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The Effects of Different Desensitizers and Their Combinations with ER, CR: YSGG Laser on Dentin Tubules, and Shear Bond Strength to Dentin#

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Research Article

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History

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ABSTRACT

Objectives: The aim of this in vitro study was to investigate the effectiveness of Sodium Fluoride, Propolis and Er, Cr:YSGG laser alone and in combination with laser applications on dentin tubules by SEM and to examine the effects of these applications on the shear bond strength (SBS) of a universal adhesive to dentin.

Materials and Methods: Dentin samples obtained by removing the buccal enamel of 72 caries-free permanent, mandibular third molars were randomly divided into six groups (n:12): Control, Sodium Fluoride (Enamelast, Ultradent), Propolis (Fanus Propolis), Er, Cr:YSGG Laser (Biolase, Waterlase), Enamelast-Er,Cr:YSGG Laser, Propolis-Er,Cr:YSGG Laser. While the agents were applied to the dentin surfaces alone in the agent groups, laser was applied after the agent applications in the combination groups. No application was performed to the control group. After storing the samples in artificial saliva for 14 days, Clearfil Tri-S Bond Universal (Kuraray) and composite resin (Estelite Sigma Quick, Tokuyama) were applied to their surfaces. SBS tests were conducted using the Universal Tensile-Compression Test System (Instron 3382, USA). The tubule plugging efficiencies of the agents were examined on two dentin samples from each group by SEM. One-way analysis of variance (One-way ANOVA) was performed for statistical analysis of the data. For $p<0.05$, the results were considered statistically significant.

Results: No statistically significant difference was found in any comparison between all the test groups. In SEM examinations, occluded dentinal tubules were observed more frequently in the groups where the applications were performed in combination with laser, compared to the application of the agents alone. Although some of the dentinal tubules were open, the tubules were generally closed.

Conclusions: Although combination applications of Er, Cr:YSGG Laser with Sodium Fluoride and Propolis showed greater dentin tubule plugging efficiency, these applications did not have a negative effect on the SBS of Clearfil Tri-S Bond Universal compared to the control group. The changes caused by the laser application on the dentin surface and the content of the adhesive system may have prevented the SBS from being adversely affected.

Keywords: Dentin Hypersensitivity, Dentin Desensitizing Agents, Propolis, Er Cr:YSGG Laser, Shear Bond Strength

Farklı Hassasiyet Gidericilerin ve ER, CR: YSGG Lazer ile Kombinasyonlarının Dentin Tübülleri Üzerine ve Dentine Makaslama Bağlanma Dayanımına Etkisi#

Bilgi
#Bu çalışma 23-25 Kasım 2021 tarihleri arasında düzenlenen 'Sivas Cumhuriyet Üniversitesi 1. Uluslararası Diş Hekimliği Kongresi'nde sözlü bildiri olarak sunulmuştur.
**Sorumlu yazar*

Süreç

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ÖZ

Amaç: Bu in vitro çalışmanın amacı Sodyum Florid, Propolis ve Er,Cr:YSGG lazerin tek başına ve lazerle kombine uygulamalarının dentin tübülleri üzerindeki etkinliğinin SEM ile incelenmesi ve bu uygulamaların bir üniversal adezivin dentine makaslama bağlanma dayanımına (MBD) etkisinin incelenmesidir.

Gereç ve Yöntemler: 72 adet çürüksüz daimi alt 3. molar dişin bukkal mine dokusu uzaklaştırılarak elde edilen dentin örnekleri rastgele altı gruba (n:12) ayrıldı: Kontrol, Sodyum Florid (Enamelast, Ultradent), Propolis (Fanus Propolis), Er,Cr:YSGG Lazer (Biolase, Waterlase), Enamelast-Er,Cr:YSGG Lazer, Propolis-Er,Cr:YSGG Lazer. Ajan gruplarında ajanlar tek başına dentin yüzeylerine uygulanırken, kombinasyon gruplarında ajan uygulamalarından sonra lazer uygulanmıştır. Kontrol grubuna herhangibir uygulama yapılmadı. Örnekler 14 gün boyunca yapay tükrük içinde bekletildikten sonra yüzeylerine Clearfil Tri-S Bond Universal (Kuraray) ve kompozit rezin (Estelite Sigma Quick, Tokuyama) uygulandı. MBD testleri Universal Çekme-Basma Test Sistemi (Instron 3382, Amerika) kullanılarak yapıldı. Ajanların tübül tıkaç etkinlikleri, her gruptan iki adet dentin örneği üzerinde SEM ile incelenmiştir. Verilerin istatistiksel analizi için Tek Yönlü Varyans Analizi (One-way ANOVA) yapıldı. $p<0,05$ için sonuçlar istatistiksel olarak anlamlı kabul edildi.

Bulgular: Tüm test grupları arasında hiçbir karşılaştırmada istatistiksel olarak anlamlı farklılık tespit edilmemiştir. SEM incelemelerinde, lazerin kombine uygulandığı gruplarda ajanların tek başına uygulanmasına göre tıkalı dentin tübüllerine daha sık rastlandı. Dentin tübüllerinin bir kısmı açık olmasına rağmen tübüller genellikle kapalıydı.

Sonuçlar: Er,Cr:YSGG Lazer'in Sodyum Florid ve Propolis'le kombine uygulamaları daha fazla dentin tübül tıkaç etkinliği göstermekle birlikte, Clearfil Tri-S Bond Universal'in MBD'si üzerinde kontrol grubuna göre olumsuz bir etki oluşturmamıştır. Lazer uygulamasının dentin yüzeyinde oluşturduğu değişimler ve kullanılan adeziv sistemin içeriği MBD'nin olumsuz etkilenmesini önlemiş olabilir.

Anahtar Kelimeler: Dentin Hassasiyeti, Dentin Hassasiyet Giderici Ajanlar, Propolis, Er Cr:YSGG Lazer, Makaslama Bağlanma Dayanımı

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Introduction

Dentin hypersensitivity (DH) is a clinical problem that occurs due to thermal, chemical, mechanical or osmotic stimuli in the dental tissue and is a very common clinical problem in the community. It is characterized by sudden, short-term and sharp pain on a specific tooth surface that cannot be explained by any dental pathology.[1,2] The rate of DH detected with a careful examination has been reported to be approximately 15%. This rate varies between 70–98% in individuals with periodontal disease.[3 4] DH occurs when the dentin tissue is opened to the oral environment as a result of erosion, abrasion and abfraction that cause tooth tissue loss, as well as factors such as gingival recession, wrong brushing habits, diet and gastroesophageal reflux.[5-7] DH is most commonly diagnosed in the canine and premolar teeth and in the cervical regions of the buccal surfaces of the teeth.[4,8] Today, the incidence of DH is increasing gradually due to reasons such as gingival recession, consumption of acidic foods and incorrect brushing habits owing the increase in periodontal diseases.[6,9,10]

The aim of the treatment of DH is to occlude the tubules opened to the oral environment and/or to prevent the formation of pain by stopping depolarization of the pulpal nerve extensions in the open dentinal tubules[11,12] Since DH is quite common in the community, there are a large number of dentin desensitizing agents (DDAs) with different contents in the market for its treatment.[1,13,14]

Sodium fluoride (NaF, 5%) varnish applications have been widely used for many years in the treatment of DH.[15] Fluoride varnishes cause calcium fluoride (CaF_2) to accumulate on the exposed dentin surface and act as a mechanical barrier to occlude open dentinal tubules.[15, 16] Although clinical studies support the successful results of fluoride in the treatment of DH, many clinical studies have suggested that fluoride has limited efficacy.[17,18] It has been suggested that its effect is limited because the barrier formed due to the small CaF_2 crystals formed by fluoride cannot provide sufficient occlusion and these crystals dissolve slowly in saliva.[4,17,19]

In addition, studies have reported the presence of components that may be cytotoxic for teeth and gingival tissues in the structure of DDAs.[16,20] Since these components are mostly applied to the cervical region at the tooth-gingival junction, their contact with the gingival tissue is often possible. Morover, since DH is a chronic condition, repeating DDA applications is often necessary in the treatment of DH. Therefore, the biocompatibility of the agents used in the treatment of DH is becoming increasingly important.

Propolis is a resinous natural substance obtained by honey bees (Apis mellifera) from various plant sources.[21,22] Propolis has been used for many years in folk medicine for the treatment of several illnesses in many countries and there is a growing interest in using it in natural and biologically active supplements. Antibacterial, antifungal, anti-inflammatory, immunomodulatory, antiviral and antioxidant properties of propolis have been demonstrated in a variety of flavonoids and phenolic acids present in its structure.[23-25]

Propolis has also shown successful results in dentistry, wound healing, caries prevention, root canal disinfection, and cavity disinfectant, and has been added to the structure of toothpastes and mouthwashes.[26-29] Furthermore, propolis has been used in the treatment of DH and its occlusive efficacy in different dentin tubule has varied.[30,31] In another study, tubular plugs were reported to be resistant to citric acid.[32] In these studies, propolis extracts dissolved in ethanol were generally preferred, and water-based propolis extracts dissolved in water have also been introduced to the market in recent years. The advantage of water-based propolis is that it is biocompatible because it dissolves in water, and thus the dark discoloration of ethanol-based propolis is not seen in water-based.[22]

In recent years, the use of lasers in the treatment of DH, as well as DDA applications in the treatment of DH, has become increasingly common.[33,34] It has been explained by theories such as providing DH removal efficiency with laser applications, occlusion of dentinal tubules by melting dentinal tubules, evaporation of dentinal fluid and suppression of nerve conduction.[19,35] It has been reported that the Er, Cr:YSGG laser provides the accumulation of insoluble salts in the dentinal tubules by evaporation of the dentinal tubule fluid, resulting in occlusion of the dentinal tubules and reduction of DH.[33,35,36] In different studies, it has been reported that laser applications showed both dentin tubule plug effectiveness alone, and combined applications with DDAs further incresed tubule plug effectiveness.[37-39]

Additionally, in cases where tooth tissues are lost after DDA applications, restorative treatments are performed with resin composites. For this reason, tubule plugs formed in the dentinal tubules after the application of desensitizers may have negative effects on the bond strength of the filling materials.[40,41] Therefore, it is important to know the effect of the DDAs and applications on bond strength. Although there are studies on bond strength in the use of sodium fluoride and laser alone in the literature, as far as we know, studies on the bond strength of propolis to dentin are very limited, and there is no information about the combined applications of sodium fluoride and propolis with laser.

For these reasons, in this in vitro study; it was aimed at investigating the effects of sodium fluoride (NaF), water-based propolis and Er, Cr: YSGG laser alone and combined application of propolis with Er, Cr: YSGG laser and NaF with laser, which are used in the treatment of DH on the shear bond strength (SBS) of a universal adhesive system to dentin.

Secondly, it was aimed to examine the tubule plug activity created by these applications on the dentin surface by scanning electron microscopy (SEM).

The initial hypotheses of this study are:

- There is no difference between the SBS values of a universal adhesive to dentin after propolis, NaF and laser application alone.
- There is no difference between the SBS values of a universal adhesive after the combined application of Propolis and NaF with laser (Propolis- Er, Cr: YSGG Laser and NaF- Er, Cr: YSGG Laser).
- There is no difference between the SBS values of a universal adhesive to dentin after the application of Propolis, NaF and Er, Cr:YSGG laser alone or in combination.
- The tubule plug activities created by these applications are similar to each other.

Materials and Methods

Ethical approval of this study was obtained from the Scientific Research Ethics Committee of Karadeniz Technical University, Faculty of Medicine with the number 2021/220 (Decision No: 27.09.2021).

In this in vitro study, the SBS of a universal adhesive (Clearfil Tri-S Bond Universal, Kuraray Noritake Dental Inc., Osaka, Japan) to dentin was investigated after sodium fluoride (5% NaF, Enamelast, Ultradent, UT, USA), which has been used for many years in the treatment of DH and water-based propolis (Fanus Propolis, Fanus, Trabzon, Turkey) and Er, Cr:YSGG laser, both alone and in combination. In addition, the plug efficiencies of these applications on the dentinal tubules were examined by SEM.

Preparation of Dentin Samples

In the study, 72 caries-free permanent lower third molar teeth extracted for orthodontic or oral reasons were used. Teeth were stored in 0.1% thymol until experiments were performed. The enamel tissues on the buccal surfaces of the teeth were removed under water cooling by a low speed precision cutting device (Micra Cut 125, low speed precision cutting device, Metkon, Bursa) and a 0.3 mm thick diamond separator (Diamond cut-off wheel B 102, ATM GMBH, Germany). Thus, the superficial dentin tissues in the buccal surfaces of the teeth were exposed. Then, dentin samples were obtained by cutting the teeth horizontally from the apical of the enamel-cementum junctions.

Dentin samples were placed horizontally in the middle of the previously prepared plastic molds and with the dentin surface facing outward. Dentin samples were obtained by pouring autopolymerizing acrylic resin (Imicryl, SC, Konya, Turkey) into the mold. Prepared samples were sanded with 600, 800, 1200, 1500 and 2000 grain silicon carbide (SiC) papers in a 200 RPM rotary sanding device (Beta Grinder Polisher, Buehler) to form a standard smear layer and obtain a smooth surface. Preparation of dentin samples, DDAs and Laser applications and all analyses were performed by a single operator.

Applications of Dentin Desensitizing Agents and Er,Cr:YSGG Laser

Dentin samples were soaked in 17% EDTA (Werax, Tunadent, Izmir, Turkey) solution for five minutes to remove the smear layer and simulate the opened dentinal tubules. The samples were washed under running water to remove residues and then sonicated with an ultrasonic cleaner in distilled water for five minutes.

72 test samples were randomly divided into six groups as n:12: Group C (Control), Group EN (5% NaF, Enamelast), Group P (Propolis), Group L (Er,Cr:YSGG Laser), Group EN-L (Enamelast-Er,Cr:YSGG Laser), Group P-L (Propolis-Er,Cr:YSGG Laser). Ten of the samples prepared for each group were used for SBS tests and two for SEM examinations. The contents and application instructions of the DDAs and Laser were given in Table 1. In Group EN-L and P-L groups, laser application was performed after the agents were applied to the dentin surface. No application was made to the control group.

All dentin samples were placed in artificial saliva (0.213 g/l $CaCl_2{\cdot}2H_2O$, 0.738 g/l KH_2PO_4, 1.114 g/l KCl, 0.381 g/l NaCl, and 12 g/l Tris buffer; pH adjusted to 7 with KOH) after DDAs and laser applications. The artificial saliva used in this study was prepared at Biochemistry Laboratory of Faculty of Medicine in Karadeniz Technical University. Dentin samples of the control group were placed in artificial saliva without applying DDAs. Artificial saliva was replaced every other day, and test samples were brushed with circular movements applied 8-10 times with a soft toothbrush under light pressure at each replacement procedure. At the end of 14 days, the samples were taken from artificial saliva, washed with water, and brushed with a soft toothbrush with circular movements applied 8-10 times, again by applying a slight force. The dentin surfaces of the test samples were washed again and dried with air-water spray for 5 seconds.

Clearfil Tri-S Bond Universal adhesive was then applied to the surfaces of the test specimens according to the manufacturer's instructions and light cured for 20 seconds (S10, 3M ESPE, St. Paul, MN, USA). Then, composite resin (Estelite Sigma Quick, A2, Tokuyama Dental, Japan) was applied to the dentin surfaces by using a specially designed plexiglass apparatus containing a cylindrical cavity with a height of 3 mm and a diameter of 2.56 mm in the middle. The composite was applied to the dentin surfaces in 2 layers with a thickness of 1.5 mm. The polymerization of composite resins was completed by applying light to each layer for 20 seconds (Table 2). SBS tests were performed after the test specimens were stored for 24 hours at 37ºC, in a 100% humidity environment.

Shear Bond Strength Test

The SBS test of the samples was performed using the Universal Tensile-Compression Test (Instron 3382, USA).

Table 1. Dentin Desensitizing Agents, Laser, Manufacturers, Contents, Lot Numbers and Application Instructions.

Materials	Manufacturer	Contents	Lot Numbers	Application Instructions
Enamelast Fluride Varnish	Ultradent Products, USA	-Sodyum fluorid (5%), -Xylitol	BHFSD	Lightly dry area to be treated. Using a painting motion, apply a thin smooth layer to as many dry tooth surfaces as possible. Gently flow cool water over the teeth.
Propolis (Water-Based Propolis)	Fanus Propolis, Turkey	-50% resin and vegetable balsam, -30% wax, -10% essential and aromatic oils, -5% pollen, -5% other substances -Polyphenols (flavonoid aglycones, phenolic acids, and their esters, phenolic aldehydes, alcohols, and ketones), sesquiterpene quinones, coumarins, steroids, amino acids,	09-650	Propolis application was performed in circular motions without applying much force for 60 seconds (32).
Er, Cr:YSGG Laser	Waterlase, Biolase, San Clemente, USA	0.25 Watt 0% water, 10% air, 20 Hz in non-contact mode (35). A 6 mm MZ6 tip with a diameter of 600 µm was used for Er, Cr:YSGG laser application.	18002402	Irradiation was performed for a total of 20 s vertically and horizontally from the 1-mm irradiation distance to the dentin tissue.

Table 2: Manufacturers, Contents, Lot Numbers and Application Instructions of Universal Adhesive and Composite Resin

Materials	Manufacturer	Contents	Lot Numbers	Application Instructions
Clearfil Tri-S Bond Universal	Kuraray Noritake Dental Inc., Japan	-BisGMA, -HEMA, -Ethanol, -10-MDP monomer -Hydrophilic aliphatic di methacrylate -Colloidal silica -Di-camphorquinone -Silan coupling agents -Water Ph:2,3	850043	1. The adhesive is actively applied to the tooth surface for 10 seconds. 2. Apply light air for more than 5 seconds until the adhesive shows no movement. 3. Light cure for 10 seconds
Estelite Sigma Quick	Tokuyama Dental, Japan	-BisGMA, -TEGDMA - 72% by weight, 71% by volume silica-zirconia fillers, -Spherical submicron fillers with an average size of 0.2 micron	W970	1. After the isolation, apply to the cavity by layering technique. 2. Light-cure from a distance of 2 mm.

After the test samples were fixed to the Instron device, a load was applied to the composite-dentin interface parallel to the acrylic surface (the speed of the test device was set to 1 mm/s). The results obtained in Newtons were converted to Megapascals (MPa). The fracture surfaces of the test samples were examined under X30 magnification with an optical microscope (Olympus Metallurgical Microscope, Tokyo, Japan) for the determination of different failure types (adhesive, cohesive and mixed).

SEM Examinations

Two test samples prepared for each group were used in SEM examinations. The agents were applied to the dentin surfaces according to the manufacturer's instructions. After waiting for 6 hours, the excess of the agents were removed from the surfaces in order to examine the dentinal tubule orifices. In combination groups, laser application was performed immediately after the agent application. After waiting for the same time, the excess agent on the dentin surfaces was removed and SEM examinations were performed. Then the dentin samples were covered with a thin layer of gold film and SEM examinations (Zeiss EVO LS 10, UK) were performed at 5 kV and magnifications with X2000 and X5000.

Statistical Analysis

SPSS for Windows 17.0 (Statistical Package for Social Sciences, SPSS Inc., Chicago, 20 IL, USA) was used for

statistical analysis. Descriptive statistics were presented as mean, standard deviation (Mean and Sd), and Min-Max values. One-way Analysis of Variance (One-way ANOVA) was performed for statistical analysis of the data. For $p<0.05$, the results were considered statistically significant.

Results

Results of Shear Bond Strength Test

Table 3 shows the mean ± standard deviation and minimum-maximum values of the SBS of the test groups. The One-way Anova results of data are given in Table 4. According to Table 4, no statistically significant difference was observed in all comparisons between the groups ($p>0.05$).

Evaluation of Optical Microscope Images of Dentin Samples

As a result of the examination of the fracture surfaces of the test samples with an optical microscope, adhesive type failure was detected in all of the specimens.

Scanning Electron Microscopy (SEM) Analysis Results

1. Group C

In the SEM images of the control group, it was determined that EDTA application removed the smear layer and dentin tubules of different diameters were exposed (Figure 1).

2. Group EN

In the SEM images of the EN group, it was observed that the dentinal tubule orifices were usually open as well as the presence of locally closed or narrowed tubules (Figure 2).

3. Group P

In the propolis group, there were usually tubule plugs besides open dentin tubules (Figure 3).

4. Group L

The dentinal tubules were generally narrowed or occluded, but open dentinal tubules were also present on Er,Cr:YSGG laser treated dentin surfaces (Figure 4).

5. Group EN-L

In the SEM images of the Enamelast-Er,Cr:YSGG laser treated group, the dentinal tubules were generally closed and the plugs on the tubule orifices were seen (Figure 5).

6. Group P-L

In the group in which the laser was applied in combination with propolis, occluded dentin tubules were more common than in the groups in which the propolis was applied alone (Figure 6).

Discussion

DH is a condition that is increasing especially in developed countries and negatively affects the life comfort of individuals. When DH is not treated, it causes disruptions in oral hygiene practices, leading to the emergence of different dental problems. Therefore, DH treatment is becoming more and more important both to improve the quality of life of patients and as a part of preventive dentistry. For these reasons, the search for a fast-acting and long-lasting treatment of DH is increasing. While new researches continue in the treatment of DH, laser applications and combined applications of laser agents are becoming more common. [19,34,35,37,39.]

Table 3. Shear Bond Strength Values of the Test Groups

	Shear Bond Strength Values (MPa)		
Groups (n:10)	**Mean ± Standard deviation**	**Min**	**Max**
Group Control (C)	10.106 (4,93)	4.96-	21.40
Group Enamelast (EN)	10.043 (3,21)	5.72-	15.75
Group Propolis (P)	7.829 (2.22)	3.90-	10.35
Group Er,Cr:YSGG Laser (L)	9.711 (2,34)	6.02-	13.65
Group EN- L	9.415 (1,01)	7.85-	10.72
Group P-L	9.182 (1.63)	6.87-	11.42

Table 4. The One-way Anova Table

Sum of Squares		df	Mean Square	F	Sig.
Between Groups	35.231	5	7.046	.868	.509
Within Groups	438.535	54	8.121		
Total	473.767	59			

*According to the One-way Anova table, no statistically significant difference was observed in all comparisons between the groups ($p>0.05$).
For $p<0.05$, the results were considered statistically significant.

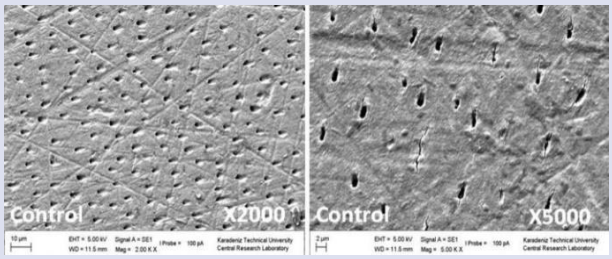

Figure 1. In the SEM images of the control group, exposed dentin tubules of different diameters were observed.

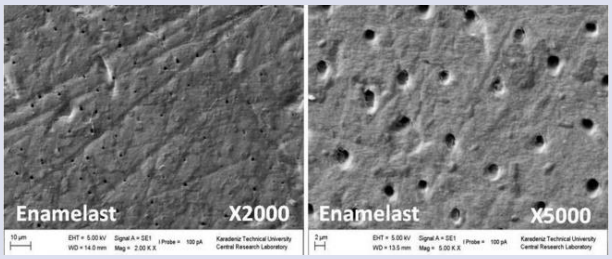

Figure 2. In the SEM images of the EN group, dentinal tubule orifices were usually open as well as the presence of locally closed or narrowed tubules.

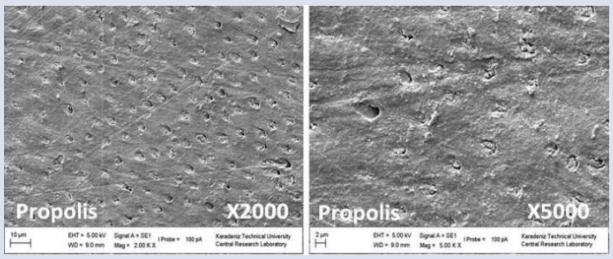

Figure 3. In the Propolis group, tubule plugs were usually observed besides open dentin tubules.

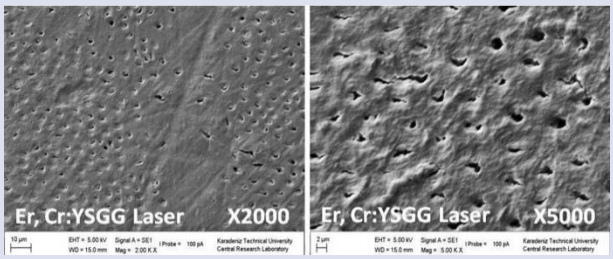

Figure 4. In the SEM images of the Er,Cr:YSGG laser group, dentinal tubules were generally narrowed or occluded, but open dentinal tubules were also present.

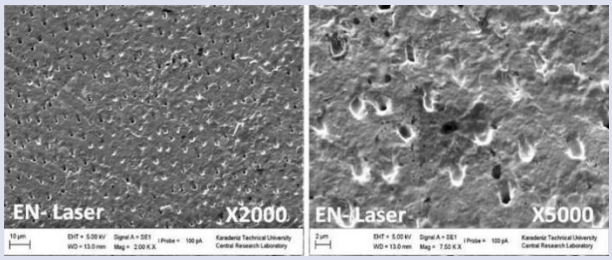

Figure 5. In the SEM images of the Enamelast-Er,Cr:YSGG laser group, the dentinal tubules were generally closed and the plugs were observed on the tubule orifices.

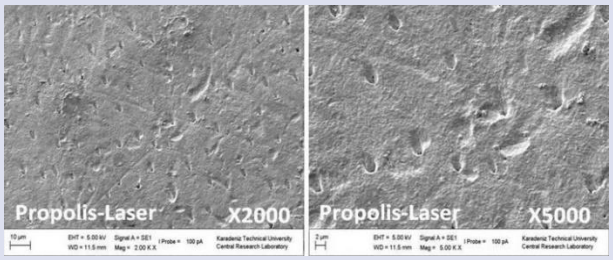

Figure 6. In the SEM images of the Propolis-Er,Cr:YSGG laser group, occluded dentin tubules were more common than in the groups in which the propolis was applied alone.

In this in vitro study, the effects of the applications of NaF (Enamelast), which has been used for many years in the treatment of DH and Propolis, which has shown successful results in different fields in medicine and dentistry and has come to the fore with its biocompatible properties, and Er,Cr:YSGG laser, which has become increasingly popular in the treatment of DH in recent years, and their combined application on the SBS of a universal adhesive system (Clearfil Tri-S Bond Universal) to dentin were investigated. Additionally, the dentin tubule plug efficiencies of these applications were examined by SEM.

According to the findings of this study, it didn't show a significant difference between the SBS values of all groups to dentin. Therefore, our first 3 hypotheses about the SBS values of a universal adhesive to dentin after different desensitizer applications were accepted. Our 4th hypothesis was "Tubule plug activities created by these applications are similar to each other." rejected because the tubule plug activities in the SEM images of the groups were different.

In this study, Enamelast application did not negatively affect the SBS compared to the control group and other groups. In SEM examinations, the dentin tubules were open in control group (Figure 1). In the SEM images of group EN, the tubules were usually open, as well as the presence of partially closed or narrowed dentin tubules (Figure 2).

There are different results in the literature regarding the bond strength of DDAs containing fluoride to dentin. In an in vitro study, it was reported that a self-etch adhesive reduces microtensile bond strength due to fluoride plugging open dentinal tubules with calcium fluoride crystals and blocking dentinal tubules.[41] In

another study, it was reported that fluoride gel reduces bond strength due to tubule occlusion.[42]

In another study, after long-term application of sodium fluoride to the dentin surface, the micro-shear bond strength of a self-etch adhesive system (Clearfil SE Bond) was found to be higher than in other experimental groups (Novamin, demineralized dentin, nonfluoridated dentin). Moreover, although the bond strength of the dentin applied with NaF after acid etching decreased compared to the self-etch system.[43] It has been suggested that the acid application stimulates alkaline phosphatases and metalloproteases activity and release in dentin and inhibits the bond strength of these enzymes, whereas fluoride released from NaF protects the hybrid layer from enzymatic degradation, which increases the bond strength.[44] In addition, it has been reported that the acidic 10-MDP momomer in the Clearfil SE Bond (self-etch adhesive system) increases the bond strength to dentin by forming insoluble salts with apatite crystals in dentin.[45] 10-MDP momomer that is included in the structure of self-etch and universal adhesive systems and which is used in the composition of Clearfil Tri-S Bond Universal, may have contributed to the increase in bond strength.

Universal adhesive systems are systems in which the adhesive procedures in self-etch systems are combined in a single system in terms of both reducing technical sensitivity and ease of application, and can be applied together or alone with acid etching when desired.[46,47] Furthermore, in mild universal adhesive systems, there was no difference in bond strength between self-etch or total-etch mode. However, in the absence of acid etching, the more superficial interactions of these materials on the dentin both reduce the risk of postoperative sensitivity and prevent the degradation of collagen fibrils. This contributes to the bond strength in the long term.[48-51] Therefore, in this study, we preferred to use Clearfil Tri-S Bond Universal, a mild universal adhesive system, in self-etch mode for reducing technical sensitivity and ease of application.

Besides, in our study, the adhesive applications performed 14 days after the fluoride application did not adversely affect the bond strength of EN compared to the other groups. The reason for the differences between the findings of other studies in the literature may be due to the chemical content of the fluoride agent used, the difference between the material methods and the tubule plug effectiveness of the applied fluoride, as well as the adhesive systems used. In our study, storing the dentin samples in artificial saliva for 14 days after EN application and brushing the dentin surfaces at regular intervals may have caused the existing tubule plugs to be removed and the dentin tubules to open. This may have prevented the adverse effects of adhesive applications.

Furthermore, in this study, removal of the excess of the EN varnish from the dentin surface for examining tubule orifices by SEM may have partially affected the tubule plugs.[52,53] Although it is not a clinical practice to remove fluoride varnish from the tooth surface, the varnish can be removed from the tooth surface over time by tooth brushing. However, performing adhesive procedures immediately after fluoride varnish has been applied to the tooth surface may damage the adhesive procedures.

Propolis, which is a natural substance in resin structure, generally showed tubule plug activity in the SEM images of this study (Figure 3). In different studies, propolis has been used in the treatment of DH and has shown dentin tubule occlusive activity.[30-32] In a study by Chen *et al.*[32], it was determined that the tubule plugs formed by propolis were resistant to citric acid application and the tubules were mostly clogged after acid application. It has been reported that the flavonoids contained in propolis gel interact with the dentin and reduce the fluid movement of the crystals formed in the dentin, thus showing DH-reducing activity. It has also been suggested that due to its resinuous structure, propolis could mechanically bond to the pores in the dentinal tubules and thus block the dentinal tubules.[30,32] In our study similar to that study, in the propolis group, plugged dentin tubules were found in addition to occasional open dentin tubules. The probable cause of tubular plugs may be the resinous structure of propolis and the crystals formed by the reaction of high concentrations of flavonoids in its structure with dentin tubules.

In a study in which ethanol-based propolis was used as an intracanal medicament and root canal disinfectant, it caused discoloration on the coronal surfaces. Although the physical and chemical properties of propolis vary considerably, its original color being amber and the minerals such as flavonoids and iron in its content may cause discoloration. In addition, ethanol may facilitate their diffusion and increase the discoloration.[54]

Therefore, in our study, 10% water-based propolis, which is colorless, was preferred in order not to cause discoloration, especially in the anterior teeth. Although water is not as good a solvent as ethanol, water-based propolis contains a fairly high concentration of flavonoids and is nontoxic. For this reason, it is an important advantage that water-based propolis does not cause discoloration, especially in cases where repetitive applications are required in the clinical treatment of DH.[22] To the best of our knowledge, this is the first study in terms of the use of water-based propolis in the treatment of dentin sensitivity.

Although the SBS of Clearfil Tri-S Bond Universal to dentin after propolis application was slightly lower than other groups, there was no statistically significant difference between the other groups ($p<0.05$). In different studies where propolis was used as a cavity disinfectant, propolis did not adversely affect the bond strength.[55,56]

In this study, Er,Cr:YSGG laser application in SEM images closed the dentinal tubules in general, although there were open tubules in places (Figure 4). Besides, the laser application did not adversely affect the bond strength in dentin samples.

In a study, it was reported that Er, Cr: YSGG laser could dissolve peritubular dentin even at low power

(0.25 Watt) and narrowed dentin tubule diameters similar to Nd:YAG laser.[36] In addition, in clinical studies examining the effect of Er, Cr:YSGG laser on DH, it has been shown that the effect can last for the medium and long term even after a single application.[35,57] In a clinical study comparing the desensitizing efficiency of different laser types, Er, Cr:YSGG laser was reported to be more effective for 6 months.[58]

In an in vitro study by Gürgan *et al.*, it has been reported that the Er, Cr:YSGG laser application to the dentin surface is affected by the power of the laser and the preparation differences depending on the adhesive type. In addition, it has been determined that SBS is higher depending on the power of the laser.[59]

Moreover, it has been suggested that laser application may have increased the bond strength to dentin due to the formation of a rough and irregular surface on the dentin surface.[60] In addition, intertubular dentin undergoes more selective ablation because it contains more water and less minerals than peritubular dentin, and it has been reported that the protrusive irregular dentin surfaces formed after ablation may have contributed to the bond strength.[61-63] In an in vitro study by Ergücü *et al.*[64], it was reported that the use of Er, Cr:YSGG laser did not adversely affect the bond strength of a total etch and self-etch adhesive to intact and caries-affected dentin.

In our study, dentinal tubules were opened by EDTA application. This may have facilitated the penetration of the laser on the tubule surfaces and inside the tubules due to the removal of the smear layer, and may have facilitated the penetration of the adhesives into the tubules. Moreover, the 10-MDP monomer in the structure of Clearfil Tri-S Bond Universal also contributes to the bond strength by forming a chemical bond with the calcium in the structure of hydroxyapatite.[45,65] Additionally, since the laser application was only used in the mode of the treatment of DH in our study, the output power of the laser may be lower than in other studies. It is because at higher power, higher bond strength could be obtained.

In this study, the combination application of Enamelast and Er, Cr:YSGG Laser did not reduce the SBS compared to the other groups. In a study, (Aqua Prep F, 35% HEMA, 2% NaF) was applied to the dentin surface alone and in combination with the Nd:YAG and Er:YAG laser, and the adhesion of lithium disilicate ceramic to the dentin surface with a self-adhesive resin cement was investigated. Although the application of Aqua Prep F in combination with Nd:YAG Laser or alone did not affect the bond strength, the agent combination with the Er:YAG laser increased the bond strength compared to the alone application of the agent. In this study, the use of different types of lasers and the use of higher power may have increased the bond strength.[66]

We could not find any study on the effect of combined applications of fluoride-containing desensitizer and Er, Cr:YSGG Laser on the bond strength. Therefore, we could not adequately discuss the effect of combined application of EN-Laser on bond strength with adhesive resins.

In our study, dentin tubule plug efficiencies were also investigated after combined EN-L application (Figure 5). The combination of EN with laser showed greater tubule plug efficacy compared to EN alone. Studies have reported that the combination of NaF with different types of lasers increases the effectiveness of dentin tubule plugs.[37,67-69] In addition, it has been suggested that laser applications with fluoride can increase the penetration of fluoride into the tubules and inhibit demineralization.[67,70]

In our study, when the effect of combined application of propolis and laser on SBS was examined, this combination did not reduce the bond strength compared to both the control group and other groups. We could not find any study on the effect of combined application of propolis with laser on bond strength. In addition, the tubule plug efficiency of propolis and laser combination was found to be higher in SEM examinations (Figure 6). Although the tubule plug efficiency of the propolis-laser combination was higher than the alone application in our study, as far as we know, there is no study examining the tubule plug effectiveness of propolis in combination with the laser. There is a need for in vitro and clinical studies where the results of this study can be discussed in detail.

One of the limitations of this study is that the effectiveness of DDA applications was not re-examined, since SEM examinations were not repeated after waiting in artificial saliva. In addition, performing bond strength tests using a single adhesive prevented the comparison of SBS values of different systems after DDA applications. Moreover, performing SBS experiments only in the early period limits the evaluation of long-term results.

Conclusions

While the combined applications of Er,Cr:YSGG Laser with Sodium Fluoride and propolis showed greater tubule plug efficiency on the dentin surface, the application of the agents alone or in combination with the laser did not create a negative effect on the SBS of Clearfil Tri-S Bond Universal adhesive compared to the control group. According to the findings of this study, it may be recommended to apply these agents in combination with laser in the treatment of DH.

In the light of this study, there is a need for studies in which different adhesive systems are used after different desensitizing agents, propolis, laser and combined applications, and the results of early and late period studies performed under different conditions could be evaluated. Moreover, in addition to the DDA applications and adhesive systems, morphological differences of dentin and the pulpal pressure in vital teeth will also affect the results. For these reasons, the results of this study should be confirmed by future clinical studies.

Conflict of Interest

The authors of this article certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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