissures: EDS and SEM Analyses

Usporedba tehnike infiltracije smolom poslije uporabe konvencionalnih preventivnih postupaka na okluzalnim fisurama s obzirom na kemijsku analizu i SEM

Abstract
Objective: To compare the subsurface mineral loss preventing capability of resin infiltration technique with topical fluoride and fissure sealant applications to demineralized occlusal fissures under simulated oral conditions. Materials and Methods: Occlusal surfaces of 64 extracted intact human third molars were demineralized. Next, the teeth were classified into four groups according to preventive applications (n = 16): G1, Specimens used as the control group with no preventive treatment; G2, Topical fluoride application (APF Gel/ DEEPAK); G3, Fissure sealant application (ClinproTM-MSealant/ 3M ESPE); and G4, Resin infiltration technique (Icon/ DMG). Chemical compositions before pH cycling were evaluated for eight specimens from each group. The remaining eight teeth from each group were subjected to pH cycling for 15 days to simulate the oral conditions. Subsequently, the specimens were fractured after immersion in liquid nitrogen and the subsurface fluoride (F), calcium (Ca), phosphorus (P) levels, and Ca/P ratio of each specimen were measured using energy dispersive x-ray spectrometer (EDS). The data were subjected to statistical analysis (p = 0.05). The effects of preventive applications to surface topography of specimens were evaluated using scanning electron microscope (SEM).

Results: There were no significant differences among the groups in subsurface F, Ca, and P levels and Ca/P ratios before or after pH cycling (p > 0.05). All three preventive applications were effective during pH cycling according to SEM observations. Conclusions: The subsurface mineral loss preventing capability of resin infiltration technique applied to occlusal fissures was comparable to topical fluoride and fissure sealant applications. Clinical significance: The resin infiltration technique could represent a valid alternative to traditionally used both preventive and restorative treatments for treating initial carious lesions on occlusal fissures, offering the advantages of better resin penetration and retention.

Introduction
Tooth decay is an infectious microbiological condition that results in local dissolution and calcified tissues destruction (1). It is experienced by more than two-thirds of all children and over 90% of all dentate adults in the United States, with a wide range of severity (2).

During caries formation, a clinically noticeable pore structure is formed by dissolving the minerals from the outer layer of the enamel, which is known as “white spot” lesions representing the earliest evidence of caries (3). These lesions are usually observed on the facial, lingual, and proximal smooth surfaces of the teeth and also on pits and occlusal fissures.

Uvod
Zubni karijes zarašno je mikrobiološko stanje koje rezultira lokalnim otapanjem i uništavanjem kalcaćiranih tkiva (1). Događa se, u različitim stupnjevima težine, kod više od dvije trećine djece i više od 90% svih odraslih osoba u Sjedinjenim Američkim Državama (2).

Tijekom nastajanja karijesa stvara se klinički uočljiva plosna struktura zbog otapanja minerala vanjskoga sloja cakline, što je poznato kao bijela mrlja koja je najraniji dokaz karijesa (3). Te se lezište obično opažaju na glatkim vestibularnim, lingualnim i aproksimalnim ploham zuba te na jamicama i okluzalnim fisurama. Okluzalne fisure su najosjetljivija mje-
Occlusal fissures are the most vulnerable sites to caries lesions because of morphology and plaque accumulation (4, 5).

White spot lesions of enamel on the occlusal fissures and other areas are generally treated by increasing remineralization through fluoridation and ensuring oral hygiene improvement (6,7) and by preventing the progression of the lesion with fissure sealant application (8).

Fluoride is one of the most important agents for the treatment of initial enamel lesions (4). It has become clear that when F ions are part of the remineralization process, the enamel integrity is restored and the enamel's resistance to further cariogenic attacks is increased (7). In comparison to smooth surface caries, fluoride treatments could not totally occlude fissure and pit caries, making them less effective on these surfaces that are more prone to caries because of their morphological conditions (1).

Fissure sealant application is a non-invasive treatment option for occlusal surfaces with non-cavitated lesions acting as a mechanical barrier between enamel surface and biofilm formation, thereby preventing caries progression (8). Although fissure sealant resins were originally designed as a preventive measure, current studies show that the resin protects the initial porous lesions on altered occlusal surfaces due to demineralization from progression and formation of cavities (8-10). The main disadvantage of this method is the insufficient etching caused by phosphoric acid on deeper parts of the fissures and the inability of the viscous resins to completely cover the fissure surfaces (1,11). Furthermore, an adequate barrier is not created inside the lesion, thereby possibly partially sealing the fissure, which also includes early caries and plaque, resulting in further caries progression (11,12).

Lately, a low-viscosity resin that can penetrate into caries lesions, which is referred to as resin infiltration technique by Kielbassa et al., has been introduced (11). The mechanisms associated with this method depend on the infiltration of subsurface lesions with low-viscosity light-curing resins after etching the highly mineralized surface layer and increasing penetration depth with a hydrochloric acid solution (11-13). This method is believed to overcome the limitations of topical F and sealant applications and is considered to be a better alternative for the treatment of initial caries lesions (11). The effectiveness of resin infiltration technique on smooth surfaces has been widely examined (3, 6, 11, 12); however, there is less information about its preventive effect on fissures (13, 14).

The present study compared the changes in subsurface chemical composition of resin infiltrant, topical F, and fissure sealant applied demineralized occlusal fissures after exposure to imitated oral conditions and evaluated the surface morphology of each specimen under a scanning electron microscope (SEM). Therefore, our hypothesis was that the protective properties of the resin infiltration technique in demineralized occlusal fissures are more effective than conventional topical F and fissure sealant applications.

Material and methods

Overall, 64 extracted healthy human third molars were used for this in vitro study. The collected teeth were disinfected in 10% formalin solution for 10 minutes, carefully

sta za pojavu karijesnih lezija zbog morfologije i nakupljanja plaka (4, 5).

Bijele mrlje na caklini okluzalnih fisura i u drugim područjima općenito se liježe poticanjem remineralizacije fluoridacijom i boljom oralnom higijenom (6, 7) te spriječavanjem napredovanja lezije primjenom smola za pečaćenje fisura (8).

Fluor je jedno od najvažnijih sredstava za liječenje početnih lezija u caklini (4). Postalo je jasno da kada su ioni fluorada dio procesa remineralizacije, obnavlja se integritet cakline i povećava njezina otpornost na daljnja kariogene napade (7). U usporedbi s karijesom na glatki površinama, aplikacija fluorora nije mogla potpuno zabrtviti fisure i jamicu, što ga čini manje učinkovitim na onim površinama koje su zbog svojih morfoloških uvjeta sklonije karijesu (1).

Primjena smola za pečaćenje fisura neinvazivna je opcija liječenja okluzalnih površina s nekavitiranim lezijama zato što djeluje kao mehanička barijera između površine cakline i stvaranja biofilma, čime se spriječava napredovanje karijesa (8). Iako su smole za pečaćenje fisura izvorno zamišljene kao preventivna mjera, aktualna istraživanja pokazuju da smola štiri od napredovanja i stvaranja kaviteta inicijalne porozne lezije na promijenjenim okluzalnim plohama zbog demineralizacije (8, 10). Glavni nedostatak te metode jest nedovoljno nagrizanje prouzročeno fosfornom kiselinom na dubljim dijelovima fisura i nemogućnost viskoznih smola da potpuno prekriju površinu fisura (1, 11). Nadalje, unutar lezije ne stvara se odgovarajuća barijera, čime se vjerojatno samo dijelomično brtvi fisura koja također sadržava inicijalni karijes i plak, što rezultira daljnjim napredovanjem karijesa (11, 12).

Nedavno je predstavljena smola niske viskoznosti koja može prodirjeti u karijesne lezije, što je poznato kao tehnika infiltracije smole prema Kielbassiju i suradnicima (11). Mehanizmi povezani s tom metodom ovise o infiltraciji potpovršinskih lezija sa svjetlosno polimerizirajućim smolama male viskoznosti nakon erozije visokomineraliziranog površinskog sloja i povećanja dubine prodiranja otopinom klorovodične kiseline (11 – 13). Pretpostavlja se da ta metoda prevladava ograničenja topikalne primjene fluorora i smola za pečaćenje, te se smatra boljom alternativom u liječenju početnih karijesnih lezija (11). Učinkovitost tehnike infiltracije smole na glatkim površinama detaljnije je ispitivana (3, 6, 11, 12), no manje je podataka o njezinu preventivnom učinku na fisurama (13, 14).

U ovom su se istraživanju uspoređivale promjene u kemijskom sastavu ispod površine nakon infiltracije smolom i topikalne primjene fluorora i smole za pečaćenje fisura na demineraliziranim okluzalnim fisurama poslije izlaganja, radi oponašanja intraoralnih uvjeta te se procjenjavala površinska morfologija svakog uzorka pod skenirajućim elektronskim mikroskopom (SEM). Zato je naša hipoteza bila da su zaštitna svojstva infiltracijske tehnike u demineraliziranim okluzalnim fisurama učinkovitija od uobičajene topikalne primjene fluorora i smola za pečaćenje fisura.

Materijali i metode

Ukupno su za ovo istraživanje in vitro upotrijebljena 64 izvadena zdrava ljudska umnjaka. Zubi su 10 minuta dezinficirani u 10-postotnoj otopini formalina, pažljivo očišćeni.
cleaned from the soft tissue, and stored in distilled water at room temperature until required for use. The root apices were sealed with epoxy resin, and all tooth surfaces, excluding the occlusal surfaces, were coated with two layers of nail polish. The occlusal surfaces of the teeth were demineralized by immersion in 8 ml of demineralization solution for 72 hours at 37°C, according to the protocol used by Ten Cate and Duijsters (15). Subsequently, the teeth were rinsed with an air/water spray for 5 seconds, dried with oil-free compressed air, and randomly assigned to four groups according to the preventive applications used (n = 16): 

G1: These specimens were used as controls and no preventive treatment was applied.

G2: The teeth were desiccated by air blowing and an acidulated phosphate F (APF) gel (APF Gel; DEEPAK, Miami, FL, USA) that contains 1.23% F ion, which was applied onto the occlusal fissures with sponge applicators. After 4 minutes, the gel was removed using cotton rolls and the teeth were rinsed/dried with air/water spray.

G3: The teeth were dried with an air-water spray, etched for 30 seconds with 37% phosphoric acid gel (Etchant gel, Prime-Dent, Chicago, IL, USA), and rinsed and air dried with compressed air for 20 and 10 seconds respectively. A light-cured and low-viscosity pit and fissure sealant (Clinpro™ Sealant/3M ESPE, St Paul, MN, USA) was applied onto the fissures along their entire extension with the tip of a probe to prevent air entrapment. Then the resin sealant was light-cured for 20 seconds using LED-curing unit (Radii Plus, SDI, Victoria, Australia).

G4: The specimens were dried with oil-free compressed air. Hydrochloric acid (HCl) etching gel (Icon etch, DMG, Hamburg, Germany) was applied with a syringe on fissures and left undisturbed for 2 minutes and the gel was rinsed with air-water spray for 30 seconds. Subsequently, 99% ethanol (Icon Dry, DMG, Hamburg, Germany) was applied for 10 seconds, and the specimens were air dried again for 10 seconds. Next, the infiltrant (Icon infiltrant, DMG, Hamburg, Germany) was applied with gentle agitation. After 3 minutes of penetration time, any excess material was removed by air blowing and the resin was light-cured for 40 seconds using LED-curing unit. The infiltration step was repeated for 1 minute to infiltrate remaining porosity followed by light curing for 40 seconds.

After completing the preventive applications according to the manufacturer’s instructions, the groups were divided into two subgroups. Eight specimens from each group were kept for evaluation of element levels, and the other eight teeth were subjected to pH cycling to mimic oral conditions (15,16). The pH cycling regime reported in previous studies was designed to approach the pH dynamics of the oral environment (Table 1) (16). The regimen was repeated for 15 days at 37°C, and the temperature was maintained by means of an incubator. The specimens were washed with distilled water and dried with oil-free compressed air between transfers from demineralizing and remineralizing solutions. The demineralizing and remineralizing solutions were refreshed every 3 days throughout the experimental period (17).

Next, the specimens were cross-sectioned perpendicular to occlusal surfaces. To obtain a clear sagittal view, vertical slits spanning from apical to the cement-enamel junction were cut through the root apical third and stained with Toluidine blue. The demineralized occlusal surfaces were then coded and randomly assigned to the experimental group (18).

G2 – the teeth were immersed in the remineralization solution for 15 days and the temperature was maintained at 37°C according to the protocol used by Ten Cate and Duijsters (15). Posilje toga zubi su 5 sekundi ispirani sprejem zrak – voda, osušeni komprimiranim zrakom bez ulja i nasumično uvršteni u jednu od četiri skupine prema primijenjenim preventivnim primjenama (n = 16):

G1 – ti su uzorci korišteni kao kontrola i nije proveden preventivni postupak.

G2 – zubi su osušeni zrakom te je na okluzalne fissure aplikatorom nanesen kisel fosfat-fluoridni (APF) gel (APF Gel; DEEPAK, Miami, FL, USA) koji sadržava 1,23 % iona fluora. Nakon četiri minute gel je uklonjen pamučnim kuglicama, a zubi isprani i osušeni.

G3 – zubi su osušeni, jetkani 30 sekunda 3-postotnim gelom fosforne kiseline (Etchant gel, Prime-Dent, Chicago, IL, SAD) nakon čega su 20 sekundi isprani i zatim osušeni komprimiranim zrakom 10 sekundo. Na fissure je cijelim duljinom vrhom sonde nanesena svjetlosno polimerizirajuća smola za pečaćenje niske viskoznosti (Clinpro™ Sealant/3M ESPE, St Paul, MN, SAD) da bi se spriječile inkluzije zraka. Zatim se smola osvijetlila 20 sekunda polimerizacijskom LED svjetiljkom (Radii Plus, SDI, Victoria, Australija).

G4 – uzorci su osušeni komprimiranim zrakom bez ulja. Gel za jetkanje na bazi klorovodične kiseline (HCl) (Icon etch, DMG, Hamburg, Njemačka) nanesen je štrkaljkom na fissure i ostavljen da djeluje dvije minute, nakon čega je 30 sekundi ispran sprejem zrak – voda. Poslije toga je apliciran 99-postotni ethanol (Icon Dry, DMG, Hamburg, Njemačka) tijekom 10 sekunde, a uzorci su ponovno sušeni na zraku 10 sekundo. Zatim je aplicirana infiltracijska smola (Icon infiltrant, DMG, Hamburg, Njemačka). Nakon trominutne infiltracije, sav višak materijala uklonjen je ispuhanjem zrakom i smola je polimerizirana svjetlosno 40 sekundo s pomoću LED svjetiljke. Postupak infiltracije ponovljen je još jednu minutu da bi se infiltrirala preostala porozna područja, nakon čega je slijedila 40-sekundna svjetlosna polimerizacija.

Nakon što su dovršeni preventivni postupci prema uputama proizvođača, skupine su podijeljene u dvije podsugrađe. Osam uzoraka iz svake skupine čuvalo se za procjenu razine elemenata, a ostale osam podvrgnuto je cikliranju pH kako bi se oponašali intraoralni uvjeti (15, 16). Režim cikliranja pH koji je proveden u ranijim istraživanjima osmišljen je da bi se simulirala dinamika pH u oralnom okruženju tablica 1 (15, 16). Ponajviše se 15 dana pri 37°C, a temperatura se održavala s pomoću incubatora. Uzorci su isprani destiliranom vodom i osušeni komprimiranim zrakom bez ulja između prijenosa iz demineralizacije i remineralizacije otopine. Otopine za demineralizaciju i remineralizaciju osvježavale su se svaka tri dana tijekom eksperimentalnog razdoblja (17).

Nakon toga uzorci su prerezani okomito na okluzijske površine. Da bi se dobio jasan sagitalni prikaz, na svakom zubu urezani su dijamantni pilom male brzine (Isomet Low Speed Saw, Buehler, Lake Bluff, IL, SAD) vertikalni prorez.
on each tooth with a low speed diamond saw (Isomet Low Speed Saw, Buehler, Lake Bluff, IL, USA) leaving the occlusal side untouched. After the immersion in liquid nitrogen for 2 minutes, the specimens were gently fracture along the preformed slits with a hammer and scalpel blade (18). The subsurface F (F), calcium (Ca), and phosphorus (P) levels and Ca/P ratio of each specimen were measured using energy dispersive spectrometer (Bruker Ax XFlash 3001 SDD-EDS, Cambridge, UK). Three measurements were taken from each specimen and the mean values for each specimen were individually calculated. The data were analyzed statistically using the SPSS 13.0 software for the changes in subsurface element levels among the groups before and after pH cycling with the One-Way ANOVA test or Kruskal-Wallis test according to the normality of the data for each element. The comparisons of each individual group before and after pH cycling were evaluated using the t-test. The statistical significance level for each test was set at $p = 0.05$.

The surface topography of all the specimens was evaluated under SEM (Zeiss EVO 50 EP SEM, Carl Zeiss, Cambridge, UK) with 1000X magnification. Acceleration voltage was 20 kV using a backscattered electron detector (BSE). The images were obtained at an extended variable pressure (XVP) mode without coating since the vacuuming conditions needed to sputter the fragile enamel specimens might have resulted in deterioration on the surfaces.

### Results

The P, Ca levels and Ca/P ratios among the groups were not statistically significant before or after pH cycling ($p > 0.05$). Although, the topical F applied group (G2) tended to exhibit an increased trend in F level, the differences among the groups were not significant ($p > 0.05$). A comparison of F, P and Ca levels and Ca/P ratios within each group before and after pH cycling revealed no significant differences ($p > 0.05$). Table 2 gives summary of the obtained results.

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**Table 2**

| Elements | G1 | G2 | G3 | G4 | p   |
|----------|----|----|----|----|-----|
| F        | 0.68 ± 0.75 | 1.53 ± 0.11 | 0.87 ± 0.09 | 1.28 ± 0.59 | 0.54 |
| P        | 16.29 ± 1.04 | 16.16 ± 0.97 | 16.29 ± 1.02 | 15.34 ± 1.60 | 0.28 |
| Ca       | 37.59 ± 3.65 | 35.94 ± 2.29 | 38.38 ± 4.09 | 36.76 ± 4.85 | 0.62 |
| Ca/P     | 2.31 ± 0.24 | 2.39 ± 0.24 | 2.36 ± 0.30 | 2.22 ± 0.12 | 0.85 |

$p$ values indicate the results of Kruskal-Wallis one-way ANOVA test ($p = 0.05$) • $p$ vrijednosti pokazuju rezultate Kruskal-Wallisova ili jednosmjernog ANOVA testa ($p = 0.05$).
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SEM observations revealed different morphological characteristics with different treatment approaches and pH cycling. After pH cycling, obvious deleterious effects were observed on enamel surfaces of occlusal fissures in G1, in which no preventive treatment was applied. The specimens treated with F (G2) or fissure sealant (G3) revealed slight changes on their morphological features, whereas there were no differences on the surface topography of the resin infiltration applied specimens (G4). Representative SEM images from each group are shown in Figures 1, 2.

Discussion

This study has determined whether infiltration of a low-viscosity light-curing resin could be an alternative method to fissure sealant and topical F applications for the prevention of mineral loss of initial fissure caries lesions. For this purpose, ca-

SEM analizom otkrivena su različita morfološka obilježja s obzirom na različite postupke tretiranja površine i cikliranje pH. Nakon cikliranja pH uočeni su očiti štetni učinci na površine cakline okluzalnih fisura u skupini G1 u kojoj nije primijenjen preventivni postupak. Na uzorcima tretiranima fluorom (G2) ili smolom za pečaćenje (G3), zabilježene su male promjene u morfološkim značajkama, a nije bilo razlika u površinskoj topografiji uzoraka infiltriranih smolom (G4). Reprezentativni SEM skenovi iz svake skupine prikazani su na slikama 1 i 2.

Rasprava

U ovom se istraživanju pokušalo ustanoviti može li infiltracija svjetlosno polimerizirajućom smolom niske viskoznosti biti alternativa metoda klasičnom pečaćenju fisura i topikalnoj primjeni fluora u prevenciji gubitka minerala u
ries-like artificial demineralization lesions were created using a similar technique mentioned in a previous *in vitro* study (17).

Although, the difference between artificial demineralization lesions and natural caries lesions may be a limitation to reflect the clinical conditions, our aim was to create standard demineralization lesions to prevent any negative effects that may arise from the heterogeneity of the specimens (13,17). It is known that an initial caries lesion in the enamel is softer than the surrounding sound tissue and is visible as white spots with increased opacity when air dried (1). There is a consensus in the literature that the basic attributes that characterize these lesions include a relatively intact, mineral-rich surface layer and a subsurface demineralized area that can be observed when the cross-section of the lesion is formed from the initial caries (1,19).

Figure 2. Scanning electron microscope views of the specimens after pH cycling (1000x). (2a) Demineralized enamel (control). Areas of superficial hypermineralized enamel erosion, characterized by an evident "localized peeling" of the surface, were observed (G1); (2b) F applied enamel surface. The appearance of honeycomb-shaped enamel, in which the prismatic orifices resulting from the pH cycling become more visible, is observed (G2); (2c) Fissure sealant applied enamel surface. An increased micro-gap is observed indicating that the junction between the fissure sealant and the adjacent enamel is weakened. It is also seen that the structure of the fissure sealant resin is deteriorated as microfracture (G3); (2d) Resin infiltrated enamel surface. An intact, interdigitating interface without micro-gaps between the resin infiltrant and the adjacent enamel is still observed. No deleterious effects of pH cycle on neighboring enamel are identified (G4).
In the present study, the subsurface element levels were evaluated because the demineralization in initial caries lesions is mostly pronounced in the subsurface layer according to the “phenomenon of subsurface demineralization” as mentioned previously (20). To evaluate the subsurface demineralization, the specimens were flash frozen by immersion in liquid nitrogen and fractured along the pre-formed slits to obtain clear and untouched cross-sections. It is also important to emphasize that all the native elements of enamel could be retained without any alteration in the specimens because of the rapid freeze procedure and the fact that the subsequent management of specimens was without the use of water (18).

The levels of Ca and P, the major components of enamel, indicate demineralization, and F is another important element that could reduce the solubility of calcium phosphate (21). Therefore, the present study evaluated the subsurface F, P, and Ca levels and Ca/P ratio using EDS, which represents a specific method for determining the concentration of chemical elements of the materials both on the surface and subsurface (22). There are several methods to evaluate demineralization process on enamel, such as microradiography (23), polarized light microscopy (24), X-Ray Photoelectron Spectroscopy (XPS) (22), and Raman spectroscopy (22). Furthermore, the X-ray energy dispersive spectroscopy (EDS) analysis is a qualitative and quantitative method to determine chemical elements compositions in various samples (22). This system bombards the sample with a high-voltage electron beam that emits a characteristic wavelength for each mineral and works by showing rays of different wavelengths that are reflected back depending on the specific mineral concentration in the sample (25). The sensitivity of this technique changes depending on the method of sample preparation for microanalysis and the atomic number of all elements found in the specimens, particularly the atomic number of the element to be identified (22). The atomic numbers of Ca, P, and F elements which were evaluated in the present study are 20, 25, and 9, respectively, and the quantitative sensitivity of EDS evaluation is higher for Ca and P than F because they possess higher atomic numbers (22). Although there have been some concerns about the efficacy of this method for analyzing the elements with lower atomic number, such as F, previously, the current SEM-EDS systems can adequately analyze such elements too (26).

Therefore, the EDS method was particularly preferred in this study for providing a certain advantage of reliable and non destructive analysis of the fragile enamel samples. There are limited data in the literature about the effect of resin infiltrant application on enamel element levels. Gelani et al. tested the ability of resin infiltrant and F application on preventing subsurface mineral loss of the artificial enamel carious lesion with the EDS and they observed less mineral loss in the groups treated with resin infiltrants than in those only treated with F (27). Similar to this study, the results of our experiment indicated some slight changes in F, Ca, and P levels and Ca/P ratio after pH cycling in all groups. However, the differences were not statistically significant.

Remineralization and arrest of initial caries lesions by topical F applications and fissure sealants have been investigated ispod površine koje se može uočiti kada se napravi presjek lezije početnog karijesa (1, 19).

In ovom istraživanju procijenjene razine elemenata ispod površine jezera demineralizacije u početnim karijenskim lezijama uglavnom pojavljuje u tom sloju u skladu s fenomenom demineralizacije ispod površine, kako je već spomenuto (20). Da bi se procijenila demineralizacija ispod površine, uzorci su naglo zamrznuti uzenjanjem u tekući dušik i preoljeni duž unaprijed oblikovanih proreza kako bi se dobili jasi i intaktni presjeci. Također je važno istaknuti da se svi nativni elementi cakline mogu zadržati bez ikakvih promjena u uzorcima zbog postupka brzog zamrzavanja i činjenice da je naknadno tretiranje uzoraka bilo bez upotrebe vode (18).

Razine kalcija i fluora, glavnih komponenta cakline, upućuju na demineralizaciju, a fosfor je drugi važan element koji bi mogao smanjiti topljivost kalcijeva fosfata (21). Zato su se u ovom istraživanju procijenjivale razine fluora, fosfora i kalcija te omjer Ca/P-a ispod površine s pomoću EDS-a, koji je specifična metoda za određivanje koncentracije kemijskih elemenata materijala na površini i ispod nje (22). Nekoliko je metoda za procjenu procesa demineralizacije na caklini, poput mikroradiografije (23), mikroskopije polariziranog svjetla (24), rendgenske fotoelektronske spektroskopije (XPS) (22) i Ramanove spektroskopije (22). Nadalje, analiza rendgenskom disperzivnom spektroskopijom (EDS) kvalitativna je i kvantitativa metoda za određivanje sastava kemijskih elemenata u različitim uzorcima (22). Tim se sustavom uzorak bombardira visokonaponskim elektronskim snopom koji emitira karakterističnu valnju dužinu za svaki mineral i djeluje tako da pokazuje zrake različitih valnih duljina koje se reflektiraju natrag, ovisno o specifičnoj koncentraciji minerala u uzorku (25). Osećajivost te tehničke miješenja se ovisno o metodi pripreme uzorka za mikroanalizu i atomskom broju svih elemenata koji se nalaze u uzorcima, posebno atomskom broju elementa koji treba identificirati (22). Atomski brojevi elemenata Ca, P, i F koji su procijenjeni u ovom istraživanju, iznose 20, 25, odnosno 9, a kvantitativa osećajivost procjene EDS-a veća je za kalcij i fosfor od fluora jer imaju veće atomski brojeve (22.). Iako je prije postojala određena sumnja u učinkovitost te metode za analizu elemenata s nižim atomskim brojem, poput fluora, sadašnji SEM-EDS sustavi mogu adekvatno analizirati i takve elemente (26).

Zato je EDS posebno pogodna metoda za ovo istraživanje jer omogućuje pouzdanu i nedestruktivnu analizu krhkih uzoraka cakline.

U literaturi su ograničeni podaci o učinku infiltracije smolom na razinu elemenata u caklini. Gelani i suradnici testirali su EDS-om svojstvo infiltracije smolom i primjenu fluora i zabilježili gubitak minerala ispod površine u umjetnoj karijenskoj leziji cakline, te su primijetili manji gubitak minerala u skupinama tretiranim infiltracijom smolom negoli u onima gdje je aplikiran fluor (27). Slično tom istraživanju, rezultati našega eksperimenta upućivali su na neke male promjene u rasinama fluora, kalcija i fosfora te u omjeru Ca/P-a nakon cikliranja pH u svim skupinama. No, razlike nisu bile statistički značajne.

Remineralizacija i zaustavljanje početnih karijenskih lezija topikalnom primjenom fluora i smolama za pečačenje fisura
in several studies (7, 8, 10, 16, 28). However, at the initial caries stage of the enamel lesion, F may be effective in remineralizing the outer “surface layer,” which is sealed off, thereby impeding the penetration and repair of deeper enamel lesions by calcium and phosphate (29). In addition, fissure sealants can only show superficial penetration into enamel lesions because they are not optimized for high penetrability (11). Since fissure sealant and F treatments can only affect the superficial region of the lesion, the dissolution of the porous structure in the subsurface lesion, which acts as a diffusion path for acids and minerals, cannot be prevented. Therefore, the infiltration technique of initial caries lesions with a low-viscous resin material has been considered as a micro invasive completion to these widely used preventive treatments (8, 11, 13).

The resin infiltration technique has been developed to stop the progress of caries by filling the porous structure of the initial caries lesions with a light-cured low-viscosity resin (11). Compared to fissure sealants, resin infiltrants show rapid capillary penetration as low-viscosity materials that form a low contact angle with enamel because of their high surface energy (3, 11). Moreover, contrary to the fact that fissure sealants remain only on the surface of the enamel as a diffusion barrier, the resin infiltration materials form a diffusion barrier in the enamel lesion and strengthen the demineralized enamel structure by replacing the lost mineral with a low-viscosity light-curing resin (6, 11).

The effectiveness of resin infiltration to prevent the progression of smooth surface caries lesions has been investigated in vitro (30), in situ (31), and in vivo (32) studies. Paris et al. (33) showed with a micro radiographic technique that, a low-viscosity resin was able to inhibit the progression of natural flat surface enamel caries lesions after 400 days storage at pH 4.95. They also reported in another study that resin infiltration was effective to prevent further demineralization of artificial smooth surface enamel caries lesions under acidic situations in situ (31). An in vivo study comparing the effect of resin infiltration of proximal lesions with non-operative measures has found that the infiltration of interproximal caries lesions was effective in reducing lesion progression (32). Since previous studies (6, 12, 30-33) have only evaluated the effect on smooth surfaces, it is not appropriate to compare these results in this study. There are only a few studies evaluating the efficacy of resin infiltration technique on occlusal surfaces and these studies have only evaluated the penetration of resin infiltrants (13, 14, 34). In addition, to our knowledge, no study has reported about the behavior of infiltrated lesions and the effects of thin resin layers on lesion surfaces and the neighboring healthy enamel.

In the present study, the surface morphology of the specimens was also evaluated using the SEM. In our study, all the preventive applications were effective to eliminate neighboring enamel destruction on some level when compared to the control group. The resin infiltration method was one step forward for reducing demineralization on the neighboring enamel compared to the conventional sealant and F application, since enamel demineralization was much milder on the SEM image of the infiltrated sample. This finding is in line with numerous in vitro studies reported by others (11, 13, 14, 27, 34).
All *in vitro* studies have some limitations. Likewise, the present study has a number of limitations. One limitation of this study was that laboratory tests could not precisely reflect clinical conditions. To reduce the influence of these factors, the pH cycling model was used in this experiment to effectively simulate the oral conditions leading to demineralization and remineralization that routinely occur in the oral environment. Another limitation is the lack of sagittal views of SEM evaluations, which would also help to interpret the subsurface element level results.

According to our findings and within the limitations of the present study, our hypothesis was partially confirmed. Being a microinvasive approach between preventive and restorative actions for the treatment of initial caries lesions on occlusal fissures, the resin infiltration technique could be a promising therapeutic method within the concept of minimum intervention dentistry. However, further *in vitro* and *in vivo* studies are needed to justify the use of such a protocol in clinical practice and assess the prognosis of infiltrated initial caries lesions under real oral conditions.

**Conclusion**

The subsurface demineralization preventing capability of resin infiltration technique, which was applied on initial defemineralized occlusal fissures, was comparable with conventional topical F and fissure sealant applications.

**Conflict of interest**

None declared

**Contribution to the paper**

S.A.H. performed the experiment, wrote the manuscript: E.E. wrote the idea, hypothesis, experimental design, performed some tests; A.O. performed statistical evaluation; S.G. worked on idea, experimental design, hypothesis and contributed substantially to discussion.

S. A. H. – osmislio eksperiment, napisao rukopis; E. E. – razradio ideju, postavio hipotezu, eksperimentalni dizajn, primijenio neke testove; A. O. – statistička analiza; S. G. – radio na ideji, eksperimentalnom dizajnu, hipoteze te znatno pridonio raspravi.

S. I. – postavio hipotezu, eksperimentalni dizajn, primijenio neke testove; A. O. – statistička analiza; S. G. – radio na ideji, eksperimentalnom dizajnu, hipoteze te znatno pridonio raspravi.
Učinak tehnike infiltracije smolom na okluzalne fisure

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