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Comparison of Surface Microhardness Values after the Applicatıon of Different Remıneralizatıon Solutions to Artificial Enamel Caries Lesions

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ABSTRACT

Background: *No published studies exist concerning the comparison of remineralization potential of ozone and fluoride agents for the management of enamel caries lesions.*

Aim: *The aim of this in vitro study was to evaluate and compare the surface microhardness (SMH) values of artificial enamel caries lesions in human teeth after different remineralization solutions application and to evaluate scanning electron microscopy (SEM) images.*

Design: *In this in vitro study, 60 extracted human premolar and molar teeth were placed in a demineralizing solution for 2 days to produce artificial carious lesions. The teeth were sectioned longitudinally and 120 specimens were obtained and then divided into 3 treatment groups (n=40): Group A: Duraphat, Group B: Elmex, Group C: HealOzone & remineralizing solution). Group A, B and C remineralization solutions were applied to specimens for 14 day pH cycle. After and before the Group A, B and C application procedure, the SMH values of the specimens were measured and SEM images were evaluated.*

Results: *The remineralization materials in Groups A, B and C significantly increased the SMH values of demineralized specimens. There was a significant difference in the SMH val-ues of specimens between Group C (HealOzone & remineralizing solution) and Group B (Elmex). Duraphat treated specimens showed a similar SMH values. However, Elmex treated specimens showed lower SMH values.*

Conclusion: *HealOzone together with remineralizing solution, Duraphat and Elmex can be used to reverse initial enamel caries lesions in human teeth.*

Keywords
Ozone, Fluoride, Demineralization, Remineralization, Surface microhardness, Enamel car-ies.

Introduction

Dental caries is one of the most prevalent chronic diseases of teeth [1]. This condition is caused by cariogenic bacteria in dental plaque, fermentable carbohydrates and an imbalance in the process of demineralization and remineralization of tooth surfaces [2]. Modifying equilibrium towards remineralization is possible with primary preventive strategies such as fluoride application, and fissure sealants if the dental enamel surface is not lost [3]. A white spot lesion is the initiation of dental caries which is defined as subsurface enamel porosity. These lesions are the first clinical sign of enamel caries and can potentially be remineralized or arrested [2,4,5].

Fluoride is the most commonly used compound to promote remineralization by forming fluorohydroxyapatite crystals [6,7]. A number of dental researchers have demonstrated that fluoride prevents and arrests dental caries lesions [8]. Non-invasive intervention with, fluoride agents, such as toothpaste, gel, foam, mouth rinse, solution, varnish and tablets, has been used for the prevention of dental caries over the past 25 years [9]. Different fluoride formulations such as sodium fluoride (NaF), sodium monofluorophosphate (Na_2PO_3F), acidulated fluorophosphate (APF), stannous fluoride (SnF_2), titanium tetrafluoride (TiF_4), amine fluoride (AmF), and silver diamine fluoride (SDF) are used as topical fluoride products in dentistry [10-12].

Ozone is a powerful oxidant and highly potent antimicrobial agent. Ozone has proved to be effective against gram-negative and gram-positive bacteria, viruses and fungi [13,14]. Ozone has been used in various fields in dentistry such as dentinal hypersensitivity, and periimplantitis; increasing the effectiveness of tooth whitening; sterilizing cavities, root canals, periodontal pockets and herpetic lesions; and promoting the healing of mucosal lesions [15,16]. In recent years, ozone has been used for treating initial enamel caries lesions as an alternative management strategy within non-invasive interventions [3]. Ozone can be delivered onto the tooth surface using two methods, either as gas or water [1]. Ozone-generating device HealOzone (Kavo, Biberach, Germany) allows the application of a high concentration of gaseous ozone (2100 ± 200 ppm) at a flow rate of 615ccs/min) to the affected area. The HealOzone application period varies from 10 seconds for the lowest CSI score to 40 seconds with the highest CSI score. HealOzone remineralizing solution (pH balancer, Cure Ozone®, USA) containing xylitol, fluoride, calcium, phosphate and zinc is applied directly to the demineralized surface. In dentistry, different pH-cycling regimen have been used to evaluate the fluoride effect in remineralization and demineralization process of dental enamel.

The aim of this *in vitro* study was to compare Group A: Duraphat (2800 ppm Sodium fluoride) (Colgate-Palmolive (UK) ltd., Guilford, Surrey, UK GC Corporation, Itabashi-Ku, Tokyo, Japan), Group B: Elmex (1250 ppm Amine fluoride) (GABA International Ag, Münchenstein, Switzerland) and Group C: HealOzone & remineralizing solution (2100 ppm HealOzone & remineralizing solution) (Kavo, Biberach, Germany pH balancer, CureOzone®, USA) (Table 1) application in the remineralization of initial enamel caries. Therefore, we compared the microhardness values of artificial enamel caries lesions in human premolar and molar teeth after sodium fluoride, amine fluoride and HealOzone+remineralizing solution application and evaluated the scanning electron Microscopy (SEM) images.

Group A: Duraphat	Colgate-Palmolive (UK) ltd., Guilford, Surrey, UK GC Corporation, Itabashi-Ku, Tokyo, Japan	2800 ppm Sodium fluoride
Group B: Elmex	GABA International Ag, Münchenstein, Switzerland	1250 ppm Amine fluoride
Group C: HealOzone & remineralizing solution	Kavo, Biberach, Germany pH balancer, CureOzone®, USA	2100 ppm Ozone & remineralizing solution

Table 1: Remineralization solutions used in the study.

Materials and Methods

Sixty human premolar and molar teeth extracted for orthodontic reasons were selected for this study. Teeth were thoroughly cleansed of periodontal tissues with hand scalers and re-inspected for intact surfaces that were free from caries, hypoplasia and white spot lesions. Then, teeth were stored in saline solution, including 0.1% thymol at 4°C. Sixty extracted teeth crowns were cut from the roots. The crown samples were longitudinally sectioned into two halves in the buccal-palatal direction with a water-cooled, double-faced diamond disk. A total of 120 halves of the crown specimens were mounted horizontally in self-cured acrylic resin (Probase ColdTM, Ivoclar Vivadent AG, Liechtenstein) in individual plastic moulds [17-19]. The enamel surface of the blocks was ground flat and polished with water-cooled carborundum discs (Microcut TM, Buehler, Lake Bluff, IL, USA), and during the process, 320, 600 and 1200 grades of abrasive paper discs were employed, respectively [19].

The enamel surfaces of 120 specimens were coated with a nail varnish (Revlon, New York, USA), leaving a 3×3 mm window [20]. A 20 mL demineralization solution was prepared and contained an aqueous solution of 0.075 mol/L glacial acetic acid, 0.002 mol/L Ca ($CaCl_2$), and 0.002 mol/L P (KH_2PO_4) (pH =4.3). Each specimen was kept in the demineralization solution for 2 days at 37°C in an incubator. After demineralization, the specimens were rinsed with deionized, distilled water and dried [21].

After demineralization, specimens were analysed for surface microhardness (SMH) values with a Micromet 5114 (Buehler Lake Bluff, IL, USA) using a Vickers diamond under a 200-g load for 5 seconds. Hardness numbers using the Vickers' hardness scale (VHNinitial) were obtained three times from each specimen, and then averaged [22]. Specimens were divided into three groups of 40 teeth according to the change in their mean SMH values.

The first group of specimens was treated with Duraphat, while the other two groups were treated with Elmex and HealOzone+remineralizing solution once every 24 hours for 14 days [23]. Duraphat and Elmex were continuously applied onto the specimen surfaces within the window area with the help of a disposable cotton tip applicator. HealOzone was applied onto the specimen surfaces within the window area for 40 seconds. Then, remineralizing solution (pH balancer, CureOzone®, USA) was applied [24,25].

After each remineralization solution treatment, specimens were rinsed with deionised, distilled water and was dried. During 14 day pH cycle, specimens were exposed to fresh demineralization and remineralization solution of the same composition. At the end of the 14 day pH cycle, SMH analyses were carried out to assess the ability of each remineralization solution to cause changes in the surface characteristics of the enamel.

Scanning electron microscopy evaluation

Following the demineralization and remineralization treatments, SEM (JEOL JSM-6335F Field EMİSSİON) was used to analyse the surface morphology and enamel thickness of the 40 specimens at the baseline level. The procedures were carried out under extended pressure (100 Pa air pressure) with LaB6 filaments. Enamel thickness was measured at three points from the dentinoenamel junction to the enamel surface by drawing tangents and an average was taken from the three readings. Three different magnification images (x750, x1500, x3500) of the specimens were examined.

Statistical analysis

The data were analysed using Number Cruncher Statistical Systems (NCSS) 2007 Statistical Software (Utah, USA). One-way ANOVA was performed to examine the effects of different remineralization solutions on SMH values. Post hoc multiple comparisons were carried out via Tukey's test. The statistical significance was set at $p<0.05$.

Results

We compared Group A: Duraphat (2800 ppm Sodium fluoride) (Colgate-Palmolive (UK) ltd., Guilford, Surrey, UK GC Corporation, Itabashi-Ku, Tokyo, Japan), Group B: Elmex (1250 ppm Amine fluoride) (GABA International Ag, Münchenstein, Switzerland) and Group C: HealOzone & remineralizing solution (2100 ppm HealOzone & remineralizing solution) (Kavo, Biberach, Germany pH balancer, CureOzone®, USA) remineralization solutions are shown in Table 1 in this study to treat initial enamel caries.

The SMH values that were measured after demineralization (SH_1) and after remineralization solutions and pH cycle (SH_2) are shown in Table 2. There was no significant difference in the microhardness values of the specimens among the groups after demineralization solution application (p=0.077). The SMH values of the specimens after remineralization solutions and the pH cycle (SH_2) were significantly higher than those of the specimens after demineraliza-tion (SH_1) (p=0.0001) in Groups A, B and C (Table 2).

	Group A (Duraphat)	Group B (Elmex)	Group C (Heal Ozone & remineral-izing solution)	F	p
SH_1	317,84 ± 15,03	313 ± 9,85	318,44 ± 10,58	2,62	0,077
SH_2	330,96 ± 12,39	328,38 ± 10,1	334,7 ± 10,89	3,37	*0,037
t	-12,05	-11,05	-14,06		
p	**0,0001	**0,0001	**0,0001		

Table 2: Surface microhardness measurement.

*p<0,05, **p<0,005. Results are given as mean ± SD/(median, CI). Significance was determined using ANOVA test (P<0.05). SH_1: surface microhardness value (after demineralization), SH_2: surface microhardness value (after remineralization solution and pH cycling).

Post hoc multiple comparisons of microhardness values among Group A (Duraphat), Group B (Elmex) and Group C (HealOzone & remineralizing solution) were carried out via Tukey's test. There was no significant difference in the microhardness values of the specimens be-tween Group A (Duraphat) and Group B (Elmex) (p>0.05). Moreover, there was no signifi-cant difference in the micro hardness values of the specimens between Group A (Duraphat) and Group C (HealOzone & remineralizing solution). However, there were significantly higher microhardness values in Group C (HealOzone & remineralizing solution) than in Group B (Elmex) (Table 3) (Figure 1).

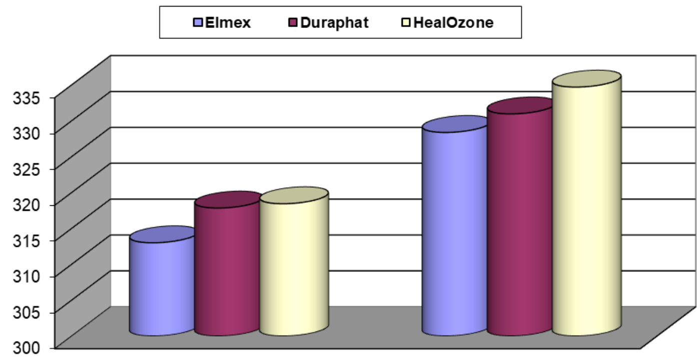

Figure 1: Microhardness values after demineralization and remineralization solutions.

Group A/Group B	(Duraphat/Elmex)	0,526
Group B/Group C	(Elmex/HealOzone & remineralizing solution)	*0,029
Group A/Group C	Duraphat/HealOzone & remineralizing solution)	0,280

Table 3: Tukey test (post hoc multiple comparisons of microhardness values among Group A, Group B and Group C) (*p<0,05).

Scanning electron microscopy results

Deterioration of surface integrity and a porous appearence was noticed after demineralization. Loss of aprismatic enamel, destruction of enamel rods, loss of prism cores and widening of inter-rod spaces were observed in demineralized enamel surfaces (Figure 2). All specimens treated with the three remineralization solutions revealed a layer of surface deposition of minerals. Specimens treated with Duraphat showed plugging of the porous defects with a decrease in cavities and micropores. Duraphat left some remnants on the surface that were not completely removed by brief washing (Figure 3).

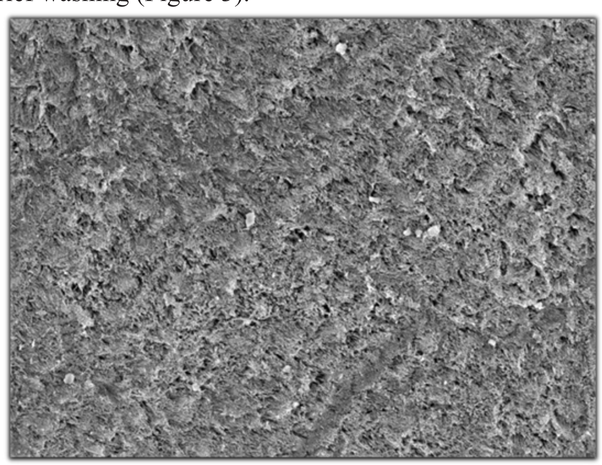

Figure 2: Postdemineralization (scanning electron microscopy image).

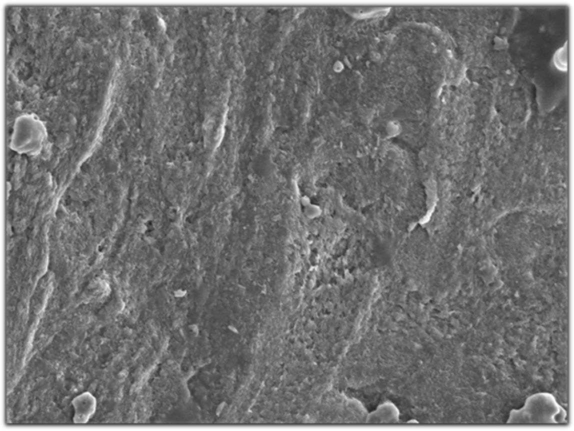

Figure 3: Group A- Postremineralization (scanning electron microscopy image).

The specimens of the Elmex solution group showed more homogeneous surfaces than did those of the other groups. Specimens treated with Elmex showed plugging of the porous defects with a decrease in cavities and micropores (Figure 4). However, compared to specimens in the other groups, specimens in the HealOzone & remineralizing solution group showed unfilled porous defects and cavities (Figure 5).

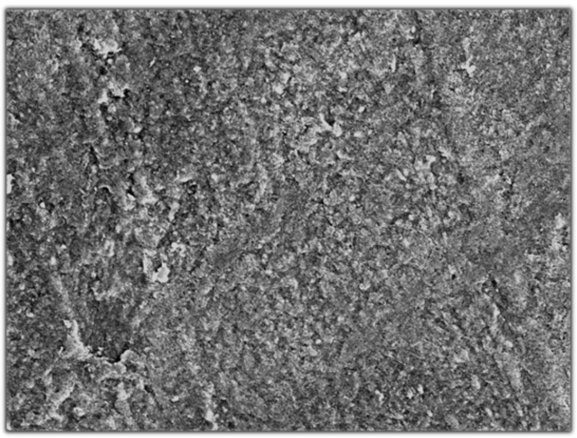

Figure 4: Group B- Postremineralization (scanning electron microscopy image).

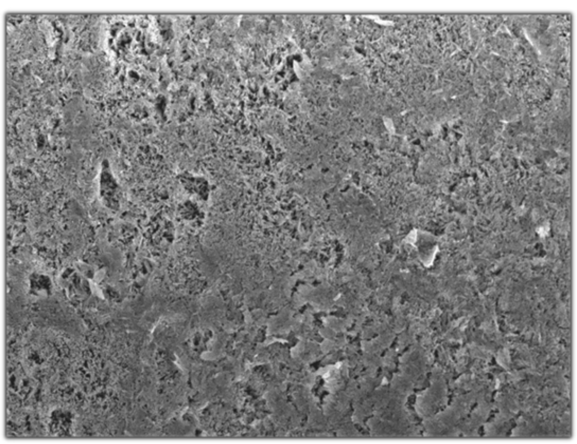

Figure 5: Group C- Postremineralization (scanning electron microscopy image).

Discussion

Preventing dental caries in its earliest stages is the current consensus in caries management [25]. Dental caries is caused by a dynamic balance between demineralization and the remineralization process. Over the last 25 years in dentistry, a minimally invasive intervention has been used that involves applying therapeutic agents for the remineralization of non-cavitated (initial) enamel caries [26]. Various therapeutic agents, such as hydroxyapatite, fluoride, casein phospho peptide amorphous calcium phosphate (CPP-ACP) and bioactive glass, have been tested and proven effective in the remineralization of initial caries [27,28].

Fluoride is one of the most effective agents in caries prophylaxis [29]. Mohd Said evaluated and compared the remineralization potential of Duraphat, MI Varnish, Embrace Varnish, Enamel Pro Varnish and Clinpro White Varnish on artificial enamel caries lesions using microhardness testing [22]. These investigators reported that compared with the use of Reductant and Patient kits, ozone has no additional effect on the inhibition of dental hard tissue demineralization. During the last decade, ozone application was recommended as a new caries preventive method [30]. Baysan and Lynch reported that ozone treatment could dramatically reduce the total number of microorganisms and reverse most root caries lesions [31]. Abu Salem stated that the use of ozone in primary fissure caries promotes caries reversal and tooth remineralization [32]. In this study, we noticed the effect of ozone and fluoride application in the remineralization of initial enamel caries using microhardness testing. We compared the remineralization potential of sodium fluoride, amine fluoride and HealOzone & remineralizing solution in initial enamel caries. As a result, specimens treated with HealOzone & remineralizing solution showed maximum remineralization values, and specimens treated with Duraphat showed similar remineralization values. However, Elmex treated specimens showed lower remineralization values.

In this study, we selected the *in vitro* model to minimize several individual factors, such as diet, saliva composition, salivary flow and buffering capacity and brushing frequency. *In vitro* enamel demineralization models have been used in many studies. Different demineralization solutions, such as acetic acid, and lactic acid, have been used in enamel demineralization model studies. In the present study, the demineralization solution was prepared as described by Yang [21]. Each specimen was kept in the demineralization solution for 2 days at 37°C.

Ozone has been applied for the duration of 10 to 120 seconds per tooth in many studies [33]. Baysan reported that ozone application to primary root caries lesions for 10 seconds produced a significant reduction in the number of *S. mutans* and *S. sobrinus* [34]. Holmes showed that regular ozone application for 40 seconds and the use of remineralizing products reversed non cavitated primary root caries [30]. Baysan and Lynch demonstrated that ozone application to primary root caries lesions for 40 seconds is sufficient to kill different concentrations of *S. Mutans*, and an application of 60 seconds eliminates *S. mutans*, *L. casei* and *A. naeslundii* [31]. Baysan and Beighton reported that ozone application to non-cavitated carious lesions does not significantly reduce the number of viable bacteria in the underlying infected dentin [35]. Atabek and Huth demonstrated that ozone application alone or combined with remineralizing solution was effective for remineralization of initial fissure caries lesions [36,37]. Tahmassebi stated that the application of ozone alone has a minimal effect on initial enamel caries, but this effect was enhanced when ozone was applied combined with fluoride [18]. In this study, ozone and remineralizing solution were applied consecutively for 40 seconds each. The evaluation of microhardness values and SEM images showed the effectiveness of the process.

In this study, 14 day pH cycle period was used to provide sufficient time to evaluate the remineralization process. Buzalaf used the same pH cycle period in his study [23].

The SMH test is a simple, quick and easy to measure non destructive method to assess the remineralization of dental enamel [38]. In tooth hardness studies, the Vickers indenter is more useful than the Knoop indenter because a square shape produced by the indentation on a nonflat surface is easily detected [39]. In this study, the Vickers SMH test was used to evaluate enamel specimens before and after remineralization solution application.

The SEM method is one of the most sensitive, time-tested techniques to evaluate the demineralization and remineralization process of carious lesions *in vitro*. In most studies, SEM specimens are coated with metals such as gold or palladium to improve image quality [40]. In this study, specimens were coated with gold for SEM imaging. Specimens were observed at x750, x1000 and x1500 magnifications to evaluate different sizes of images. Nevertheless, There are a limited number of studies in the literature regarding the effects of ozone on enamel and its potential to inhibit demineralization and enhance remineralization *in vitro*. Further studies are required to identify the mechanism of action of ozone on demineralized enamel.

Conclusıon

Summarizing the above findings within the limitations of the present study, we concluded that Group A (Duraphat), Group B (Elmex) and Group C (HealOzone & remineralizing solu-tion) showed significant enhancement of the SMH values of the specimens. All the tested agents have a good effect on the remineralization of initial enamel caries. The results of this study confirmed the effectiveness of HealOzone & remineralizing solution on specimens that demonstrated maximum remineralization values. Compared with HealOzone & remineralizing solution treated specimens, Duraphat treated specimens showed a similar enamel remineralization effect. However, Elmex treated specimens showed lower remineralization values. HealOzone application in combination with remineralizing solution can be used to reverse non-cavitated initial enamel carious lesions. Hence, the remineraliza-tion materials used in this study had a significant remineralization effect when applied to artificial dental caries lesions of human teeth.

This study is important to paediatric dentists, since:

- There are limited studies about the effects of ozone therapy in remineralizing the enamel caries *in vitro*. No published studies exist concerning the comparison of remineralization potential of ozone and fluoride agents for the management of enamel caries lesions. Therefore this study is both unique and pioneer among the studies published in this field.
- Ozone application is extremely time efficient for clinician and patient alike, and the patient's visit is completely painless and non traumatic which make it particularly of interest for use in paediatric dentistry.
- It should also taken into consideration that this paper calls attention to the high therapeutic potential of ozone treatment protocols as an alternative to fluoride application in dental schools or clinical training. Further studies are required before ozone can be accepted as an alternative to fluoride for the remineralization of enamel caries.

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