

PAPER DETAILS

TITLE: The effects of cavity disinfection on the nanoleakage of compomer restorations: an in vitro study

AUTHORS: Ipek ARSLAN,Ozgul BAYGIN,Tamer TUZUNER,Fatih ERDEMIR,Aykut CANAKCI,Fatih Mehmet KORKMAZ

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The effects of cavity disinfection on the nanoleakage of compomer restorations: an in vitro study*

Purpose

Cavity disinfection, in addition to routine caries removal methods, is recommended to eliminate the microorganisms. The aim of this study was to compare the effect of various systems Er,Cr:YSGG lasers, diode lasers and FotoSan and agents Corsodyl; Cervitec and Cervitec Plus and Fluor Protector—on the nanoleakage of compomer restorations when used for cavity disinfection.

Materials and Methods

A total of 40 intact human deciduous molar teeth containing BlackV cavities (3×2×1.5 mm) on the buccal and lingual surfaces parallel to the cemento-enamel junction were randomly divided into 8 groups according to the cavity disinfection methods. The antibacterial agents and systems were applied according to the manufacturer's instructions. Restorations were completed using a compomer. The restored teeth were then subjected to thermocycling for 500 cycles in a water bath at 5°C and 55°C with a dwell time of 30 seconds. After the thermocycling procedures, 1-mm sticks were obtained from the center of each cavity to prepare for the nanoleakage test. After the teeth were sectioned, they were immersed in 50 wt % ammoniacal silver nitrate solution for 24 hours and dipped in photo-developing solutions for 8 hours with fluorescent light irradiation. The samples were examined under a scanning electron microscope (SEM). The non-parametric Kruskal-Wallis and Mann-Whitney U test ($p < 0.05$) were applied.

Results

The Er,Cr:YSGG laser group showed significantly less nanoleakage than all of the tested groups ($p < 0.01$). The diode laser, Fluor protector and FotoSan groups showed similar nanoleakage to that of the control group ($p > 0.05$). The Corsodyl ($p < 0.01$) and Cervitec ($p < 0.001$) groups showed significantly higher nanoleakage than the control group.

Conclusion

Er,Cr:YSGG laser irradiation which showed lower nanoleakage scores from either control or tested groups can be recommended for cavity disinfection. Additionally, a diode laser and FotoSan, which have antibacterial effects and no negative effect on leakage, can be used for cavity disinfection.

Keywords: Er,Cr:YSGG laser; Diode laser; FotoSan; Antibacterial agents; Nanoleakage

Introduction

Traditional restorative dentistry aims to remove all infected tooth structures and obturate the area with biocompatible filling materials (1). However, none of the currently used caries removal methods eliminate all of the microorganisms in the cavities consistently (2). Several studies have shown the existence of bacteria in dentin even after using caries detector dyes (3,4). Researchers have proved that fermentative microorganisms remained viable under non-antiseptic restorations for as long as 139 days (5).

Ipek Arslan¹ ,
Ozgul Baygin² ,
Tamer Tuzuner² ,
Fatih Erdemir³ ,
Aykut Canakci³ ,
Fatih Mehmet Korkmaz⁴ 

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ORCID IDs of the authors: I.A. 0000-0002-8648-3554;
O.B. 0000-0003-2220-7654; T.T. 0000-0001-5817-5928;
F.E. 0000 0002 0449 9801; A.C. 0000 0001 5244 6467;
F.M.K. 0000-0001-9324-2014

¹Recep Tayyip Erdoğan University, Faculty of Dentistry,
Department of Pediatric Dentistry, Rize, Turkey

²Karadeniz Technical University, Faculty of Dentistry,
Department of Pediatric Dentistry, Trabzon, Turkey

³Karadeniz Technical University, Faculty of Engineering,
Department of Metallurgical and Materials Engineering,
Trabzon, Turkey

⁴Karadeniz Technical University, Faculty of Dentistry,
Department of Prosthodontic, Trabzon, Turkey

Corresponding Author: Ipek Arslan

E-mail: sareipekarslan@gmail.com

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Thus, cavity disinfection, in addition to routine caries removal methods, is recommended to eliminate the microorganisms and reduce potential secondary caries, pulp sensitivity and pulp inflammation before restoring the cavities (6, 7).

The treatments of carious lesions has been changing as the knowledge about the caries process has increased (8). Some authors have advised that new approaches should remove only infected dentin and provide an opportunity for the affected dentin to undergo remineralization (8,9). The main problem with this approach is the remaining cariogenic bacteria. Thus, cavity disinfection has gained importance with new approaches (8,10).

In the literature regarding cavity disinfection, various systems and agents are suggested. Understanding the disinfection mechanisms and their effects on the sealing ability of restorative material is essential in the selection of disinfection methods (11).

Chlorhexidine (CHX) is a commonly used cavity disinfection agent in dental procedures. Its disinfection effect occurs upon its binding to the amino acids in microorganisms, and its effects can continue for several hours. Due to these properties, CHX is an excellent antibacterial agent (12,13). However, in the literature, there is disagreement concerning the effects of chlorhexidine on the sealing ability of resin restorative materials. Some researchers have argued that chlorhexidine has no adverse effects on bond strength and leakage (14,15). Conversely, some studies have reported that chlorhexidine increases leakage scores (16,17).

Currently, combinations of chlorhexidine with other antibacterial agents—e.g., fluoride or thymol—are commercially available. In the present study, Corsodyl gel containing 1% CHX digluconate, Cervitec gel (combination of 0.2% CHX digluconate and sodium fluoride gel) and Cervitec Plus Varnish (combination of 1% CHX diacetate and 1% Thymol) were used. Many studies have reported that these combinations showed less antibacterial activity than CHX (18,19). Wallman *et al.* (19) reported that CHX gel was more effective in reducing MS in saliva compared with Cervitec. The other study showed that CHX-containing dentifrice was more effective in reducing MS in saliva compared with Cervitec (19).

Fluoride is the most popular anticaries agent in dentistry. Its antibacterial activity has been demonstrated many times (20–22). Fluoride is not designed specifically for cavity disinfection, but some properties of fluoride such as its ability to inhibit active growth of cariogenic bacteria, remineralize the affected dentin, and increase the microhardness of dentin (8, 23) were thought to make it usable as a cavity disinfectant. Some recent studies have tested it for cavity disinfection (8, 24).

Photoactivated disinfection (PAD), also known as antimicrobial photodynamic therapy, is a disinfection method that can be used in both restorative and endodontic treatment to eliminate microorganisms. Its disinfection principle is based on a photosensitizer, which is irradiated by a specific wavelength of light (25). After irradiation, singlet oxygen is produced that causes bacterial cell wall rupture and faster antibacterial effects (26). Previous studies have demonstrated the reduction by 95–99.9% of the viable cell count with PAD (27,28).

Laser therapy is a disinfection system that is effective against oral bacteria, associated or not with a photosensi-

tiser. The antibacterial action of a laser is related to its thermal effects and photodisruption (29). Despite its well-known antibacterial action, studies concerning the use of lasers for cavity disinfection are limited. One previous study proved its effectiveness against caries-related bacteria (30).

The ideal cavity disinfectant should provide both strong antimicrobial action and not interfere with the sealing ability of restorative materials (31). When the sealing ability is disrupted, marginal leakage may occur. The occurrence of leakage between restorative material and teeth may decrease the longevity of restoration (32). Nanoleakage is described as the diffusion of nanoscale ions or molecules in the hybrid layer of the restoration (33). Silver nitrate (AgNO₃), which is detectable by both SEM and TEM, is used to evaluate nanoleakage (34).

The aim of this study was to compare the effect of various systems—Er,Cr:YSGG lasers, diode lasers and FotoSan, which is a PAD system—and agents—Corsodyl; Cervitec and Cervitec Plus, which contains CHX in their combination; and Fluor Protector—on the nanoleakage of compomer restorations when used for cavity disinfection. The null hypotheses tested were as follows: 1) The systems and agents that were used in the study would have no effect on nanoleakage; 2) nanoleakage would not differ between the systems or agents.

Materials and Methods

Specimen preparation

Ethical approval of the present study was obtained from the Ethics Committee of Karadeniz Technical University, Faculty of Medicine (Protocol # 2015/149). A total of 40 intact human deciduous molar teeth extracted for exfoliation or orthodontic reasons were collected and cleaned with pumice. The teeth were stored in 0.5% Chloramine T aqueous solution following the extraction. Standardized class V cavities (3×2×1.5 mm) were prepared on the facial and lingual/palatinal surfaces of each tooth with a diamond bur (Diatech Swiss Dental Instruments, Switzerland; 881-012-8 ml), parallel to the cemento-enamel junction. Next, the teeth were randomly divided into eight experimental groups of 10 teeth, each according to cavity disinfection method. The antibacterial agents and systems were applied according to the manufacturer's instructions (Table 1).

Restoration

After disinfection, all samples were restored with a compomer (Dyract eXtra, Dentsply, Germany) according to the manufacturer's instructions. Prime&Bond NT (Dentsply, Germany) were used as bonding agent. Finishing was achieved by using flexible polishing discs. The restored teeth were then subjected to thermocycling for 500 cycles in a water bath at 5°C and 55°C with a dwell time of 30 seconds. After the thermocycling procedures, 1-mm sticks were obtained from the center of each cavity to prepare for the nanoleakage test.

Preparation for nanoleakage test

Two layers of nail varnishes were applied to sticks up to 1 mm from the restoration margins. The specimens were then

Table 1. Agents and systems used for cavity disinfection and application forms

Agents-Systems	Application Forms
Group 1: Control	<ul style="list-style-type: none"> No disinfection process applied.
Group 2: Corsodyl (GlaxoSmithKline USA)	<ul style="list-style-type: none"> 1% CHX Digluconate gel was applied to the dentin for 1 minute Excess gel was removed from the cavity with a clean cotton pellet
Group 3: Cervitec Gel (Ivoclar, Schaan, Liechtenstein Germany)	<ul style="list-style-type: none"> A combination of 0.2% CHX Digluconate and Sodium Fluoride gel was applied to the dentin for 2 minutes.
Group 4: Cervitec Plus (Ivoclar, Schaan, Liechtenstein)	<ul style="list-style-type: none"> A combination of 1% CHX Diacetate and 1% thymol varnish (Ivoclar Vivadent) was applied to the dentin for 2 minutes.
Group 5: Fluor Protector (Ivoclar, Schaan, Liechtenstein)	<ul style="list-style-type: none"> A 1% difluorosilane varnish was applied to dentin for 1 minute.
Group 6: PAD (FotoSan, CMS Dental, Denmark)	<ul style="list-style-type: none"> A fotosensitizer containing 0.01% toluidine blue was applied to the dentin. The teeth were irradiated with red light (660 nm wavelength and 100 mW)
Group 7: Diode laser (Biolase, San Clemente, CA)	<ul style="list-style-type: none"> The dentin surfaces were irradiated with a diode laser with a wavelengths of 940 nm, 1- W power output, and 20-Hz frequency. A sapphire tip, 600 µm in diameter and 6 mm in length was used to deliver the laser light.
Group 8: Er:Cr:YSGG laser (Waterlase MD; Biolase, San Clemente, CA)	<ul style="list-style-type: none"> The dentin surfaces were irradiated with an Er,Cr:YSGG laser with a wavelength of 2780 nm, 1-W power output, and 20-Hz frequency. A sapphire tip, 600 µm in diameter and 6 mm in length, was used to deliver

immersed in 50 wt% AgNO₃ solution in the dark chamber according to Tay *et al.* (35) for 24 hours and then were rinsed with running water for 5 minutes, dipped in photodeveloping solutions for 8 hours with fluorescent light irradiation to reduce the silver or diamine silver ions to metallic silver (36) and again washed with running water for 5 minutes.

SEM/EDX and Elemental Mapping analyses

The sticks were embedded into acrylic resin prior to polishing. The specimens were polished with descending grits of silicone carbide papers (600, 1200 and 2500) and diamond polishing paste then conditioned with 5% phosphoric acid for 5 sec and immersed in ethanol solution (70%) for 10 sec. They were coated with a thin layer of gold (sputtering) and analyzed using SEM in the backscattered mode. Quantitative analyses of AgNO₃ uptake into the hybrid layer were performed as a percentage with EDX analyses.

Elemental mapping of the samples was performed using SEM-EPMA. The elements in the samples were marked with different colors.

Statistical analysis

Statistical analyses were performed with SPSS 15.0 for Windows (SPSS Inc., Chicago, Ill, USA). Shapiro-Wilks test was used to evaluate the distribution of the data. The Non-parametric Kruskal-Wallis and Mann-Whitney U tests ($p < 0.05$) were applied. The group that caused the difference was identified with the Mann Whitney U test.

Results

AgNO₃ accumulations in all samples were seen in SEM images, EDX and elemental mapping analyses. In some SEM images, cracks were visible in the materials but this was not

Table 2. The effect of the agents and systems used in the study on the nanoleakage values of compomer restorations

	Ag (%) Mean Value	Standard Deviation	Ag (%) Max Value	Ag (%) Min Value	Comparison with control
Group 1	42.7 ^{b,c}	5.75	53	35	-----
Group 2	73.4 ^{a,c}	11.06	80	45	0,000
Group 3	58.1 ^{a,b,c,d}	10.35	67	42	0,001
Group 4	49.5 ^{b,c}	14.21	67	29	0,126
Group 5	48.0 ^{b,c}	15.01	67	22	0,286
Group 6	37.9 ^{b,c}	12.14	54	21	0,692
Group 7	43.0 ^{b,c}	4.05	53	39	0,378
Group 8	20.5 ^{a,b}	5.19	26	15	0,000

Kruskal Wallis and Mann-Whitney U multiple comparison test were used with the significance level of 0.05.

^aGroups that are statistically different from the Group 1 ($p < 0.01$), ^bGroups that are statistically different from the Group 2 ($p < 0.01$),

^cGroups that are statistically different from the Group 8 ($p < 0.01$), ^dGroups that are statistically different from the Group 6 ($p = 0.001$)

taken into consideration as an important result because the samples were subjected to vacuum conditions. SEM images and elemental mapping showed that AgNO₃ uptake were generally noted at the base of the hybrid layer.

The resin-dentin interfaces in the eight groups were analysed with SEM-EDX; AgNO₃ deposition was observed. Eight groups were analysed using line scanning: Si, C, and

Ca element peaks were detected. The means, minimum and maximum uptake values (%) of AgNO₃, standard deviation and p values compared with the control group are shown in Table 2. The Corsodyl ($p < 0.01$) and Cervitec ($p < 0.001$) groups showed significantly higher nanoleakage than the control group. The Er,Cr:YSGG laser group showed significantly less nanoleakage than the control group ($p < 0.001$).

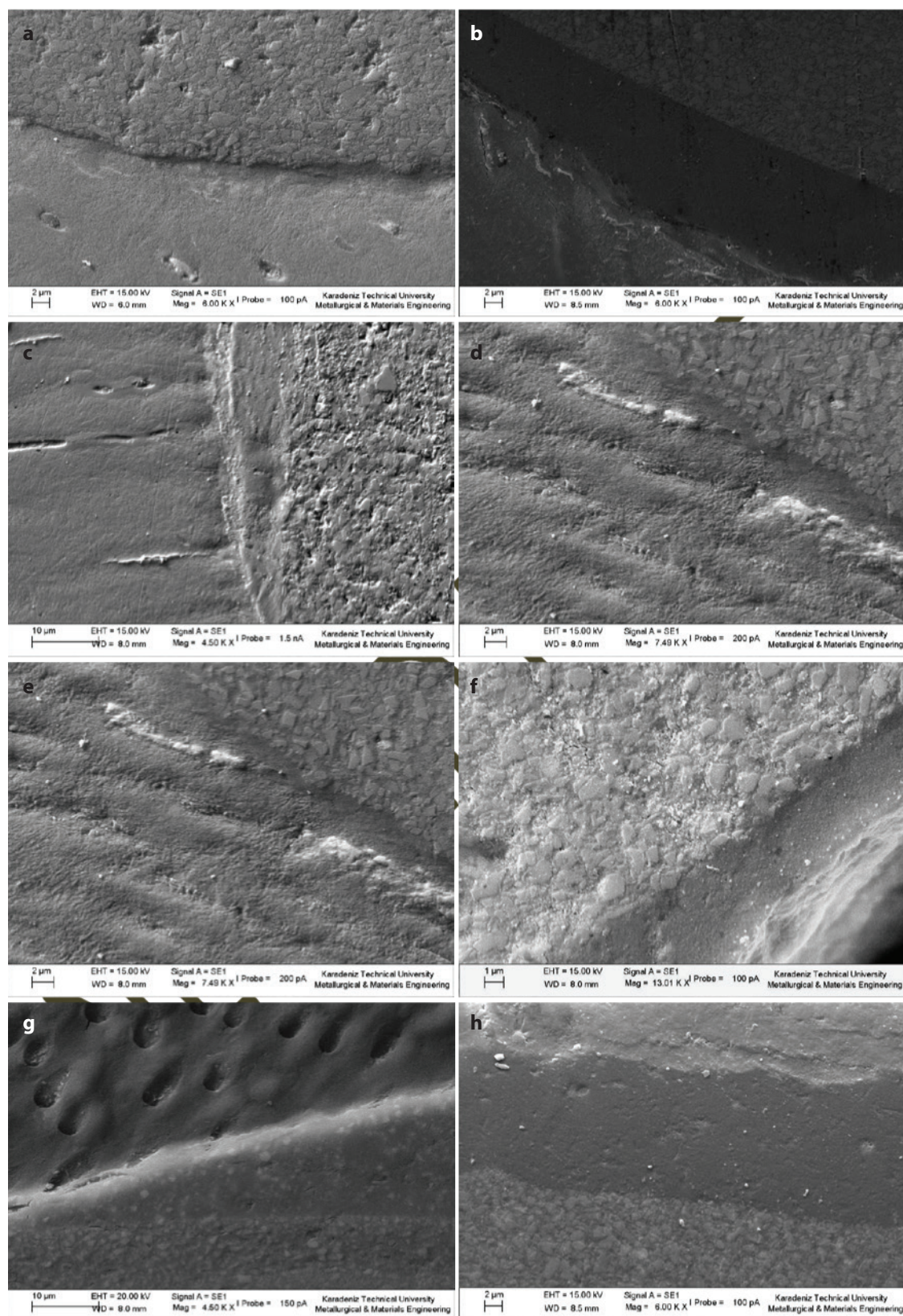


Figure 1. Representative backscattered SEM images of groups. a: Control group, b: Corsodyl group, c: Cervitec group, d: Cervitec Plus group, e: Fluor Protector Group, f: FotoSan group, g: Diode Laser group, h: Er, Cr:YSGG Laser group.

The Cervitec Plus, Fluor Protector, FotoSan and diode laser groups showed similar nanoleakage results to the control group ($p>0,1$). The Corsodyl group showed higher nanoleakage than all the tested groups ($p<0.01$). The Er,Cr:YSGG laser group showed significantly less nanoleakage than all the tested groups ($p<0.01$). The SEM images, as well as the findings on elemental analysis and SEM-EDX analysis of the groups, are given in Figures 1-3.

Discussion

The presence of bacteria in the smear layer of the restored tooth is the major cause of secondary caries and failure of restoration (37,38). None of the currently used caries removal methods eliminate all the microorganisms in the cavities (2). Thus, cavity disinfection procedures are recommended to eliminate these residual bacteria (6,7). One of the main problems with cavity disinfection is increased leakage between dentin and resin restorative material by interfering

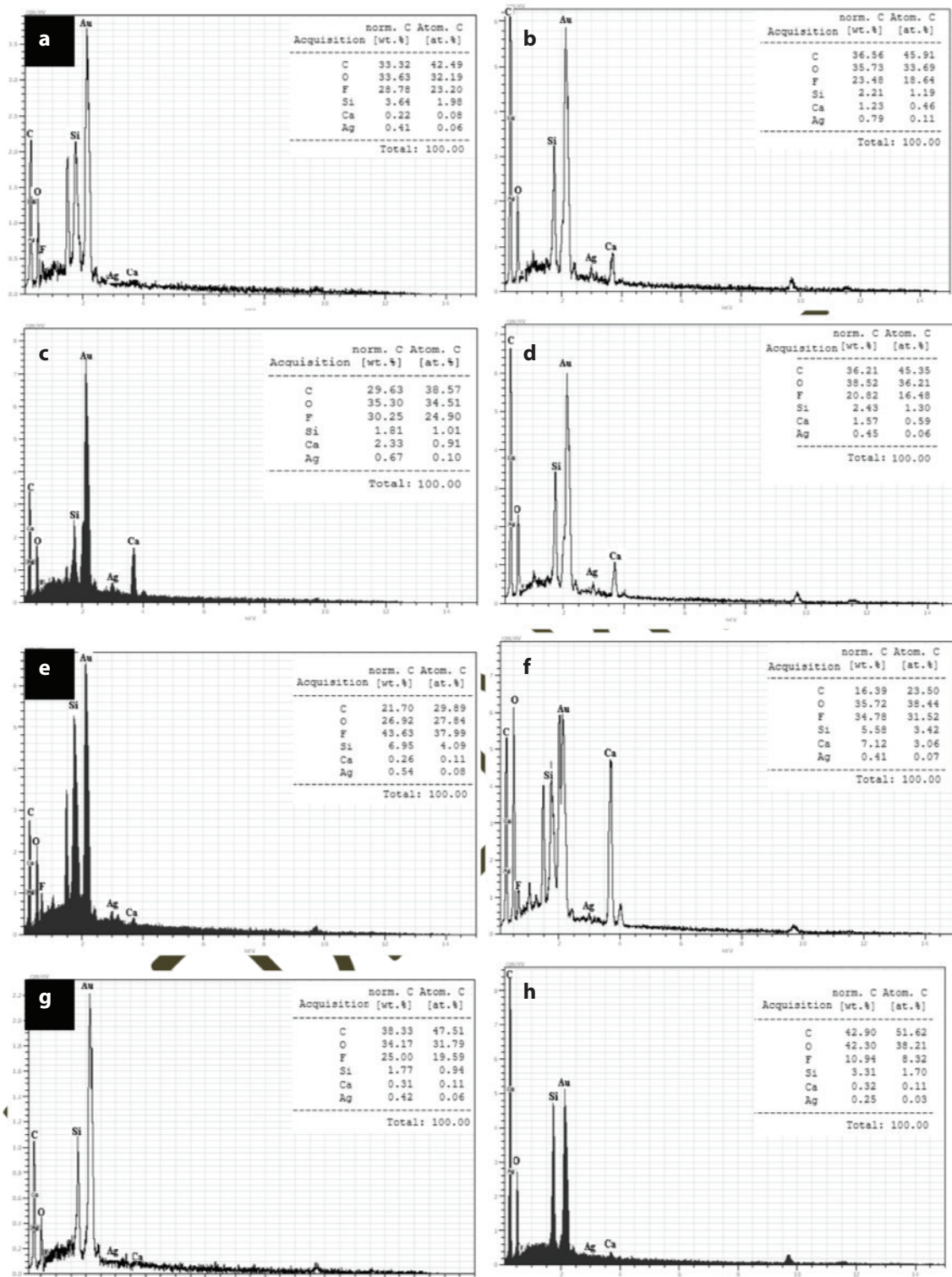


Figure 2. The Ag ion uptake percentages of groups. a: Control group, b: Corsodyl group, c: Cervitec group, d: Cervitec Plus group, e: Fluor Protector Group, f: FotoSan group, g: Diode Laser group, h: Er, Cr:YSGG Laser group.

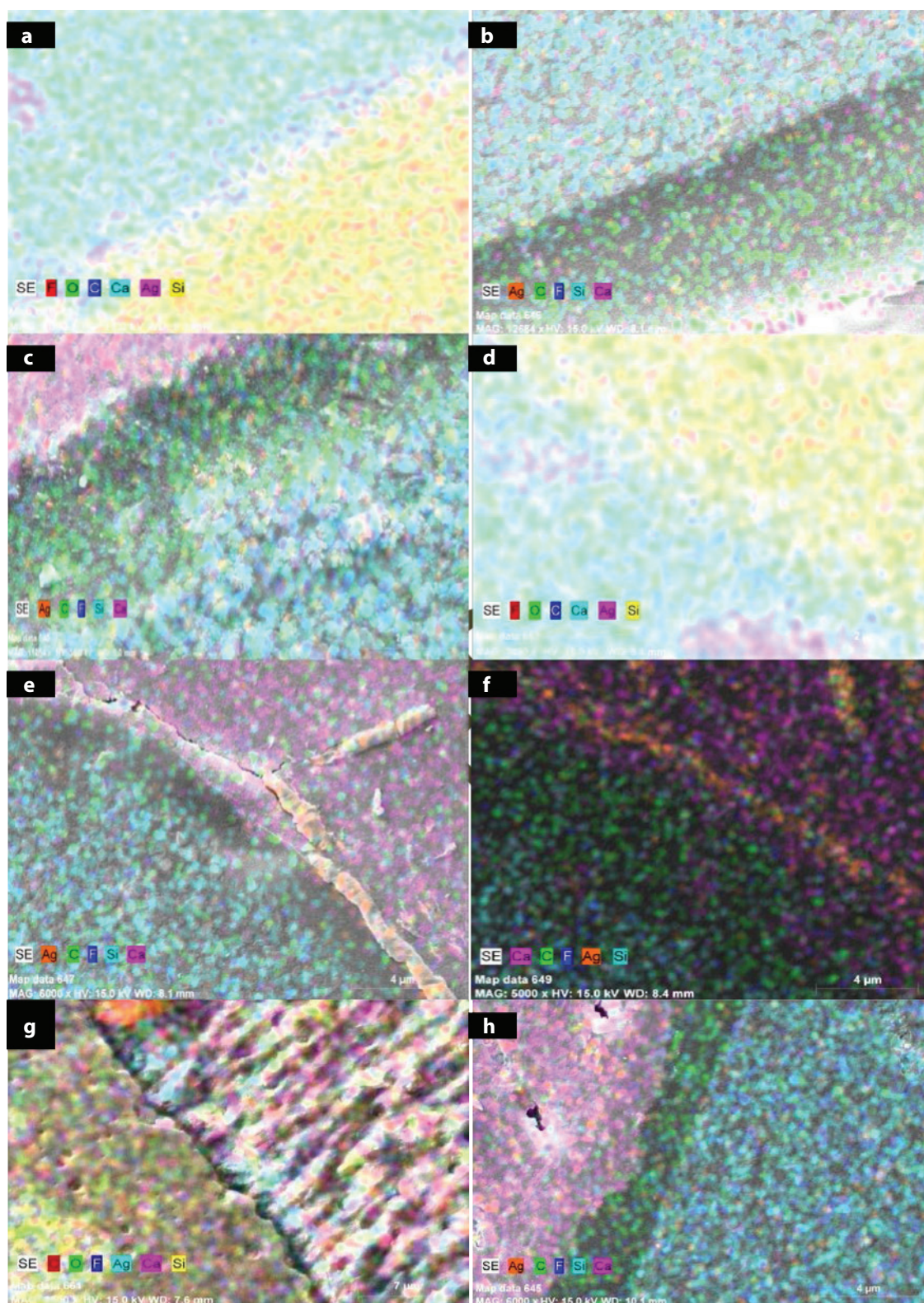


Figure 3. Distribution of the ions were shown with mapping. Colours shown at the bottom of the SEM images represent the ions written in. a: Control group, b: Corsodyl group, c: Cervitec group, d: Cervitec Plus group, e: Fluor Protector Group, f: FotoSan group, g: Diode Laser group, h: Er,Cr:YSGG Laser group.

with the sealing ability (16). Because of these reasons; with the aim of comparing the effect of various systems; Er,Cr:YSGG lasers, diode lasers and FotoSan, which is a PAD system and agents; Corsodyl Cervitec and Cervitec Plus, which contains CHX in their combination; and Fluor Protector on the nanoleakage of compomer restorations when used for cavity disinfection, the present study was designed.

Leakages have been mostly investigated at the micro scale (39). However, the diameter of the stained particles used in microleakage studies was larger than that of the bacteria; thus, the leakage was not detected accurately. So, researchers have searched for new methods (40). Nanoleakage refers to the nanosize leakage that occurs around collagen fibrils in the hybrid layer. In nanoleakage studies, staining is per-

formed using AgNO₃ solution. The AgNO₃ solution particles are approximately 0.59 nm in size. The sizes of the bacteria that live in the mouth vary between 0.5 and 1 nm. Thus, AgNO₃ is considered a suitable staining solution for leakage studies (41). Until now, the effects of cavity disinfection on nanoleakage have not been investigated. All the studies conducted previously were microleakage studies.

Currently, various cavity disinfectants have been used and include chlorhexidine, fluoride gels, sodium hypochloride, benzalkonium-based solutions, propolis and *Aloe vera* (42,43). Technological devices like lasers or PAD systems may be alternative procedures for cavity disinfection (27,30).

Chlorhexidine is the accepted gold standard antibacterial agent that is commonly studied when used as a cavity disinfectant (17). Speculation exists concerning the effects of chlorhexidine on the sealing ability of resin-restorative materials. Some researchers have found that chlorhexidine does not have an adverse effect on the bond strength (14,15). On the other hand, studies have demonstrated controversial results that chlorhexidine increases leakage scores (16,17). The studies that found chlorhexidine increased leakage scores used self-etched adhesive systems. This situation can be attributed to negative interactions between chlorhexidine and the self-etched adhesive systems (17).

In the present study, an increased nanoleakage score was found in the chlorhexidine group. This result also concurs with studies using the self-etched adhesive system (11,17) such as PrimeBond NT, which was used in this study.

The combinations of chlorhexidine with other antibacterial agents, such as fluoride or thymol, are commercially available. In the present study, Cervitec gel (chlorhexidine digluconate and sodium fluoride) and Cervitec Plus varnish (chlorhexidine diacetate and thymol) were used. Although the Cervitec groups showed significantly higher nanoleakage than the control group ($p < 0.001$), the Cervitec Plus group showed similar nanoleakage results to the control group ($p > 0.1$). This situation could be explained with the concentration differences between the Cervitec and Cervitec Plus groups. Cervitec has a gel form, but Cervitec Plus has a varnish form.

The studies concerning the effect of fluoride on leakage are limited, and in most of the studies, fluoride was used as a desensitiser or remineralising agent (44,45). Selveraj *et al.* (45) used silver diamine fluoride/potassium iodide (SDF) for dentin pretreatment. They reported that SDF minimized the leakage score. In the present study, the Fluor Protector group showed similar nanoleakage scores to the control group. This can be explained by the differences between the fluoride contents. In another study, Nystrom *et al.* (46) applied 0.71% tin fluoride to class V restorations that comprised 50% cement and 50% enamel and restored the teeth with a composite by using a total etch adhesive. No significant differences were detected compared with the control group when the microleakage values were examined. No significant differences were detected between the Fluor Protector that contained fluoride and the control group in present study ($p = 0.286$). However, higher nanoleakage values were detected compared with the Cervitec Gel and control groups ($p = 0.001$). The cause might be fact that Cervitec were in gel forms, and the Fluor Protector was in a varnish form. Additionally, many differences were found in their contents.

Penetration into dentin tubules is one of the important factors for the selection of cavity disinfection methods. Chemical agents can penetrate only up to 130 μm into dentin, although bacterial penetration is 1100 μm . (47) Thus, lasers and PAD with high penetration capabilities gain importance (48, 49). Odor *et al.* (50) gave a possible explanation about the penetration of laser beams to dentin. According to their explanations, enamel and dentin are capable of acting as a light collecting and transmitting device. Therefore, emitted laser light to the deeper layers of dentin can be actualized (48).

Although PAD has strong antibacterial efficacy, limited data are available concerning its effects on marginal leakage. Oskee *et al.* (51) used a PAD, Nd:YAG and diode laser for cavity disinfection. They declared that diode lasers and PAD had no detrimental effects on marginal leakage. In another study, no adverse effects of PAD on marginal leakage were found, in accordance with the present study, which used FotoSan as a PAD system (52).

Laser irradiation on dental hard tissues has been widely studied in dentistry (8,12). Several advantages, such as the creation of an acid-resistant surface, antibacterial activity and enhanced bonding capacity, were shown in previous studies (30, 53). In the present study Diode and Er,Cr:YSGG laser were used. The limited studies were found about the diode laser usage for cavity disinfection (51, 54). They found that diode laser had no adverse effect on marginal leakage parallel to the results of the present study. After Er:YAG laser treatment, a honeycomb pattern similar to that following phosphoric acid application was observed (55). An increased bonding capacity can be explained with this pattern. It was reported in a previous study that Er,Cr:YSGG laser etching does not eliminate the need for acid etching (56). In the present study, a self-etched bonding system was used; thus, no additional acid etching was used. In the present study, decreased nanoleakage scores after Er,Cr:YSGG laser irradiation can be explained by the honeycomb pattern detected following phosphoric acid application; thus, the sealing ability of the restoration material may increase. Consistent with the results of this study, Baygin *et al.* (53) found decreased microleakage scores after Er,Cr:YSGG laser irradiation.

According to the results of the present study, the first null hypothesis, 'the systems and agents that were used in the study would have no effect on nanoleakage' was rejected. Corsodyl and Cervitec usage increased and Er,Cr:YSGG laser usage decreased the nanoleakage scores compared with the control group. Also, the second null hypothesis, 'nanoleakage would not differ between the systems or agents' was rejected. The Corsodyl use showed higher nanoleakage than in all the tested groups and the Er,Cr:YSGG laser group showed significantly less nanoleakage than all the tested groups.

Conclusion

Under the limitations of this study, Er,Cr:YSGG laser irradiation, which showed lower nanoleakage scores than either the control or tested groups, can be recommended for cavity disinfection. Also Diode laser, FotoSan and Fluor Protector which have no adverse effect on nanoleakage scores can be alternative system for cavity disinfection.

Türkçe Öz: Kavite dezenfeksiyonunun kompozit restorasyonların nanosızıntısına etkileri: taramalı elektron mikroskopu ve enerji dağılımı x-ray analizi. Amaç: Rutin çürük uzaklaştırma yöntemlerine ek olarak kavite dezenfeksiyonu mikroorganizmaların eliminasyonu için önerilmektedir. Bu çalışmanın amacı farklı sistemlerin; Er,Cr:YSGG lazer, diod lazer, FotoSan ve ajanların; Corsodyl, Cervitec, Cervitec Plus, Fluor Protector kavite dezenfeksiyonu amacıyla kullanımında kompozit restorasyonların nanosızıntısına etkilerinin incelenmesidir. Gereç ve yöntem: Bukkal ve lingual yüzlerinde mine sement sınırına paralel sınıf V kaviteler (3×2×1.5 mm) bulunan 40 adet insan süt azı dişi kavite dezenfeksiyon yöntemine göre 8 gruba ayrıldı. Antibakteriyel ajan ve sistemler üretici firmaların önerileri doğrultusunda uygulandı. Tüm örnekler kompozit dolgu materyali ile restore edildi. Restore edilen dişlere 30 saniye 5°C-55°C su banyosunda 500 tur termal siklus uygulandı. Termal siklus işlemlerinden sonra, nanosızıntı testleri için her bir kavitenin orta hattından 1 mm'lik kesit alındı. Örnekler bölündükten sonra %50'lik amoniyal gümüş nitrat solüsyonunda 24 saat, florosan ışık altında fotoğraf solüsyonunda 8 saat bekletildi. Örnekler SEM ile incelendi. İstatistiksel analizlerde non parametrik Kruskal Wallis ve Mann Whitney U Test'leri uygulandı. Bulgular: Er,Cr:YSGG lazer grubunda diğer gruplara göre anlamlı derecede daha az nanosızıntı görüldü ($p<0.01$). Diod lazer, Fluor Protector ve Fotosan grupları kontrol grubuna göre benzer nanosızıntı skorları gösterdiği bulundu ($p>0.05$). Corsodyl ($p<0.01$) ve Cervitec ($p<0.001$) gruplarında ise nanosızıntı miktarı kontrol grubuna göre daha yüksek olduğu tespit edildi. Sonuç: Antibakteriyel etkinliği bulunan ve hem kontrol hem de test gruplarına göre daha düşük nanosızıntı değerleri gösteren Er,Cr:YSGG lazer kavite dezenfeksiyonunda önerilebilir. Ayrıca antibakteriyel etkinliği bulunan ve sızıntıyı etkilemeyen diod lazer ve FotoSan da kavite dezenfeksiyonunda önerilebilecek yöntemler arasında yer alabilir. Anahtar kelimeler: Er,Cr:YSGG Lazer; Diod Lazer; FotoSan; Antibakteriyel Ajan; Nanosızıntı.

Ethics Committee Approval: Ethical approval of the present study was obtained from the Ethics Committee of Karadeniz Technical University, Faculty of Medicine (Protocol # 2015/149).

Informed Consent: The informed consents were provided by the participants.

Peer-review: Externally peer-reviewed.

Author contributions: IA and OB designed the study. IA, FE and AC participated in generating the data for the study. IA and OB participated in gathering the data for the study. TT and FMK participated in the analysis of the data. IA wrote the majority of the original draft of the paper. IA and OB participated in writing the paper. All authors approved the final version of this paper.

Conflict of Interest: The authors had no conflict of interest to declare.

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