

SCANNING ELECTRON MICROSCOPIC ANALYSES OF THE EFFECTS OF DIFFERENT DISINFECTANT METHODS ON DENTINAL STRUCTURE

Taskin Gurbuz¹, Fatih Sengul², Tevfik Demirci², Munevver Coruh³

1. Associate Professor, Department of Pediatric Dentistry, Faculty of Dentistry, Ataturk University, Erzurum, Turkey.

2. Assistant Professor, Department of Pediatric Dentistry, Faculty of Dentistry, Ataturk University, Erzurum, Turkey.

3. Research Assistant, Department of Pediatric Dentistry, Faculty of Dentistry, Ataturk University, Erzurum, Turkey.

Abstract

The purpose of this study was to evaluate the prepared dentin surface and to determine whether or not it is possible to seal the dentinal tubules, after different cavity disinfectant applications.

Thirty mandibular molars were sectioned parallel to the occlusal plane to expose the mid-coronal dentin. All of the teeth were divided into five groups (n = 6 per group): (1) in group OZ, the dentin surfaces were exposed to ozone gas from the Ozonytron XP delivery system, (2) in group ED, the dentin surfaces were irradiated with an Er:YAG laser, (3) in group ND, the dentin surfaces were irradiated with an Nd:YAG laser, (4) in group CHX, the dentin surfaces were treated with a 2% chlorhexidine solution, and (5) in the control group, no treatment was applied.

Significant differences among the test groups were also observed in the scanning electron microscopy evaluation (P < .05). Scanning electron microscopy showed that the effect of laser energy on dentin varied from cratering, poring, fissuring, fracturing and cracking up to melting; also, the dentinal tubules were not sealed, in contrast with the control group.

No formation of smear layer and open dentinal tubules were obtained by using Er:YAG laser irradiation.

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Introduction

The infected tooth structure removed and achieve complete sterilization of the cavity preparation can prevent microleakage, pulp sensitivity, pulp inflammation and secondary caries.^{1,2}

To remove all the bacteria from the cavity preparation and to reduce the potential for residual caries, an antibacterial solution has been suggested in addition to the physical removal of carious dentin for the disinfection of dentinal cavities.¹ There are several methods and approaches described in the literature for

disinfecting the preparations. A thorough understanding of the interactions of these disinfection methods and their effects on sealing restorations is crucial in the selection of disinfection agent.

Chlorhexidine contains chlorhexidine gluconate that binds to the amino acids in the dentin and continues to kill bacteria for several hours, making it a good antibacterial agent.³ In addition to chlorhexidine, the Nd:YAG, Er:YAG laser and, more recently, gaseous ozone have been considered an alternative treatment to disinfect the dentinal cavities. It has been reported that ozone has a strong oxidizing power with a reliable micro-bicidal effect.⁴ Previous studies suggested that ozone treatment kills microorganisms via a mechanism involving the rupture of their membranes.⁴⁻⁶ However, some researchers have stated that chemical disinfection methods have an inhibitory effect on the bond strength of adhesive techniques.⁷

The objective of this study was to evaluate the effect of desensitizing agents and

*Corresponding author:

Assoc. Prof. Dr.Taskin GURBUZ
Atatürk Universitesi
Dis Hekimligi Fakultesi
Pedodonti Ana Bilim Dali
Erzurum, TURKEY

E-mail: gurbuzt25@yahoo.com

laser irradiation on the dentin surface were evaluated using a scanning electron microscope (SEM).

Materials and methods

A total of 30 extracted noncarious human molar teeth were used in this study. The teeth were cleaned with a toothbrush and water and then stored in sterile saline. A diamond bur with a water coolant was used to section the teeth parallel to the occlusal plane to expose the mid-coronal dentin. To create a standardized smear layer, each of the dentin surfaces was polished along the cut surface with the same series of wet silicon carbide disks (#600, #800, and #1000) and rinsed under water for 60 s. Following preparation, the specimens were randomly divided into five experimental groups of 6 specimens each according to the cavity disinfection method:

(G1) In group OZ, the dentin surfaces were exposed to ozone gas using the Ozonytron XP delivery system (OzonyTron XP-Biozonix, Munich, Germany) with a GI probe for 60 s, at 1 mm from the cavity surface.

(G2) In group ED, the dentin surfaces were irradiated with the Er:YAG laser (Fotona Lightwalker, Slovenia) emitting photons at a wave-length of 2940 nm with a pulse duration of 100 μ s. Laser energy was delivered through a sapphire tip, 600 μ m in diameter and 6 mm in length positioned perpendicular to the dentin surface. A power of 1,20 W at 10 Hz was used in focus mode at a focal distance of 1–2 mm. The laser was applied to cavity surfaces five times with an application time of 10 s at intervals of 5 s.

(G3) In group ND, the dentin surfaces were irradiated with an Nd: YAG laser (Fotona Lightwalker, Slovenia) with 120 mJ/20 Hz for 20 s at a wave-length of 1064 nm with a fiber-optic tip scanning the cavity surface.

(G4) In group CHX, (Drogsan, Ankara, Turkey), 2% CHX solution was applied to dentin for 20 s with a cotton pellet. The cavity surfaces of the teeth were then dried with air for 10 s.

(G5) In group control, the specimens were not treated with any cavity disinfection method.

SEM Investigation

One half of each tooth was prepared for SEM investigation of the dentin surface. The

samples were dehydrated in an ascending series of ethanol. Completely dehydrated specimens were mounted separately on aluminum stubs, coated with gold/ palladium, examined by using an SEM (JSM-6400; JEOL, Tokyo, Japan) operating at 23 kV, and micrographs were obtained.

A trained operator (T.G.), blinded to treatment group, evaluated the SEM images by using the scoring scale of Hülsmann et al.⁸ The amounts of debris and smearing were each graded between 1 and 5 at 2000X magnification.

Statistical Analysis

The scores of the SEM evaluation were compared by using the Kruskal- Wallis and Mann-Whitney *U* tests.

Results

The significant differences were found between the groups SEM evaluation results ($P < .05$). When removing debris from dentin surfaces there were significant differences between CHX and the control group ($P < .05$).

While Nd: YAG laser and the control group had significant differences in debris removing ($P < .05$), there was not any significant difference in the removal of smear layer ($P > .05$).

In the Er: YAG applied dentin surface samples, cratering, poring, fissuring, and fracturing has been found in the SEM images. Also, when compared with the control group open dentinal tubules were observed.

The results for the mean debris and smear layer scores are shown in Table 1. The lowest scores for the debris and smear layer were obtained in G2 and G4 ($P < .05$).

Representative SEM photomicrographs from each group displaying the changes on the dentin surface are shown in Figure 1.

Groups	Debris (mean \pm standart deviation)	Smear layer (mean \pm standart deviation)
G1 (Ozone)	3.33 \pm 0.816 ^{bc}	2.5 \pm 1.049 ^{ab}
G2 (Er:YAG laser)	1.33 \pm 0.816 ^a	1.17 \pm 0.408 ^a
G3 (Nd:YAG laser)	3 \pm 1 ^b	2.83 \pm 1.169 ^b
G4 (CHX)	2.67 \pm 0.516 ^{ab}	2.33 \pm 0.816 ^{ab}
G5 (Control)	4.4 \pm 0.548 ^c	3.8 \pm 0.447 ^b

Table 1. Mean values and standard deviations for debris and smear layer scores.

If marked by the same letter, the difference between the groups is statistically insignificant ($P > .05$).

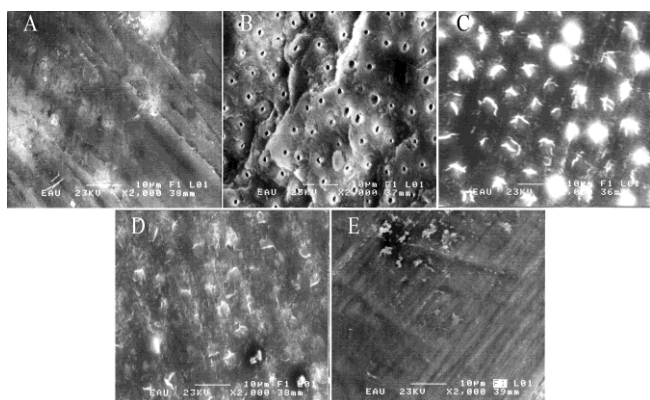


Figure 1. SEM images of dentin surface (original magnification 2000X). (A) More than 50% of the root canal wall covered by debris; complete root canal wall covered by a homogenous smear layer and no open dentinal tubules in a G1 sample. (B) Clean dentin surface, only few small debris particles, cracks; no smear layer, and dentinal tubules open in a G2 sample. (C) Few small agglomerations of debris, small amount of smear layer, and some dentinal tubules open in a G3 sample. (D) Many agglomerations of debris covering less than 50% of the root canal wall, homogenous smear layer covering the root canal wall, and only few dentinal tubules open in a G4 sample. (E) Complete or nearly complete dentin surface covered by debris and heavy smear layer covering the complete dentin surface in a G5 (control) sample.

Discussion

Restorative procedures such as cavity preparation are used to remove the infected dentin and make space for the restorative materials. The successes of these procedures depend on the effective removal of infected dentin, prior to the placement of the restorative materials.⁹ Martin et al. determined that the presence of bacteria in dentin and proximity to the pulp have clearly been associated with pulpal inflammation.¹⁰ To reduce the potential for residual caries, an antibacterial solution or technologies like laser and ozone may be alternative treatment approaches for disinfecting the dentin after the cavity is prepared.¹ Therefore, an effective cavity disinfection method is essential for increasing the longevity of restorations.

Ozone is a naturally occurring compound of three oxygen atoms. Ozone, in its gaseous or

aqueous phase, has been shown to be a powerful and reliable antimicrobial agent against bacteria, fungi, protozoa, and viruses.¹¹ Baysan et al. determined that there was a significant reduction in *S. mutans* and *S. sobrinus* in ozone-treated samples.¹² Therefore, the application of ozone on dental hard tissues prior to restoration has recently been proposed for disinfecting the cavity surfaces.¹² However, the effect of an ozone application on dental hard tissues prior to restoration has been poorly investigated. Ozone is a strong oxidizing agent that can react with almost every organic material. In the case of a dentin surface, the possibility exists that the treatment altered organic surface constituents, such as collagen.¹³ This changed surface may have negatively altered the bond strength of the self-etch adhesive. Gürsoy et al. reported that the dentine tubules more marked occlusion were seen in ozone treated group.¹⁴ In this study ozone applied dentin surfaces obtained higher debris scores similar to the results of the researchers. Also, smear layer were widely found on dentin surfaces.

Er:YAG and Nd:YAG lasers can be used in many clinical procedures, such as caries ablation, reduction of bacterial contamination, and treatment of root canals, reduction of dentinal hypersensitivity, remineralization of incipient dental caries, and pits and fissure sealing.¹⁵⁻¹⁷ In addition, previous studies found that the Er: YAG and Nd:YAG lasers altered and modified the dentin and enamel surfaces.^{14, 18}

Researchers reported that dentin surface of cavities using Er:YAG laser showed no formation of smear layer and open dentinal tubules.^{18, 19}

When examining SEM photographs of dentin irradiated by an Nd:YAG laser, melting and resolidification of dentin was observed.²⁰ In accordance with the results of the researchers in this study open dentinal tubules and the lowest scores for the debris and smear layer were found in the Er:YAG laser applied samples. Furthermore, in Nd: YAG laser treated samples dentinal tubules were occluded.

Dalkilic et al. determined that a Nd:YAG laser and 2% chlorhexidine did not change the microtensile bond strength of two-step, self-etch adhesive system to dentin. Moreover, ozone decreased the microtensile bond strength of two-step, self-etch adhesive to dentin.²¹

The use of chlorhexidine-containing

products as a cavity disinfectant after tooth preparation could help to reduce the potential for residual caries and post-operative sensitivity.¹ However, some researchers have reported that the positive benefits would be negated if the solution decreased the composite resin bond strength to dentin. SEM examination by Meiers and Kresin showed that cavity disinfectants applied to dentin surfaces were resistant to acidic conditioning.¹ This acid-resistant layer might inhibit the impregnation of the adhesive resin to the dentin surface. In our study, less debris and smear layer were observed in CHX samples compared to the ozone and Nd: YAG laser group. When applying desensitizing agents dentist should be careful in choosing the agents which are effective in removal of the smear layer because they are directly related with the adhesive materials bonding.

Conclusions

Cavity disinfectant application and laser irradiation may influence dentin surfaces. Er:YAG laser application has removed the smear layer and opened the dentin tubules. Ozone gas application has been found to be inadequate in removal of debris and smear layer of dentin surfaces. Er:YAG laser showed better results than Nd:YAG laser in disinfection of the cavity.

Declaration of Interest

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