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EFFECTS OF DIFFERENT THERAPEUTIC METHODS ON ARTIFICIAL ENAMEL LESIONS

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ABSTRACT

Background and Aim: The aim was to assess the healing effects of sodium fluoride (NaF) varnish, ozone, caseinphosphopeptide-amorphous calciumphosphate (CPP-ACP) crème, and resin-infiltration on artificial enamel lesions.

Materials and Methods: Eighty caries-free mandibular molars were embedded in acrylic resin, and buccal enamel surfaces were grounded. Enamel surfaces were coated with nail varnish, leaving 8x6 mm windows. Artificial enamel lesions were performed with a demineralization solution (pH 4.5). Specimens were randomly divided into five groups (n=16): (1) artificial saliva (negative control), (2) NaF varnish (positive-control), (3) ozone, (4) CPP-ACP crème, (5) resin infiltration. Subsequently, specimens were subjected to pH-cycling for 10 days. The surface microhardness and roughness measurements were determined before (T_0), after lesion formation (T_1), and at the end of the 14th day period of pH cycling (T_2). The data were analyzed with repeated measurements ANOVA, one-way ANOVA followed by Bonferroni tests ($p<0.05$).

Results: The microhardness values at T_2 were higher than T_1 and lower than T_0 in each group ($p<0.05$). Moreover, T_2 microhardness values revealed significant differences among the groups ($p<0.05$). CPP-ACP crème and resin-infiltration applications increased the microhardness of lesions significantly more than the artificial saliva, ozone or NaF varnish did ($p<0.05$). In respect to surface-roughness, T_2 values were lower than T_1 and higher than T_0 ($p<0.05$), except for the resin infiltration group in which a slight increase was observed at T_2 ($p>0.05$).

Conclusion: CCP-ACP crème and resin-infiltration applications are more effective for the healing of artificial enamel lesions in comparison to NaF varnish and ozone applications.

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Keywords: Artificial Enamel Lesions, Caries Treatment, Microhardness, Surface Toughness

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FARKLI TERAPÖTİK YÖNTEMLERİN YAPAY MİNE LEZYONLARI ÜZERİNE ETKİLERİ

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ÖZ

Amaç: Yapay mine lezyonları üzerine sodyum florür (NaF) vernik, ozon, kazeinfosfopeptit-amorf kalsiyumfosfat (CPP-ACP) krem ve rezin-infiltrasyonun iyileştirici etkisini incelemektir.

Gereç ve Yöntem: Çürüksüz 80 alt molar diş, akrilik rezine gömüldü ve bukkal mine yüzeyleri aşındırıldı. Mine yüzeylerinde, demineralizasyon/remineralizasyon döngüsünden önce 8x6 mm boyutlarında pencereler bırakarak yüzeyler tırnak cilası ile kaplandı. Yapay mine lezyonları demineralizsyon solüsyonu (pH:4.5) ile oluşturuldu. Örnekler rastgele 5 gruba ayrıldı (n=16): (1) Yapay tükürük (negatif-kontrol) (pH: 7.0), (2) NaF vernik (pozitif-kontrol), (3) ozon, (4) CPP-ACP krem, (5) rezin-infiltrasyon. Daha sonra, örnekler 10 gün pH-döngüsüne tabi tutuldu. Mine yüzeylerinin mikrosertlik ve pürüzlülük ölçümleri lezyon oluşumundan önce-(T_0), yapay mine lezyonları oluşturulduktan sonra-(T_1) ve pH döngüsünün 14. gününün ve tedavi prokollerinin sonunda-(T_2) yapıldı. Veriler, tekrarlayan ANOVA, tek yönlü ANOVA ve ardından Bonferroni testleri ile analiz edildi ($p<0.05$).

Bulgular: Her grupta T_2'deki mikrosertlik değerleri T_1'den daha yüksek ve T0'dan daha düşük bulundu ($p<0.05$). T_2'de mikrosertlik değerleri gruplar arasında anlamlı farklılık gösterdi $p<0.05$). CPP-ACP krem ve rezin-infiltrasyon uygulamaları yapay mine lezyonlarının mikrosertliğini yapay tükürük, ozon ve NaF vernikten daha fazla arttırmıştır ($p<0.05$). Yüzey pürüzlülüğü bakımından, T_2'de hafif bir artış gözlenen rezin-infiltrasyon grubu hariç ($p>0.05$), T_2 değerleri T_1'den düşük ve T_0'dan düşük bulundu ($p<0.05$).

Sonuçlar: CCP-ACP krem ve rezin-infiltrasyon uygulamaları yapay mine lezyonlarının iyileştirilmesinde NaF vernik ve ozon uygulamalarına göre daha etkilidir.

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Anahtar Kelimeler: Yapay Mine Lezyonları, Çürük Tedavisi, Mikrosertlik, Yüzey Pürüzlülüğü

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INTRODUCTION

The hardest tissue in human body is tooth enamel. There is a continuous balance in enamel between demineralization and remineralization which is a natural dynamic process. This dynamic process has effects on caries initiation, progression and reversal.[1] Whether this balance is interrupted and demineralization process becomes dominate, in any event, it may lead to the existence of caries lesions in enamel and dentine.[2] The earliest manifestation of dental caries is a white spot lesion formation, which still has the potential to remineralize. Hence, if appropriate conditions are provided, the process can be shifted toward remineralization, thus healing the lesion. Enamel consists of hydroxyapatite crystals, which are in a dynamic equilibrium with calcium and phosphate ions in a neutral medium. Acids, which have intrinsic or extrinsic sources, cause demineralization; some acids are produced when certain bacteria colonize on the tooth surface and metabolize carbohydrates, and some are introduced into the mouth as a part of food or drink. Demineralization can be reversed if the pH is neutralized and there are sufficient bioavailable calcium and phosphate ions in the immediate environment.[3]

Approaches to the management of dental caries have changed dramatically in recent years, evolving from the traditional, largely restorative treatment approach to a preventive approach with non-invasion or minimal invasion. In view of a better understanding of the caries process, modern management approaches should aim towards preventing the disease, managing the caries risk, and detecting caries lesions as early as possible in order to avoid invasive treatment, but, when treatment is indicated, to use the least invasive methods.[4] As the "minimal intervention" concept becomes widely practiced, dental practitioners are taking increasing interest in various products and methods with tooth surface protection and anti-cariogenic or remineralization effects.[5]

Several approaches have been proposed for the noninvasive management of non-cavitated caries lesions, also known as initial or early caries lesions (from the first signs of demineralization through to the presence of a dentine lesion without cavitation). These treatments include the use of sodium fluoride (NaF),[6] casein phosphopeptide-amorphous calcium phosphate (CPP-ACP),[7] ozone,[8] or resin infiltration.[9]

NaF is a widely recognized remineralizing agent that interacts with oral fluids on the enamel surface and subsurface and combines with calcium and phosphate ions to form carbonate substituted hydroxyapatite and fluorapatite. The effectiveness of its topical application as a cariostatic agent has been well established, and professional topical NaF applications are commonly used to arrest the progression of active caries.[2]

Several studies have reported that CPP-ACP promotes remineralization through the release of free calcium and phosphate ions and works effectively against enamel demineralization.[10, 11, 12] Currently, some studies report that application of CPP-ACP prevented decalcified enamel lesions during fixed orthodontic therapy[13, 14] and also could be used for treating dentinal hypersensitivity.[15]

Ozone has been introduced as an alternative strategy to aid in reversal of incipient caries lesions after the promotion of minimal intervention dentistry to treat caries. Ozone can be delivered onto the tooth surface using two methods, either as gas or water.[8] It is a strong oxidizing agent, is highly bactericidal, and has been used in dentistry to treat primary root caries,[16] occlusal caries,[17] and dentinal hypersensitivity.[18] Ozone has also been shown to remineralize dentinal lesions. It has strong oxidizing potential, damages the carious lesion biomolecules, and opens dentinal channels, thus enhancing remineralization by increased.[17] The remineralization effect of ozone on open carious lesion is well established,[19] but the literature on the effect of ozone in reversing initial caries lesions with intact enamel surface is limited, and the mechanism behind it is still ambiguous.

Another noninvasive alternative treatment is based on experiments conducted by Robinson et al.[20] on caries infiltration with resorcinol-formaldehyde resin. This concept has been modified and commercially developed in Germany for the management of non-cavitated caries lesions; the porosities of an enamel lesion are infiltrated with a low viscosity resin, a technique known as resin infiltration.[9, 21] Resin infiltration is a promising therapeutic method since its approach is between preventive and restorative actions in the treatment of non-cavitated carious lesions.[22] Resin infiltration was developed to obstruct the diffusion pathways for acids in order to protect internal enamel. The low-viscous resin can penetrate deep porosities and creates a diffusion barrier on the surface and within the enamel, thus occluding pathways for acid entry into the enamel. Other indications for resin infiltration related to the presence of tissue porosity, namely amelogenesis imperfecta, molar incisor hypomineralization, fluorosis and white spots, have also been suggested.[23]

The efficacy of these therapeutic methods in arresting caries lesions has been investigated in vitro, [24] in situ, [8] and in vivo [25] studies separately. Nevertheless, the comparison of healing effects of all these methods on early enamel lesions needs to be further studied.

With the aim of improving the surface properties of early enamel lesions, the objective of this study was to evaluate the effect of using NaF varnish, ozone, CPP-ACP crème, and resin infiltration on artificial early enamel lesions after a pH cycling model including microhardness and surface roughness properties. The null hypotheses were (1) the treatments tested would not alter the surface properties of the early enamel lesions, (2) no difference would be seen among the treatments in improving artificial enamel lesions, (3) demineralization subsequent to treatments would not influence the surface properties of treated early enamel lesions.

MATERIALS AND METHODS

Specimens Preparation

The Institutional Office of Human Research Ethical Committee of the Medical School of Hacettepe University approved this study (research protocol #FON 12/19-36). All subjects provided informed written consents. A total of 80 extracted human mandibular third molars from the range of 20-30-year-old patients were obtained from the clinic of perfusion of remineralization agents the Oral & Maxillofacial Surgery, School of Dentistry, Hacettepe University, Ankara, Turkey. The teeth were rinsed with tap water immediately after extraction and then stored in 4°C deionized water that contained 0.05% thymol till use. Teeth were checked visually and using LED light to detect any defects or micro-cracks on their enamel surface. Teeth with cracks, defects, or visible stains on the lingual and facial surfaces were excluded. The crowns were sectioned separately from the roots, and the buccal halves of the crowns were used for the study. Each buccal surface was embedded in the self-cured acrylic resin using standard silicone molds. The aprismatic layer of enamel was removed from all specimens using a polishing machine (Buehler Polisher, Buehler, Illinois, USA), and specimens were flat polished with 600-2000 grid silicon-carbide abrasive papers (Fuji Star, Sankyo Rikagaku, Saitama, Japan) under water-cooling. Polished specimens were individually sonicated in distilled water for 10 s to remove the residual abrasives while not disrupting structure and properties.

Formation of Artificial Early Enamel Lesions

A rectangular window (approximately 8 mm x 6 mm) was marked on each specimen using a carbon pencil, and the remaining surface was painted with a color-free acid-resistant nail varnish (Revlon Nail Enamel, Oxford, NC, USA) using a brush under a microscope. The specimens were kept in a demineralization solution ($CaCl_2$: 12 mM, KH_2PO_4: 10 mM, Lactic acid: 50 mM, NaCl: 100 mM) with 4.5 pH at 37°C for 48 h in an incubator to perform artificial enamel lesions.[26] Specimens were rinsed under running tap water for approximately 1 min, dried with air for 5 min and then inspected under a light microscope (SV 6, Zeiss, Germany) at x40 magnification to evaluate the demineralization surfaces.

pH Cycling Model

The pH cycling model was the modified form of that originally described by Featherstone et al.[27] The specimens were submitted to a pH cycling regimen and were kept individually in a demineralization solution (2.0 mM calcium, 2.0 mM phosphate, 0.030 ppm F, in 75 mM acetate buffer, pH: 4.3) for 3 h and in a remineralization solution (1.5 mM calcium, 0.9 mM phosphate, 150 mM of KCl, 0.050 ppm F in 20 mM cacodylic buffer, pH: 7.4) for 20 h each day. After each cycle, the specimens were rinsed with distilled water and kept in artificial saliva (Na_3PO_4-3.90 mM, $NaCl_2$-4.29 mM, KCl-17.98 mM, $CaCl_2$-1.10 mM, $MgCl_2$-0.08 mM H_2SO_4-0.50 mM, $NaHCO_3$-3.27 mM, distilled water, pH: 7.0). This cycle was repeated daily for 10 days. All solutions (demineralization and remineralization) were freshly prepared for each cycle, and separate containers were used for each group throughout the experimental period. The pH of the demineralization and remineralization solutions were measured before every cycle and contained thymol to avoid fungal growth. The experiment was carried out at 37°C.

Treatment Methods

The specimens were randomly divided into five groups: (1) artificial saliva (negative control), (2) NaF varnish (Colgate Duraphat Varnish/Colgate-Palmolive Company, New York, USA) (positive control), (3) ozone (Ozonytron®X/Biozonix, Munich, Germany), (4) CPP-ACP crème (Tooth Mousse/GC Corp., Tokyo, Japan), and (5) resin infiltration (Icon/DMG, Hamburg, Germany). NaF varnish, ozone, and resin infiltration treatments were applied one time only after the formation of artificial lesions, and the treatments were not repeated during the pH cycling regime, whereas CPP-

ACP crème treatment was applied once daily after the last demineralization challenge of each day.

Artificial saliva (negative control) group.

The specimens of the negative control group underwent pH cycles only and was kept in the artificial saliva during the study. The artificial saliva solution was refreshed daily, and the specimens were kept in the incubator at 37°C.

NaF varnish (positive control) group.

NaF varnish (Duraphat, Colgate/Palmolive, New York, NY, USA) containing 5% NaF (22,600 ppm F) was applied with a small, single tufted brush. The varnish was left on the specimens' surface for 4 min after the application.

Ozone.

Ozone was generated and applied with the GI probe of a high frequency ozone generator OzonytronX (Biozonix, München, Germany). The GI probe was centrally positioned on the enamel surfaces of the specimens with 1 mm distance. The activated ozone concentration was adjusted to 100 µg/ml for 60 sec at the speed of ≈ 0.4 ml/sec in level 5.

CPP-ACP crème.

As a topical coating, a water-based, sugar-free, 10% w/w CPP-ACP nanocomplexes with bioavailable calcium and phosphate containing crème (Tooth Mousse/GC Corp., Tokyo, Japan) was freshly squeezed from the tube and placed in direct contact with the specimens using an applicator brush for 3 min.

Resin infiltration.

The lesions were resin infiltrated according to the manufacturer's instructions by the following procedure using Icon (DMG, Hamburg, Germany): (1) the Icon-Etch (15% hydrochloric acid) was applied to the lesion for 2 min; (2) surface was rinsed and dried for 30 sec; (3) surface was dehydrated twice by treating with Icon-Dry (99% ethanol) for 30 seconds and air dried; (4) icon infiltrant resin (triethylene-glycol-dimethacrylate-based resin, camphoroquinone (TEGDMA) was applied two times, the first time for 3 min and the second time for 1 min. Both applications were light cured for 40 sec with a LED curing unit (Radii plus, 1500 mW/cm^2, SDI, Bayswater, Victoria, Australia); (5) specimens were polished with aluminum oxide abrasive papers (4000 grit; FEPA-P, Struers) for 20 sec.

Surface Microhardness (SMH) Measurements

Microhardness was assessed using a Vickers hardness-testing machine (Micro Hardness Tester, HMV 2, Shimadzu, Tokyo, Japan) and expressed as the length of the indentations (µm). The indentations were made under a 200-gf load for 15 s. Five indentations, spaced with 100 µm, were made for each specimen and the mean was calculated. The SMH measurements was determined before the lesion formation (T_0). The same SMH measurement procedure was followed after the creation of the artificial enamel lesions (T_1) and at the end of the 14th day of pH cycling and treatment protocols (T_2).

Surface Roughness (Ra) Measurements

Surface roughness was characterized by the average roughness (Ra), which represents the arithmetical average value of all absolute distances of the roughness profile from the center line within the measuring length. The surface roughness of each specimen was measured using a profilometer (Mahr M1, Göttingen, Germany). Five readings were recorded on each specimen with a cut-off length of 0.25 mm, a tracing length of 0.8 mm, and a stylus speed of 0.1 mm/second. The machine was calibrated after every five samples to ensure reliable readings. The same Ra measurement procedure was applied to access each specimen roughness at before the lesion formation (T_0), after the creation of the artificial enamel lesions (T_1) and at the end of the 14th day of pH cycling and treatment protocols (T_2).

Statistical Analysis

All data were processed by the SPSS PASW statistics 15.0 software (SPSS Inc., Chicago, IL, USA). Since measurements were normally distributed as points in parametric tests each time, a repeated measurements ANOVA test was used for within groups comparison at different time points at a significance level of 0.05. We also used a one-way ANOVA test to compare groups in each time points separately. When the significant difference was obtained, the Bonferroni test was used for pairwise comparisons.

RESULTS

The average SMH values of the specimens in each group measured at the different evaluation periods during the experiment are shown in Table 1. The SMH values of the specimens before lesion formation (T_0) was not significantly different among the groups ($p>0.05$). After

Table 1. Mean surface microhardness (SMH) values and standard deviations of each group at different evaluation periods (n=12).

Treatment Methods	Evaluation Periods			
	T_0	T_1	T_2	p
Artificial saliva	530.5 (2.1) ^{aA}	267.2 (2.4) ^{aB}	389.1 (1.3) ^{aC}	0.022
NaF varnish	523.1 (2.6) ^{aA}	276.4 (3.2) ^{aB}	392.2 (3.1) ^{aC}	0.031
Ozone	525.5 (3.3) ^{aA}	261.7 (2.1) ^{aB}	387.5 (3.4) ^{aC}	0.027
CPP-ACP crème	523.9 (2.9) ^{aA}	287.5 (2.6) ^{aB}	447.4 (2.9) ^{bC}	0.029
Resin infiltration	520.5 (2.8) ^{aA}	295.4 (4.1) ^{aB}	479.5 (2.1) ^{bC}	0.033
p	1.000	1.000	1.000	

[a] Same lowercase letter in same column indicates no significant difference (p>0.05).
[A] Same uppercase letter within individual rows indicates no significant difference (p>0.05).

formation of the artificial enamel lesions, the SMH values of the specimens in all groups decreased (p<0.05), and no significant differences were seen between the groups (T_1) (p>0.05). The SMH values measured at T_2 were higher than T_1 and lower than T_0 in each group (p<0.05). The highest SMH value after treatments was measured in the resin infiltration treatment group (479.5±2.1), which was statistically similar to the CPP-ACP crème treatment group (447.4±2.9) and significantly higher than the artificial saliva (389.1±1.3), ozone (387.5±3.4) and NaF (392.2±3.1). CPP-ACP crème and resin infiltration treatments increased the microhardness of artificial early enamel lesions significantly more than the artificial saliva, ozone, and NaF varnish did (p<0.05).

The average surface roughness values of the specimens in each group measured at the different evaluation periods during the experiment are shown in Table 2. The surface roughness values of the specimens before the lesion formation (T_0) was statistically similar in all groups (p>0.05). After formation of the artificial enamel lesions (T_1), the surface roughness values of the specimens in all groups increased (p<0.05,) and no significant differences were seen among the groups (p>0.05). The surface roughness values of the specimens after treatments (T_2) in each group decreased (p<0.05). The surface roughness values measured at T_2 were lower than T_1 and higher than T_0 in each group (p<0.05). The lowest surface roughness value after treatments was measured in the resin infiltration treatment group (351.5±18.4), which was significantly lower than that of the artificial saliva (377.1±23.9), ozone (374.4±25.6), NaF (372.5±28.0), and CPP-ACP crème (366.8±20.4) (p<0.05). The resin infiltration treatment decreased the surface roughness of artificial early enamel lesions significantly more than the artificial saliva, ozone, NaF varnish, and CPP-ACP crème did (p<0.05).

DISCUSSION

In modern dentistry, it has been widely accepted that no cavity design or restorative material is required to cure a non-cavitated carious lesion. The original anatomy, strength, and esthetics of the tooth might be lost forever in case of restoration. The continuum of replacement dentistry, with repeatedly enlarged restorations and increased damage of hard tissues, has led to the development of multiple prevention strategies that center on prompt treatment of the disease at an early stage and include measures that remineralize, arrest, and/or reverse the caries process after initiation of clinical signs.[28] In the present study, a NaF varnish, high frequency ozone, topical crème containing CPP-ACP, and resin infiltration were evaluated to find their healing effect on artificial enamel lesions in terms of change in surface microhardness and surface roughness. Surface microhardness and roughness measurements were used to analyze their therapeutic effects since they provide a relatively simple, sensitive, nondestructive, and rapid quantitative method in demineralization and remineralization studies.[29, 30]

Microhardness testing is considered to be a relatively simple and reasonably reliable method for the provision of indirect information about the mineral content changes

Table 2. Mean surface roughness (Ra) values and standard deviations of each group at different evaluation periods (n=12).

Treatment Methods	Evaluation Periods			
	T_0	T_1	T_2	p
Artificial saliva	344.5 (15.2) ^{aA}	419.3 (27.2) ^{bB}	377.3 (23.9) ^{aC}	0.044
NaF varnish	346.1 (18.3) ^{aA}	428.5 (23.9) ^{bB}	372.5 (28.0) ^{aC}	0.039
Ozone	359.4 (19.6) ^{aA}	412.3 (22.1) ^{bB}	374.4 (25.6) ^{aC}	0.047
CPP-ACP crème	351.4 (10.8) ^{aA}	429.3 (21.5) ^{bB}	366.8 (20.4) ^{aC}	0.040
Resin infiltration	345.2 (19.0) ^{aA}	417.9 (29.4) ^{bB}	351.5 (18.4) ^{bC}	0.023
p	1.000	1.000	1.000	

[a] Same lowercase letter in same column indicates no significant difference (p>0.05).
[A] Same uppercase letter within individual rows indicates no significant difference (p>0.05).

of hard dental tissues. The Vickers surface microhardness technique has been utilized as an indirect mineral content assessment method in several laboratory models, simulating the effect of application of various commercial products in vitro.[31] Furthermore, surface roughness assessment is an important aspect, since it may not only affect aesthetic properties but also reflects the bacterial adhesion and plaque formation potentials in the oral environment, which helps to elucidate the surface morphological changes induced by different remineralization agents.[32]

In the present study, flat and polished specimens were used in an attempt to standardize specimens and remove natural variations on surface enamel between teeth and between different tooth sites and types which may result in different responses to acid dissolution. Additionally, a microhardness assessment requires flat polished surfaces to enable accurate measurements. Thus, the area subjected to demineralization did not represent the ideal enamel surface since removal of the outer surface layer made the enamel more susceptible to demineralization.

One of the in vitro models used to study the effects of remineralization agents in reducing the progression of caries was a pH cycling model that simulated the conditions in the oral cavity. These pH cycling experiments (including demineralizing periods) could mimic the clinical dynamics more adequately; remineralization-only models offer the opportunity to effectively monitor caries-preventive products on dental hard tissues on a short-time basis, thus simulating a best-case scenario, even if the breadth of relevant biological aspects is limited.[33]

In this study, the initial mean SMH values of the enamel surfaces ranged from 530.05 to 520.5. After being subjected to demineralization solution, the SMH values significantly decreased and surface roughness apparently increased after the therapeutic treatments, as depicted in Tables 1 and 2.

The negative control group showed the smallest values of remineralization based on the limited availability of calcium and phosphorous ions compared to the remineralization effect of other regimens applied in accordance to Cochran et al.[34] In addition, the formula for the artificial saliva used in the present study did not contain a fluoride component, which could explain the limited ability for remineralization by the control group. When demineralized enamel was observed to harden in the presence of saliva, remineralization of non cavitated early enamel lesions was reported.[35] Saliva can act on the acids themselves (via buffering or neutralization), on the bacteria (via inhibition of the metabolic process involved in acid production), and on the enamel (by maintaining chemical supersaturation in the adjacent plaque fluid).[22] In this study, the remineralization potential of saliva was observed as demineralized specimens immersed in artificial saliva showed increased microhardness and decreased surface roughness values. Nevertheless, when compared to the other treatments for early enamel lesion recovery, this improving effect was too limited.

In the presence of fluoride, the remineralizing effect of saliva was shown to be enhanced. The remineralization action of highly concentrated fluorides, such as those found in NaF varnishes, was observed in previous studies that showed the

prevention of the progression of incipient enamel caries.[36, 37] Duraphat NaF varnish was used as the positive control in this study since it is a commonly used fluoride varnish that has been tested in similar studies and is well defined in terms of fluoride release and caries prevention.[38, 39] The NaF application on the early enamel lesions incorporated into enamel crystals, thereby forming a fluoroapatite-like mineral that improved the ability of the enamel to resist further acid challenge. In the present study, the remineralization action of fluoride in the artificial enamel lesions was also observed, but NaF varnish application was considered less effective than the ACP-CPP crème and resin infiltration applications. Torres et al.[29] reported that the frequency of application of NaF varnish was more important than its concentration. In this study, a higher fluoride concentrated NaF varnish (5% NaF - 22,600 ppm F) was applied only once before pH cycling. A mainstay in caries prevention and remineralization is frequent exposure to low levels of fluoride.[40] Higher fluoride concentrations can cause rapid mineral precipitation on the enamel surface and obturation of the surface enamel pores that communicate with the underlying demineralized lesion. This process can further limit remineralization of the subsurface demineralized enamel.[41]

CPP-ACP technology delivers ACP compounds to the tooth structure that readily solubilize to calcium and phosphate ions when coming in contact with saliva, creating a supersaturated state of calcium and phosphate around the tooth.[42] ACP is stabilized by CPP casein-derived peptides. CPP contains the amino acid cluster sequence –Ser(P)-Ser(P)-Ser(P)-Glu-Glu– and has been reported to bind amorphous calcium phosphate, forming small clusters of CPP-ACP. This helps to prevent these clusters from reaching the critical size for precipitation, thereby stabilizing calcium phosphates in solution in close proximity to the tooth, making it available when needed. These nanocomplexes act as calcium and phosphate reservoirs when incorporated into the dental plaque and on the tooth surface.[43] Several studies have reported that CPP-ACP has a reparative effect on a demineralized enamel surface.[12, 44] The results of the current study are in accordance with Hamba et al.[2] and Shen et al.[45] who reported that the CPP-ACP paste had a comparatively modest reparative effect on enamel demineralization in comparison to NaF solutions.

There is conflicting evidence regarding the clinical effectiveness of ozone treatment on early enamel lesions.[46] In an in-situ study by Duggal et al.,[46] the effect of ozone on the inhibition of mineral loss from human enamel and dentine under a cariogenic challenge was investigated (14 days study regime) using microhardness testing. The authors concluded that ozone had no additional effect on the inhibition of dental hard tissue demineralization as compared to the use of high concentrations of fluoride (Reductant and Patient Kit). According to the results of our study, ozone application created a parallel remineralization effect on early enamel lesions with artificial saliva (negative control) group in terms of SMH and surface roughness. In most of the previous studies, ozone has been applied for a duration of 10 to 120 seconds per tooth. In this study, ozone was applied for 60 seconds to each enamel specimen.[46, 47] This was carried out once before the pH cycling period, and this application duration could be the limitation of this study. Tahmasseb et al.[26] applied ozone four times during the pH cycling period. According to their result, the remineralization of the artificial enamel lesions was not affected by the application duration.

The resin infiltration technique is an innovative approach investigated in previous studies, showing good results in hampering the progression of enamel caries.[48, 49] The infiltration technique, in contrast to the application of sealant, in which the diffusion barrier remains on the enamel surface, creates a diffusion barrier inside the enamel lesion and possibly strengthens the demineralized enamel structure with the resin matrix, preventing cavitation.[48] In the present study, the resin infiltration technique exhibited significantly higher microhardness than all other tested groups (Table 1) and showed that the resin infiltration technique results in significantly rising surface properties in the lesion structure. This reflects the ability of the low-viscosity resin to fill the spaces between the remaining crystals of the porous lesion and reharden the demineralized tissue, improving its mechanical strength due to resin composition. This result showed that resin infiltration was a promising technique with which to treat early enamel caries, contrary to Shaik et al.[50] who found that a CPP-ACP product had a better remineralization performance than NaF and resin infiltration products on the demineralized enamel.

Despite the different forms of compounds and application methods incorporated in these products, all therapeutic regimens examined showed higher improvement potential compared to their controls (demineralized) in terms of increase in SMH and reduction in surface roughness values. It was found that the resin infiltration group showed the

highest improving results compared to all other groups, in terms of highest SMH and lowest surface roughness values. This could be attributed to the resin (TEGDMA) content of this product.

Although all tested treatments were capable of enhancing the surface properties of demineralized enamel, it should be emphasized that any strategy to reduce the progression of carious lesions should be based on the control of caries as a biofilm-dependent disease, and caries should be prevented by means of oral hygiene education, including tooth brushing and dietary control.[6]

Finally, it was suggested that CPP-ACP crème and resin infiltration methods could provide various range of therapeutic effect on remineralization of artificially demineralized enamel lesions. So, all the null hypotheses of this study were rejected. According to SMH and Ra results, the application of CPP-ACP, resin infiltration and NaF varnish application on artificially demineralized enamel lesion surfaces could improve the surface properties. Further in vitro and in vivo studies are required to determine the efficacy of these therapeutic treatments on early enamel lesions in clinical conditions.

CONCLUSION

Within the limitation of this in vitro study it was concluded that resin infiltration and CPP-ACP crème are effective materials in treating the early enamel lesions, however resin infiltration seems to be superior than all the other tested treatment methods in terms of SMH and surface roughness.

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