

# Evaluation of Dentin Tubule Plugging Efficiencies and Effects on Dentin Surface Roughness of Dentin Desensitizing Agents, the Er,Cr:YSGG Laser, and Their Combination After Erosion-abrasion Cycles: An *In Vitro* Study

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## Clinical Relevance

Combined laser-DDA treatments could be more effective than DDA treatments alone for dentin hypersensitivity (DH) treatment, particularly in challenging oral conditions, such as erosion and abrasion. These applications may help obtain longer-lasting and more satisfying results in the treatment of DH.

## SUMMARY

**Objectives:** The purposes of this *in vitro* study were to evaluate the tubule plugging efficiencies and effects on the surface roughness of dentin of different dentin desensitizing agents (DDAs; Teethmate Desensitizer, Kuraray; Gluma Desensitizer, Kulzer; Clinpro White Varnish, 3M

ESPE; Enamelast, Ultradent) and the Er,Cr:YSGG laser (Biolase, Waterlase), both alone and in combination with DDAs, after application and after an erosion-abrasion cycle.

**Methods and Materials:** For surface roughness examinations, superficial buccal dentin specimens were divided into 10 groups: the control, Teethmate

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Desensitizer, Gluma Desensitizer, Enamelast, Clipro White Varnish, Er,Cr:YSGG Laser, Teethmate Desensitizer-Laser, Gluma Desensitizer-Laser, and Enamelast-Laser, and Clinpro White Varnish-Laser groups. Profilometric analyses and scanning electron microscopy (SEM) examinations were performed after applications and after a 5-day erosive-abrasive cycle. For the statistical analysis of surface roughness measurements, 2-way analysis of variance (ANOVA), 1-way ANOVA, and Tukey *post hoc* test were used.

**Results:** Among the treatments, only DDAs alone did not cause increase in surface roughness after application. All of the laser applications increased the surface roughness of dentin, and after the erosion-abrasion cycle, all of the test groups had increased surface roughness. However, SEM images showed that morphological changes were less frequently observed in all of the experimental groups than in the control group. In addition, all of the laser-DDA combinations had stronger tubule occlusion effects than did DDAs alone, even after erosion-abrasion.

**Conclusions:** All of the test treatments showed protective effects on dentin surfaces against the negative effects of erosion-abrasion. The addition of the laser to DDA applications increased tubular plugging efficiencies of DDAs, and the tubule plugs of the combination treatments were resistant to the erosion-abrasion cycle.

## INTRODUCTION

Dentin hypersensitivity (DH) is defined as short-term and sharp pain arising from exposed dentin in response to thermal, evaporative, tactile, osmotic, or chemical stimuli that stops after stimulus removal and is not associated with other dental defects or diseases.<sup>1-3</sup> DH has a reported prevalence of between 4% and 74% in the general population.<sup>4</sup> The prevalence varies between 72% and 98% among individuals with periodontal disease.<sup>2</sup> It is most often found in permanent canines and premolars in both dental arches. The cervical facial region of the teeth is the most affected region.<sup>4,5</sup>

DH occurs when dentin tubules are exposed to the oral environment as a result of the loss of enamel and/or root surface.<sup>5</sup> Dentin can become exposed for various reasons. In some developmental tooth anomalies, the enamel tissue, which normally covers the dentin anatomically, cannot contact the cementum at the cervical area, so the dentin tissue is exposed.<sup>6</sup> Gingival

recession is another important reason for dentin exposure. Gingival recession and subsequent exposure of the root surface lead to the exposure of the dentinal tubules.<sup>7</sup> Enamel or cementum covering dentin surface can disappear as a result of abrasion, attrition, abfraction, or erosion.<sup>5,6</sup> The consumption of acidic foods and beverages, which are frequently included in today's diets, can also cause enamel loss and exposure of dentin tissue. Gastric acid, with internal causes such as recurrent vomiting, regurgitation, and reflux, can also come into contact with teeth and expose dentin.<sup>8</sup> *In vitro* and clinical studies of DH have shown that acid erosion and tooth brushing can open and widen the dentinal tubules, resulting in the emergence of DH, an increase in its severity, or a decrease in the effectiveness of treatment.<sup>2,9,10</sup>

The most widely accepted theory for DH is the hydrodynamic theory suggested by Brännström and others.<sup>11,12</sup> According to this theory, the fluid inside the dentinal tubules is affected by thermal, physical, or osmotic changes, and these fluid movements stimulate baroreceptors and cause DH.<sup>6</sup> One of the main strategies for treating DH is to occlude the dentinal tubules and thus prevent fluid flow.<sup>13</sup> There are many treatment alternatives for DH, and desensitizing agents with different effect mechanisms have been placed on the dental market.<sup>14</sup> Dentin desensitizing agents (DDAs) can be applied by dentists or by patients at home. Today, the most commonly used DDAs by dentists include dentin tubule occlusive agents and tubule sealant agents.<sup>15</sup> In addition, lasers have been used as an alternative to these agents.<sup>16</sup>

Fluoride varnish applications, which are among the treatments applied by dentists to treat DH, are widely used. Fluoride varnishes contain high fluoride concentrations that can create a mechanical barrier on exposed dentin.<sup>17</sup> Sodium fluoride (NaF, 5%) is used clinically for DH treatment.<sup>18</sup> Topical NaF applications allow for the deposition of calcium fluoride (CaF<sub>2</sub>) on the tooth surface, blocking open dentinal tubules and thus reducing dentin permeability.<sup>18-20</sup> Although clinical studies have supported the beneficial results of fluoride, several clinical studies have suggested that fluoride has limited efficacy.<sup>21,22</sup> The slow dissolution of the formed CaF<sub>2</sub> precipitates in saliva, and the small size of these crystals (approximately 0.05 μm) can cause the barrier to become transient.<sup>4,23</sup>

The combination of NaF with chemicals such as tricalcium phosphate (TCP) has been developed since NaF application is not fully effective in occluding the diameter of the dentinal tubules of sensitive teeth and requires repeated applications. Studies have reported that adding TCP to fluoride increases fluoride

retention in both enamel and dentin and facilitates remineralization.<sup>24,25</sup> In addition, according to the manufacturer of a product containing 5% NaF and TCP, the combination causes the release of calcium and fluoride ions when in contact with saliva,<sup>26</sup> and it has been shown to cause the partial occlusion of dentinal tubules and thereby decrease DH in different studies.<sup>26,27</sup>

Calcium phosphate-containing desensitizers have become a popular topic for biological material research in recent years because of their biocompatibility, bioactivity, and crystal structure similar to that of human teeth.<sup>28</sup> Calcium phosphate-containing desensitizers contain tetracalcium phosphate and dicalcium phosphate anhydrous (DCPA), which can spontaneously form hydroxyapatite (HA). This type of desensitizer has been shown to form a calcium phosphate-rich layer on the dentin surface and thereby decrease dentin permeability. It has also been reported to significantly decrease DH by providing remineralization of early enamel lesions.<sup>29,30</sup> Short- and long-term clinical studies have shown that calcium phosphate-containing desensitizers are effective in reducing DH.<sup>31,32</sup>

Hydroxyethyl methacrylate (HEMA) and glutaraldehyde-containing desensitizers block dentin tubules and show rapid and long-term activity. While HEMA physically occludes the dentinal tubules, glutaraldehyde causes coagulation of plasma proteins in the dentinal tubules. Glutaraldehyde primarily reacts with serum albumin in dentinal tubular fluid, causing albumin to precipitate. It then reacts a second time with albumin and results in the polymerization of HEMA. HEMA contributes to the formation of deep resin tags within dentinal tubules owing to its hydrophilic property.<sup>4,33</sup> Scanning electron microscopy (SEM) and confocal laser scanning microscopy studies have shown that a desensitizer containing HEMA and glutaraldehyde blocked dentinal tubules through protein coagulation.<sup>32,34</sup> Clinical and *in vitro* studies have shown that its success rate in reducing DH varies between 5% and 27%.<sup>28,32,34</sup>

With the development of laser technology, a new treatment option has emerged for DH. The Er,Cr:YSGG laser is a medium-power laser that can be used in soft and hard tissues without damaging the pulp and surrounding tissues because of the specific properties of its wavelength (2.78  $\mu\text{m}$ ). The Er,Cr:YSGG laser can cut enamel and dentin because of its high absorption in water and its strong absorption by hydroxyl radicals in the HA structure.<sup>35,36</sup> This laser causes insoluble salts to accumulate in the dentinal tubules by evaporation of the dentinal tubular fluid. There have been clinical and *in vitro* studies reporting that this accumulation enables the occlusion of dentinal tubules and the reduction of

DH.<sup>37-40</sup> Considering the increase in the prevalence of DH in recent years, alternative treatments are needed that provide long-term efficacy. For this reason, the combined application of DDAs with lasers has been explored for the treatment of DH.<sup>41</sup>

Studies have shown the effectiveness of the combined use of DDAs and lasers in the treatment of DH.<sup>28,39</sup> However, it has been reported that laser applications can cause cracks and irregularities on dentin surfaces.<sup>28</sup> Moreover, factors such as erosion and abrasion can change the surface properties of tooth tissues, which can increase surface roughness.<sup>42</sup> A significant increase in surface roughness causes plaque retention and an increase in bacterial adhesion on dental tissues, creating a surface prone to caries formation.<sup>42,43</sup> Caries formation is one of the most important processes affecting the survival rate of teeth in the mouth.

A limited number of studies have investigated the effects of DDAs, laser application, and the combined application of DDAs and lasers on surface roughness after erosion-abrasion cycles.<sup>26,39</sup> Moreover, to the authors' knowledge, no comprehensive study comparing all of these agents has been conducted.

Therefore, the aim of this study was to investigate the effects of DDA applications with different contents (calcium phosphate; HEMA and glutaraldehyde; 5% NaF and TCP 5%) and Er,Cr:YSGG laser, which can cause insoluble salts to accumulate in the dentinal tubules via the evaporation of the dentinal tubular fluid and thereby enable dentinal tubule occlusion and DH reduction and DDA–laser combinations on dentin surface roughness and tubule plugging efficiency.

Null hypotheses of this study were as follows:

1. DDA applications, laser application, and combination applications to the dentin surface do not have significant effects on surface roughness.
2. DDA applications, laser application, and combination applications do not have significant effects on surface roughness after an erosion-abrasion cycle.

## METHODS AND MATERIALS

In this *in vitro* study, calcium phosphate-containing, HEMA- and glutaraldehyde-containing, 5% NaF- and TCP-containing, and calcium- and fluoride-releasing DDAs and Er,Cr:YSGG laser, which enables dentinal tubule occlusion and DH reduction, were applied separately or in combination to dentin specimens.

## Power Analysis

Power analysis revealed that the minimum sample size required to detect a significant difference was 9 per

group (90 in total) assuming a type I error ( $\alpha$ ) of 0.05, a power (1- $\beta$ ) of 0.8, and an effect size of 2 for after the erosion-abrasion cycle. We used 12 specimens for each group.

### Specimen Preparation

A total of 140 intact, permanent third molar teeth without caries or cracks extracted for orthodontic or oral reasons were used for this study. Before the extractions, the patients were informed that their teeth would be used for research purposes, and a consent form was read and signed by each patient. After extraction, soft tissue residues and bone particles on the teeth were removed with a periodontal curette. The teeth were maintained in 0.1% thymol solution before the experiments. The buccal surfaces of the teeth were cut vertically in the mesiodistal direction under water cooling with the aid of a low-speed precision cutting device (Micra Cut 125, Metkon, Bursa, Turkey) and 0.3-mm-thick diamond discs (Diamond cut-off wheel B 102, ATM GMBH, Mammelzen, Germany). The enamel tissue was removed, and the superficial dentin tissue was exposed. Then the teeth were cut horizontally from the apex of the enamel-cementum junction to obtain dentin specimens, which were embedded in autopolymerizing acrylic resin (Imicryl, SC, Konya, Türkiye) for use in the experiments. Test specimens were sanded using 600, 800, 1200, 1500, and 2000 grit silicon carbide abrasive papers in a polishing machine (Beta Grinder Polisher, Buehler, IL, USA) with a 200-RPM rotation speed to form a standard smear layer and obtain a smooth surface.

### Application of DDAs, Laser, and DDA-Laser Combinations

Dentin specimens were maintained in 17% ethylenediaminetetraacetic acid (EDTA; Werax, Tunadent, Izmir, Turkey) solution for 5 minutes to open the dentinal tubules and remove the smear layer. The specimens were washed under running water to remove residue and sonicated in distilled water for 5 minutes with an ultrasonic cleaner.

A total of 140 specimens were divided into 10 test groups ( $n=14$ ) (control, TMD [Teethmate Desensitizer, Kuraray Noritake Dental Inc, Okayama, Japan], GD [Gluma Desensitizer, Heraeus Kulzer, GmbH & Co, Hanau, Germany], EN [Enamelast, Ultradent, South Jordan, UT, USA], CWV [Clinpro White Varnish, 3M ESPE, St Paul, MN, USA], L [Er,Cr:YSGG laser, San Clemente, CA, USA], TMD-L [Teethmate Desensitizer–Er,Cr:YSGG laser], GD-L [Gluma Desensitizer–Er,Cr:YSGG laser], EN-L [Enamelast–Er,Cr:YSGG laser], and CWV-L [Clinpro White

Varnish–Er,Cr:YSGG laser]). Two specimens from each group were used for SEM analysis.

DDAs were applied to the TMD, GD, EN, and CWV groups according to the manufacturers' instructions. Er,Cr:YSGG laser application was applied to group L. Er,Cr:YSGG laser application was performed at 0.25 W, 20 Hz, and 12.5 mJ. Laser irradiation was performed in noncontact mode with a pulse width of 140  $\mu$ s using a 6-mm MZ6 tip with a 600- $\mu$ m diameter operated in 0% water and 10% air. A total of 20 seconds of irradiation was applied vertically and horizontally (10 seconds each) from the 1-mm irradiation distance to the dentin surface.

In the TMD-L, GD-L, EN-L, and CWV-L groups, DDAs were first applied to the dentin specimens, then Er,Cr:YSGG laser application was performed. No applications were performed in the control group. Manufacturer instructions are reported in Table 1.

### Erosion-Abrasion Cycle

For all of the groups, a modified 5-day erosion-abrasion model proposed by Scaramucci and others was used.<sup>9</sup> A 0.3% citric acid solution ( $\text{pH} \approx 2.45$ ) was used to simulate erosion in the mouth. The specimens were immersed in citric acid solution at room temperature for 2 minutes four times per day without stirring. After each episode of erosion, the specimens were immersed for 60 minutes in artificial saliva (0.213 g/l  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.738 g/l  $\text{KH}_2\text{PO}_4$ , 1.114 g/l KCl, 0.381 g/l NaCl, and 12 g/l Tris buffer; pH adjusted to 7 with KOH), rinsed with distilled water, and gently dried with absorbent paper. The erosion-abrasion cycle procedures are reported in Table 2.

A brushing mechanism was created with a pressure of 2 N to brush the teeth. Tooth brushing was performed twice per day for 15 seconds in the middle of the first and last remineralization periods using electric brushes (Oral-B Professional, Braun, Frankfurt, Germany). Oral-B Sensitive (Oral-B Professional) was used as the brush head. Brushing was performed with a slurry made from Colgate Maximum Anti-caries Protection dentifrice and artificial saliva (1:3 w/w) (Colgate-Palmolive, SP, Brazil) for all of the groups. Total exposure time of the specimens to the dentifrice slurries in each brushing episode was 2 minutes. Only one operator performed the tooth brushing procedures. During the night, specimens were stored in a humid environment at 4°C.

### Profilometric Analysis

A contact profilometer device (Marsurf PS10, Mahr, Göttingen, Germany) was used for surface roughness measurements. First, profilometric



Table 1: Manufacturer Instructions

DDAs	Manufacturer	Composition	Lot Number	Application Instructions
Teethmate Desensitizer	Kuraray Noritake Dental Inc	Powder: tetracalcium phosphate, dicalcium phosphate anhydrous Liquid: water, preservative	041154	Mix the powder and liquid (15 s) carefully, apply with the applicator, rub for 30 s, and rinse with water.
Gluma Desensitizer	Heraeus Kulzer	35% 2-hydroxyethyl methacrylate, 5% glutaraldehyde	K010516	Apply to clean dentin with a cotton pellet or brush and allow it to dwell for 30-60 s. Air dry and rinse.
Clinpro White Varnish	3M ESPE	Sodium fluoride (5%), tricalcium phosphate, xylitol	NA56453	Mix according to the dosage guide and apply to clean and dry dentin.
Enamelast	Ultradent	Sodium fluoride (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.
Er,Cr:YSGG laser	Biolase		18002402	A total of 20 s of irradiation was performed vertically and horizontally from the 1-mm irradiation distance to the dentin.

Abbreviations: DDA, dentin desensitizing agents; TCP, tricalcium phosphate.

analysis was performed after EDTA treatment for sample standardization of all of the groups. Other measurements were performed after DDA applications and at the end of the erosion-abrasion cycle. The dentin specimens were measured in three different areas, and the averages were calculated. The results are expressed in micrometers. The device sensor scans an area of 1.5 mm.

### SEM Evaluation

Twenty dentin specimens were prepared as one sample from each group and one sample from each group

subjected to an erosion-abrasion cycle to be examined by SEM (EVO LS 10, Zeiss, Oberkochen, Germany). Dentin specimens were covered with a thin layer of gold film. SEM examinations were performed at 5 kV with magnifications between 2000 × and 7500 ×. The effectiveness of the DDA lasers and combination applications on dentinal tubule occlusion was examined by SEM. Then the examined dentin samples were broken vertically, and the effects of the agents on the interface morphology of the dentinal tubules were examined.

Table 2: Erosion-Abrasion Cycle Procedures

Steps	Procedures	Application
1	Erosion Remineralization Tooth brushing Remineralization	Citric acid (2 min) Artificial saliva (30 min) Exposure to artificial saliva and toothpaste slurry for 2 min; 15 s of active brushing Artificial saliva (30 min)
2	Erosion Remineralization	Citric acid (2 min) Artificial saliva (60 min)
3	Step 2 repeated.	
4	Step 1 repeated.	
5	Kept in a humid environment at 4°C overnight.	

## Statistical Analysis

SPSS for Windows 17.0 (Statistical Package for Social Sciences, SPSS Inc, Chicago, IL, USA) was used for statistical analysis. Descriptive statistics are reported as the mean and standard deviation. The Shapiro-Wilk test was used to determine whether the data conformed to a normal distribution. After ensuring normality, two-way ANOVA (group x time) was applied for repeat samples. One-way ANOVA and Tukey *post hoc* test were used to analyze differences between groups within each time frame. In addition, the *t*-test was used for paired samples while analyzing time-dependent changes within each group. Differences at the level of  $p < 0.05$  were considered statistically significant.

## RESULTS

### Profilometric Analysis Results

Interactions are reported in Table 3. According to the interaction table, significant differences were found among the groups, and significant differences were found within the groups according to time ( $p < 0.001$ ).

*Comparisons of Surface Roughness Among Baseline (T0), After Application (T1), and After the Erosion-Abrasion Cycle (T2) Within Groups* — The mean and standard deviation values of surface roughness and significant differences within groups for all of the evaluation periods are shown in Table 4. For the control group, because the T0 and T1 surface roughness values were the same, no comparison was performed. There was a significant difference between T1 and T2 measurements ( $p < 0.001$ ) in the control group. Surface roughness increased in the control group after the erosion-abrasion cycle (Table 4).

While there was no significant difference between T0 and T1 measurements in the TMD, GD, EN, or CWV group ( $p > 0.05$ ), there were significant differences between T1 and T2 measurements ( $p < 0.001$ ), with surface roughness increasing after the erosion-abrasion cycle in these groups (Table 4).

There was a significant difference between T0 and T1 measurements in each of the L, TMD-L, GD-L, CWV-L, and EN-L groups ( $p < 0.05$ ; EN-L,  $p < 0.001$ ). Surface roughness values increased in all of the laser-applied groups. In addition, the differences between T1 and T2 measurements were significant in these groups ( $p < 0.001$ ). Surface roughness increased after the erosion-abrasion cycle.

*Comparisons of the Surface Roughness Among Groups After Application (T1) and After the Erosion-Abrasion Cycle (T2)* — There were no statistically significant differences in baseline value among the groups ( $p > 0.05$ ). Statistical comparisons of T1 and T2 values among the groups are shown in Table 5.

After application, there was no significant difference in surface roughness among the TMD, DDA-only, and L groups ( $p > 0.05$ ). However, compared with the TMD group, the TMD-L group showed a significant increase in surface roughness ( $p < 0.05$ ). After the erosion-abrasion cycle, there was no significant difference between the control and DDA-only groups ( $p > 0.05$ ). The surface roughness values of the L and TMD-L groups were significantly increased compared to that of the TMD group after the cycle ( $p < 0.001$ ).

After application, there was no significant difference in surface roughness among the GD, DDA-only, and L groups ( $p > 0.05$ ). Group GD-L showed a significant increase in surface roughness compared with group GD after application ( $p < 0.05$ ). After the erosion-abrasion cycle, surface roughness of L and GD-L groups was significantly increased compared to that of the GD group ( $p < 0.001$ ).

There was no significant difference in surface roughness after application between the EN group and the DDA-only groups ( $p > 0.05$ ). The increases in surface roughness in the L ( $p < 0.05$ ) and EN-L ( $p < 0.001$ ) groups relative to the values in the EN group were significant. After the erosion-abrasion cycle, the surface roughness of the L and EN-L groups significantly differed from that of the EN group ( $p < 0.001$ ).

Source	Type III Sum of Squares	df	Mean Square	F	Significance
Intercept	25.263	1	25.263	9209.027	$p < 0.001$
Group	1.470	9	0.163	59.553	$p < 0.001$
Error (Group)	0.302	110	0.003		
Time	6.325	1.344	4.706	2572.677	$p < 0.001$
Time x Group	1.590	12.096	0.131	71.850	$p < 0.001$
Error	0.270	147.835	0.002		

Abbreviation: ANOVA, analysis of variance.

Table 4: Comparisons of the Surface Roughness of Different DDAs, Laser, and Combination Applications Within Groups<sup>a</sup>

Group <sup>b</sup>	Baseline (T0)	After Application (T1)	After Erosion-Abrasion (T2)	Within-Group Evaluation
Control	0.174 (0.032) A	0.174 (0.032) A	0.360 (0.021) B	T1/T2 $p < 0.001$
TMD	0.148 (0.031) A	0.154 (0.035) A	0.290 (0.031) B	T1/T2 $p < 0.001$
GD	0.147 (0.032) A	0.152 (0.030) A	0.309 (0.028) B	T1/T2 $p < 0.001$
EN	0.141 (0.038) A	0.146 (0.036) A	0.300 (0.026) B	T1/T2 $p < 0.001$
CWV	0.145 (0.028) A	0.148 (0.038) A	0.315 (0.033) B	T1/T2 $p < 0.001$
L	0.179 (0.022) A	0.197 (0.033) B	0.705 (0.068) C	T0/T1 $p = 0.013$ $p < 0.05$ T1/T2 $p < 0.001$
TMD-L	0.180 (0.038) A	0.209 (0.042) B	0.604 (0.078) C	T0/T1 $p = 0.016$ $p < 0.05$ T1/T2 $p < 0.001$
GD-L	0.176 (0.027) A	0.201 (0.028) B	0.681 (0.076) C	T0/T1 $p = 0.034$ $p < 0.05$ T1/T2 $p < 0.001$
EN-L	0.158 (0.031) A	0.221 (0.051) B	0.541 (0.078) C	T0/T1 $p < 0.001$ T1/T2 $p < 0.001$
CWV-L	0.176 (0.031) A	0.201 (0.035) B	0.417 (0.045) C	T0/T1 $p = 0.022$ $p < 0.05$ T1/T2 $p < 0.001$

Abbreviations: CWV, Clinpro White Varnish; DDA, dentin desensitizing agents; EN, Enamelast; GD, Gluma Desensitizer; L, laser; TMD, Teethmate Desensitizer.

<sup>a</sup>Values with different uppercase letters within a row indicate statistically significant differences at different time intervals (among baseline, after application, and after erosion-abrasion cycle) within a group, as determined by Bonferroni test. The significance of mean differences was evaluated at the 0.05 level.

<sup>b</sup>DDA-only applications (TMD, GD, EN, and CWV) did not cause an increase in surface roughness after application (T0-T1;  $p > 0.05$ ). However, the L and combined DDA-laser applications (TMD-L, GD-L, CWV-L, and EN-L) caused increases in surface roughness ( $p < 0.05$ ; EN-L,  $p < 0.001$ ). After the erosion-abrasion cycle, surface roughness was increased in all of the test groups, and there were significant differences between T1 and T2 measurements in all groups ( $p < 0.001$ ).

After application, the surface roughness values did not significantly differ between the CWV group and the DDA-only groups ( $p > 0.05$ ). Surface roughness was significantly increased in the CWV group compared with that in the L and CWV-L groups ( $p < 0.05$ ). After the erosion-abrasion cycle, the surface roughness of the L and CWV-L groups was significantly increased compared to that of the CWV ( $p < 0.001$ ).

The L group showed a significant increase in surface roughness compared with that of the EN and CWV groups after application ( $p < 0.05$ ). In addition, after application, surface roughness did not significantly differ among any of the combination groups ( $p > 0.05$ ).

After the erosion-abrasion cycle, group L showed a significant increase in surface roughness compared to all of the other groups except the GD-L group ( $p < 0.001$ ).

Among the combined-application groups, the GD-L group had the highest surface roughness, and there were significant differences between the GD-L group and the CWV-L ( $p < 0.001$ ), EN-L ( $p < 0.001$ ), and TMD-L ( $p < 0.05$ ) groups. In addition, among the combined-application groups (CWV-L, TMD-L, GD-L, and EN-L), the CWV-L group had the lowest surface roughness ( $p < 0.001$ ).

### SEM Results

In the SEM images, in contrast to the control group and the other DDA-only groups, the TMD group generally showed widespread tubular occlusion on the buccal surfaces. In the interface examinations, the plugs were observed to extend into the tubules. In the

Table 5: Comparisons of the Surface Roughness of Different DDAs, Laser, and Combination Applications Among the Groups<sup>a</sup>

Group	After Application (T1) <sup>b</sup>	After Erosion-Abrasion (T2) <sup>c</sup>
Control	0.174 (0.032)	0.360 (0.021) With L ( $p<0.001$ ) With TMD-L ( $p<0.001$ ) With GD-L ( $p<0.001$ ) With EN-L ( $p<0.001$ )
L	0.197 (0.033) With EN ( $p=0.03$ ; $p<0.05$ ) With CWV ( $p=0.04$ ; $p<0.05$ )	0.705 (0.068) With TMD ( $p<0.001$ ) With GD ( $p<0.001$ ) With EN ( $p<0.001$ ) With CWV ( $p<0.001$ ) With TMD-L ( $p<0.001$ ) With EN-L ( $p<0.001$ ) With CWV-L ( $p<0.001$ )
TMD	0.154 (0.035) With TMD-L ( $p=0.13$ ; $p<0.05$ )	0.290 (0.031) With TMD-L ( $p<0.001$ )
TMD-L	0.209 (0.042)	0.604 (0.078) With GD-L ( $p=0.02$ ; $p<0.05$ ) With CWV-L ( $p<0.001$ )
GD	0.152 (0.030) With GD-L ( $p=0.44$ ; $p<0.05$ )	0.309 (0.028) With GD-L ( $p<0.001$ )
GD-L	0.201 (0.028)	0.681 (0.076) With EN-L ( $p<0.001$ ) With CWV-L ( $p<0.001$ )
EN	0.146 (0.036) With EN-L ( $p<0.001$ )	0.300 (0.026) With EN-L ( $p<0.001$ )
EN-L	0.221 (0.051)	0.541 (0.078) With GD-L ( $p<0.001$ ) With CWV-L ( $p<0.001$ )
CWV	0.148 (0.038) With CWV-L ( $p=0.02$ ; $p<0.05$ )	0.315 (0.033) With CWV-L ( $p<0.001$ )
CWV-L	0.201 (0.035)	0.417 (0.045)

Abbreviations: CWV, Clinpro White Varnish; DDA, dentin desensitizing agents; EN, Enamelast; GD, Gluma Desensitizer; L, laser; TMD, Teethmate Desensitizer.

<sup>a</sup>In the columns, significant p-values according to Bonferroni test, indicating differences among different groups within the same time period, are indicated.

<sup>b</sup>After application (T1), surface roughness did not increase among the DDA-only groups ( $p>0.05$ ), but there was a significant difference between each DDA group and the corresponding DDA-laser combination group (TMD-TMD-L, GD-GD-L, CWV-CWV-L,  $p<0.05$ ; EN-EN-L,  $p<0.001$ ). In addition, there was no significant difference in surface roughness among all of the DDA-laser combination groups after application ( $p>0.05$ ).

<sup>c</sup>After the erosion-abrasion cycle (T2), there was no significant difference among the DDA-only groups, but there was a significant difference between each DDA group and the corresponding DDA-laser combination group ( $p<0.001$ ). In addition, the L group showed higher surface roughness than all other groups except the GD-L group ( $p<0.001$ ).



images obtained after the erosion-abrasion cycle, some tubule plugs had been removed, but most of the tubule plugs remained, and the levels of surface deterioration and tubular enlargement were quite low compared to those of the control group (Figures 1 and 2).

The SEM images of the GD group revealed a small number of plugs on the buccal surfaces, which did not completely block the tubules. In the images, most of the tubules were open. In the interface images, although plugs were observed in the dentinal tubule orifices, no plugs were observed extending into the tubules. Although open dentinal tubules were generally observed after the erosion-abrasion cycle, surface deformations and irregularities were far less common in the GD group than in the control group (Figures 1 and 2).

In the SEM images of the EN and CWV groups, the dentin tubules were generally open on the buccal surfaces, but there were closed or narrowed tubules in some areas. In the interface images, plugs in the tubule orifices were observed; some of the plugs extended into the tubules in the EN group, and the presence of an occluding layer on the surface in the CWV group was observed. The SEM images showed that after the erosion-abrasion cycle, the tubules in the EN and CWV groups were generally open, but the levels of deterioration and tubular enlargement on the surfaces were far lower than those in the control group (Figures 1 and 2).

In the laser-treated groups, the SEM images showed that the dentinal tubules were generally narrowed, with

some occluded and open dentinal tubules also present. In addition, local short cracks and irregularities were observed on the dentin surface. On the interface images, there were plugs in the dentinal tubules and local depression areas on the surfaces. After the erosion-abrasion cycle, there were still plugged dentinal tubules, and local short cracks and irregularities were still observed on the dentin surface, but they were not more abundant than they were after application, and the extent of degradation and abundance of irregularities on the surface was lower than those in the control group (Figures 1 and 2).

In the SEM images of the TMD-L-treated groups, most of the dentinal tubules were closed and narrowed; rarely, open dentinal tubules were also present. In the interface images, some of the tubule orifices were obstructed, and the plugs progressed toward the inner surface of some dentinal tubules. After the erosion-abrasion cycle, closed or narrowed dentinal tubules were still predominant in the SEM images, but open dentinal tubules were also observed. Surface irregularities were far fewer, and degradation was much lower than those in the control group (Figures 2 and 3).

In the GD-L-treated group, the SEM images revealed closed and narrowed dentinal tubules and partially open dentinal tubules. In the interface images, tubule plugs were apparent in the tubule orifices. The SEM images obtained after the erosion-abrasion cycle showed that closed dentinal tubules remained. Surface irregularities and degradation were far less widespread in the GD-L group than in the control group (Figures 2 and 3).

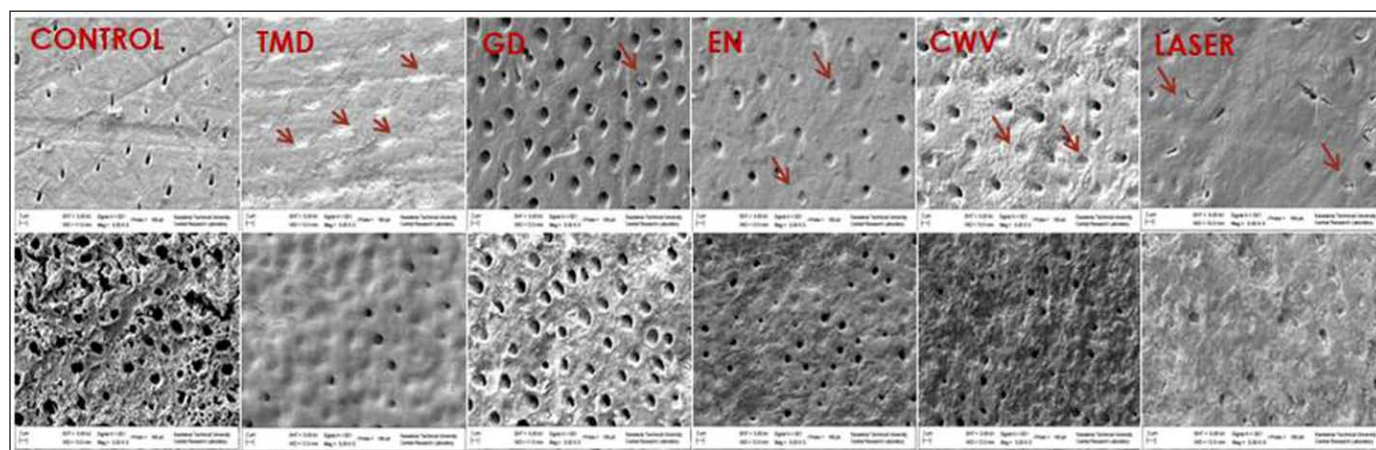


Figure 1. SEM images of the DDA-only groups and laser-applied groups (the upper images were after the application, and the lower images were after the erosion-abrasion cycle of the same group). According to the after application (upper) images, tubules were occluded in the TMD group. Although there were locally tubular plugs in the GD group, no completely occluded tubules were observed. Although partially occluded tubules were present in the EN and CWV groups, the majority of them were open. There were mostly narrowed, partially occluded tubules, in addition to local cracks and irregularities in the laser group. After the erosion-abrasion cycle (lower images), irregularities on the surface were less frequently observed than in the control group. Arrows indicate occluded dentinal tubules. CWV, Clinpro White Varnish; DDA, dentin desensitizing agents; EN, Enamelast; GD, Gluma Desensitizer; L, laser; SEM, Scanning electron microscopy; TMD, Teethmate Desensitizer.

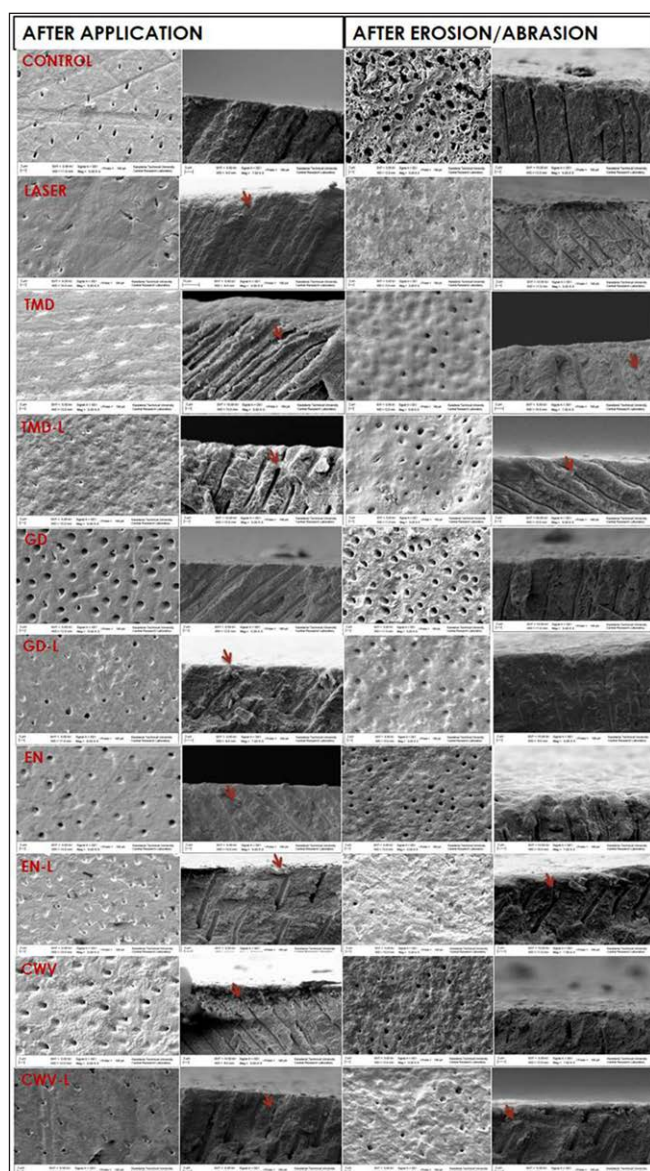


Figure 2. Comparisons of buccal and interface SEM images after application for all of the groups (only DDA, laser, and combined groups). There were more occluded dentinal tubules in combined applications. Arrows indicate plugs that run inward to the tubules in the interface images. CWV, Clinpro White Varnish; DDA, dentin desensitizing agents; EN, Enamelast; GD, Gluma Desensitizer; L, laser; SEM, scanning electron microscopy; TMD, Teethmate Desensitizer.

In the SEM images of the EN-L and CWV-L groups, the dentinal tubules were generally closed, and plugs in the tubule orifices were observed. In the interface images, tubular plugs were still present on the interface images of both groups. In the SEM images of these groups after the erosion-abrasion cycle, most of the tubule plugs continued to occlude the dentinal tubules, and the extents of surface irregularities and

degradation were far lower than those in the control group (Figures 2 and 3).

## DISCUSSION

DH is a common condition in the general population and is an increasing problem, especially in developed countries. Although people are able to delay the loss of teeth by maintaining their oral hygiene, the risk of developing DH is increasing because of various factors.<sup>44</sup> Today, DH is an important problem that should be emphasized in dentistry because of its increasing prevalence, negative effects on patient quality of life, and the problems it poses for oral hygiene practices.

In the present study, DDAs with different contents (TMD, GD, CWV, EN) and the Er,Cr:YSGG laser were applied to dentin surfaces alone and in combination (TMD-L, GD-L, CWV-L, EN-L). First, surface roughness changes in dentin tissue and tubule plugging effectiveness were investigated following the application of DDAs on dentin surfaces alone or in combination with laser. Then, using the erosion-abrasion cycle model, the changes in the surface roughness and tubule plugging efficiency of all of the test groups after an erosion and abrasion cycle were investigated. No significant increase in surface roughness was observed in the DDA-only groups (TMD, GD, EN, CWV) after application compared with the baseline values ( $p > 0.05$ ), whereas in the Er,Cr:YSGG laser group (L) and combined-application groups, the increases in surface roughness from baseline to after application were significant ( $p < 0.05$ ). Therefore, our first null hypothesis that DDA applications, laser application, and combination applications to the dentin surface do not significantly affect surface roughness was partially rejected.

Examination of the SEM images revealed a predominance of occluded dentin tubules in the TMD and L groups; although open dentin tubules were predominant in the EN and CWV groups, there were also closed dentin tubules in these groups. In the GD group, although there were tubule plugs and narrowed dentin tubules, no completely closed dentin tubules were apparent in the images. The increases in surface roughness in the groups treated with the Er,Cr:YSGG laser (TMD-L, GD-L, EN-L, and CWV-L) after application were significant compared with the baseline values ( $p < 0.05$ ). However, in the SEM images, more occluded dentinal tubules were observed in the combined-application groups than in the DDA-only groups.

The erosion and abrasion cycle decreases the tubule plugging effectiveness of DDAs and causes the removal of tubule plugs. Therefore, it complicates



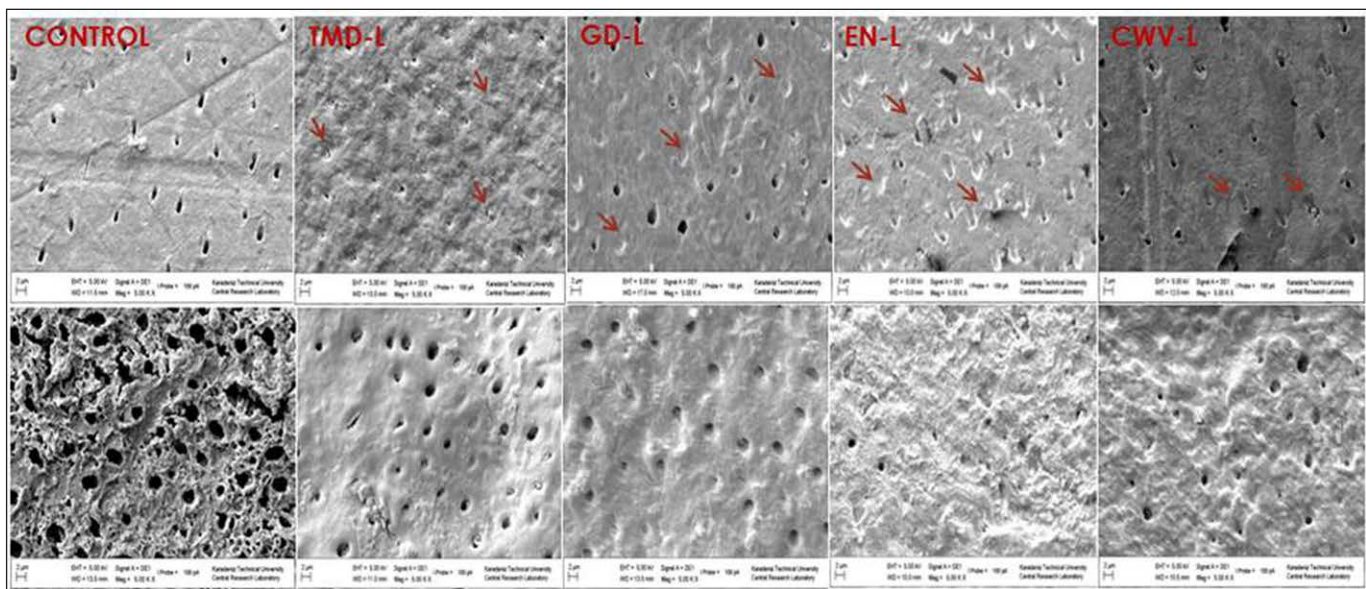


Figure 3. SEM images of the groups of lasers combined with DDAs (the upper images were after the application groups, and the lower images were after the erosion-abrasion cycle in the same group). It was observed that occluded dentinal tubules were the majority after the applications for all of the combined groups. After the erosion-abrasion cycle, fewer irregularities were observed on the surface for all of the combined groups compared to the control group. Arrows indicate occluded dentinal tubules. CWV, Clinpro White Varnish; DDA, dentin desensitizing agents; EN, Enamelast; GD, Gluma Desensitizer; L, laser; SEM, scanning electron microscopy; TMD, Teethmate Desensitizer.

the effectiveness of long-term DH treatment and could cause the reemergence of DH by opening the tubules after treatment.<sup>9,10</sup> In addition, the changes in roughness caused by erosion and abrasion on the dentin surface can increase the risk of dental caries.<sup>42,43</sup> For these reasons, determining how DDA-treated dentin is affected by erosion and abrasion could provide important insight into the clinical effectiveness of DDAs.

In the present study, the increases in surface roughness after the erosion-abrasion cycle compared with after application were statistically significant in all of the groups ( $p < 0.001$ ). The DDA only, the Er,Cr:YSGG laser, and their combined application could not prevent an increase in roughness on the dentin surface. Therefore, our second null hypothesis that DDA-only applications, laser application, and combined DDA laser applications do not significantly affect surface roughness after an erosion-abrasion cycle was rejected.

The SEM images revealed that despite the increases in surface roughness after the erosion-abrasion cycle, the morphological changes and irregularities on the dentin surface were far fewer in all of the treatment groups than in the control group.

TMD is a calcium phosphate-based desensitizing agent. The advantage of TMD is that the supersaturation of saliva with Ca and  $\text{PO}_4$  contributes to further HA crystal growth in the TMD layer on the tooth surface over the long term.<sup>32,45</sup> In the present study, while there

was no significant difference in surface roughness between the TMD group and the control group after application ( $p > 0.05$ ), there was a significant increase after the erosion-abrasion cycle compared to the after-application measurements ( $p < 0.001$ ). TMD could not prevent the increase in surface roughness on the dentin surface after the erosion-abrasion cycle. SEM images of the TMD group after application showed that the dentin tubules were mostly occluded, consistent with studies in the literature.<sup>29,46,47</sup> In the SEM images, occluded dentinal tubules were more common in the TMD group than in the other DDA-only groups.

In an *in vitro* study by Ishihata and others, SEM images showed that all of the dentinal tubules were closed following TMD.<sup>48</sup> However, in an *in vitro* study by Machado and others, TMD only partially occluded the dentinal tubules. In that study, SEM images after a 5-day erosion-abrasion cycle showed that the deposits in the tubule orifices of the dentinal tubule had been removed after the cycle.<sup>47</sup> In contrast, in the present study, the TMD group still exhibited closed tubules after the erosion-abrasion cycle. Although TMD could not prevent the increase in surface roughness after the erosion-abrasion cycle, it reduced the surface deformation compared to that in the control group, as shown in the SEM images.

The ability of TMD to maintain tubule occlusion and reduce the formation of irregularities on the surface could be attributable to the chemical composition of

TMD. Calcium and phosphate ions dissolved from TTCP and DCPA are precipitated as HA during the setting of the material, which can form an erosion-resistant layer depending on the solubility of the HA formed. In addition, the clinical efficacy of TMD has been supported by short- and long-term follow-up studies under normal clinical conditions.<sup>31,32,49</sup> According to the findings of this study, TMD can maintain its tubular plugging efficacy under challenging clinical conditions such as erosion-abrasion.

GD is a desensitizing agent containing 5% glutaraldehyde and 35% HEMA. Glutaraldehyde is a biological fixative, and it has been suggested that it reacts with plasma proteins in dentin fluid and obstructs dentinal tubules.<sup>50</sup> In this study, a significant increase was observed in the surface roughness of the GD group after the erosion-abrasion cycle compared with the values after application ( $p < 0.001$ ), and there was no significant difference from the control group ( $p > 0.05$ ). In the SEM images of the GD group, partial tubule plugs were apparent in the dentin tubule orifices, but no dentin images showed completely occluded tubules. In addition, occluded dentinal tubules were not seen in this group after the erosion-abrasion cycle.

In an *in vitro* study, Kolker and others detected a thin layer on dentin surfaces after GD was applied but reported that most of the dentinal tubules were opened.<sup>51</sup> In another *in vitro* study conducted to determine the resistance of GD to acid erosion, the application of an acidic solution (Coca-Cola, pH: 3.15) after GD application on the cervical dentin surface was reported to cause the complete dissolution of the GD.<sup>52</sup> Another *in vitro* study revealed that open dentinal tubules were predominant in a GD group after treatment, similar to the pattern in the control group; however, SEM images revealed that the diameter of the tubules had narrowed. In that study, after a 5-day erosion-abrasion cycle, open dentinal tubules were predominant, but a layer covering the tubule orifices was observed. After the cycle, it was shown that the dentin surface with GD had lower dentin permeability than the control surface and some chemical and mechanical resistance.<sup>53</sup>

In the present study, GD application could not prevent the increase in surface roughness after the erosion-abrasion cycle. However, after the erosion-abrasion cycle, surface deformations and irregularities, as observed in the SEM images, were far fewer in the GD group than in the control group. These findings suggested that GD could protect the dentin surface from erosion, although there were no completely occluded dentinal tubules in GD group. Most likely, the preventive effect of GD is due to the reaction of GD with plasma proteins in the tubules. In addition, its

fixative effect on dentin tissue could have protected the dentin surface from erosion.

EN is a desensitizing agent containing 5% NaF. As a result of the reaction between NaF and calcium ions,  $\text{CaF}_2$  crystals are formed, which accumulate in the dentinal tubules.<sup>54</sup> Varnishes containing high concentrations of fluoride are the most widely used desensitizing products and provide highly satisfactory results in the short term after application.<sup>55</sup> The short-term effectiveness of fluoride varnish has been demonstrated in the literature, but its long-term results have been questioned. Saliva can dissolve  $\text{CaF}_2$  crystals, and pain from sensitive teeth can reappear.<sup>54-56</sup> A 6-month clinical study showed that NaF cannot prevent DH in the long term and that DH can reappear.<sup>57</sup> The low effectiveness of NaF in the long term can be attributed to its insufficient adhesion to dentinal tubules and the small diameter of the  $\text{CaF}_2$  crystals formed (approximately  $0.05 \mu\text{m}$ ).<sup>57</sup>

In our study, the SEM images of the EN-treated groups showed that the dentinal tubule orifices were generally open, but there were closed or narrowed tubules in some areas. After the erosion-abrasion cycle, most of the tubules were open, but some closed tubules remained. The surface roughness of the EN-treated specimens was not significantly different from that of the control group after the erosion-abrasion cycle ( $p > 0.05$ ). Although EN did not prevent the surface roughness increase after the erosion-abrasion cycle, it partially protected the surface, as evidenced by the SEM images showing the presence of closed dentin tubules and fewer irregularities in the EN group compared to the control group.

In a study by Alencar and others, NaF varnish was applied to the eroded surface, and surface roughness and SEM images were examined after a 3-day erosion-abrasion cycle.<sup>8</sup> Partial occlusions of the dentinal tubules were observed in the SEM images. However, noncontact profilometer images showed that NaF varnish was unable to fully protect the dentin surface during the erosion-abrasion cycle.<sup>5</sup> In contrast, Garofalo and others reported that NaF varnish can significantly reduce dentin loss, although it cannot provide significant tubular occlusion after a 5-day erosion-abrasion cycle.<sup>26</sup> A similar study showed that NaF varnish failed to maintain tubule plugging efficacy after a 5-day erosion-abrasion cycle.<sup>47</sup> In our study, the fluoride varnish was removed from the dentin surface after being maintained on the dentin surface for 6 hours, in accordance with the manufacturer's instructions; this removal could decrease the effectiveness of the varnish. In *in vitro* studies, after its application to dental hard tissues, varnish is commonly removed from the surface before



analysis. However, tubule plugs could be damaged during varnish removal from dentin surfaces.<sup>59,60</sup> Since fluoride varnish was not removed from the tooth surface clinically in this manner, the protective capacity of the varnish might have been increased.

CWV is a varnish containing TCP and 5% NaF. It has a higher  $\text{CaF}_2$  precipitation potential than other varnishes because of the presence of calcium in its formula. Karlinsey and others reported that the addition of TCP to fluoride toothpaste increased fluoride retention in both enamel and dentin and facilitated remineralization.<sup>24</sup> Another *in vitro* study showed that the diameters of dentinal tubules narrowed significantly after CWV application.<sup>27</sup> In the present study, SEM images of the CWV group showed that although some of the dentinal tubule orifices were narrowed or completely occluded, most of them were open. The tubular plugging effectiveness of CWV could be reduced because of its removal from the dentin surface after waiting for 24 hours as per the manufacturer's instructions. Although the surface roughness of the CWV-applied group was not significantly different from that of the control group after the erosion-abrasion cycle ( $p>0.05$ ), the surface irregularities observed via SEM were decreased in this group compared to the control group. In addition, in the SEM images, closed dentin tubules remained after erosion-abrasion. These findings are similar to those of Garofola and others. In their study, after erosion-abrasion cycles, closed dentinal tubules were found in CWV-applied samples, but the number of open dentinal tubules did not differ from that in the control group. In the profilometric examination, it was revealed that CWV could not protect the dentin surface against erosive wear, possibly because of its low adhesion to dentin.<sup>26</sup> In our study, although CWV application did not prevent the increase in surface roughness after the erosion-abrasion cycle, it enabled the dentin surfaces to be less affected by erosion.

Different types of lasers can be used in the treatment of DH. The usage of Nd:YAG and  $\text{CO}_2$  lasers has been limited because of their thermal side effects.<sup>38,61,62</sup> Therefore, there has been a tendency toward the use of alternative laser types in the treatment of DH.<sup>63</sup> In the present study, because of the specific properties of its wavelength (2.78  $\mu\text{m}$ ), a medium power type Er,Cr:YSGG laser that can be used in soft and hard tissue without damaging the pulp and surrounding tissues was used. The Er,Cr:YSGG laser uses not only existing water in tissue but also exogenous water for ablation. It has been reported that exogenous water has a greater effect than endogenous water in dentin ablation.<sup>35-37,64</sup> Therefore, an Er,Cr:YSGG laser

was used without water in this study. The results of a previous study showed that carbonization occurred even at 0.5 W when the Er,Cr:YSGG laser was used without water.<sup>65</sup> Since this situation can cause a rougher surface, in this study, the energy settings were chosen to be lower than the threshold at which carbonization, melting, and surface roughness could occur, so the laser was used at 0.25 W. A rougher surface can promote plaque accumulation and discoloration on tooth surfaces and increase caries risk.

The high absorption of the Er,Cr:YSGG laser emission wavelength (2.78  $\mu\text{m}$ ) in water causes the accumulation of insoluble salts in the dentinal tubules by evaporating the tubular fluid. It has been reported that this accumulation enables the occlusion of dentinal tubules and reduction of DH.<sup>37,38</sup> In the study by Gholami and others, it was shown that an Er,Cr:YSGG laser could dissolve peritubular dentin and partially or completely occlude dentinal tubules therefore reducing the symptoms of hypersensitivity in patients.<sup>40</sup> In the SEM images in the present study, in accordance with the literature, the dentinal tubules of Er,Cr:YSGG laser-applied samples were generally closed and narrowed, but local short cracks and irregularities were present on the surface. Depression areas were observed on interface examinations after application. The profilometric analysis results indicated that the surface roughness increased after laser application compared to the baseline level ( $p<0.05$ ).

SEM images of the Er,Cr:YSGG laser group after the erosion-abrasion cycle showed that closed dentin tubules were in the majority. In addition, local short cracks and irregularities were rarely seen on dentin surfaces and were no more common than they were after application, and degradation and irregularities on the surface were less common in this group than in the control group. In addition, after the erosion-abrasion cycle compared to after application, there was a significant increase in the surface roughness values of the laser group ( $p<0.001$ ). Additionally, after the erosion-abrasion cycle, the surface roughness of the laser group was greater than that of all of the DDA and combination DDA-laser groups except GD-L.

The effectiveness of laser application can be affected by many factors, such as laser wavelength, energy output, and dentin surface conditions (dry or wet surface). Laser application can increase surface roughness as well as cause dentinal tubule occlusion, with ablative and dissolving effects on dentin tissue. Therefore, cracks and irregularities can occur on the dentin surface. These cracks can render the dentin surface more susceptible to erosion and abrasion and cause a significant increase in surface roughness

after the cycle. In addition, although laser application increased the surface roughness of dentin surfaces after the cycle, dentinal tubule plugs were still widely observed, and the degradation and irregularities on the surface were far less widespread than those in the control group. Although the surface roughness was increased by laser application, the tubule plugs formed by the laser resisted erosion-abrasion. These findings show that lasers can be effective for DH treatment in difficult oral conditions.

In the present study, in addition to its use alone, the Er,Cr:YSGG laser was used in combination with DDAs, and tubular plugging efficiencies and surface roughness were investigated. Compared to those in the DDA-only groups, occluded dentin tubules were more abundant in the groups treated with DDA in combination with laser. In addition, in the combination DDA and laser groups, tubule plugging activity persisted after the erosion-abrasion cycle, as shown in the SEM images. Consistent with the literature, this study indicates that the combination of DDA treatment with laser irradiation is significantly more effective in dentinal tubule occlusion than DDA alone.<sup>57,66</sup>

In an *in vitro* study, TMD and GD in combination with Er:YAG was found to be more advantageous than laser or DDAs alone. The occlusion rates of dentinal tubules were higher in the DDA-laser combination groups. Most dentinal tubule occlusions were observed under laser application combined with GD. However, the application of GD or TMD alone did not damage the dentin surface, unlike laser treatment. As shown by atomic force microscopy observations, the groups treated with DDA in combination with Er:YAG laser showed a very rough surface characterized by grooves, and prominent cracks and craters were apparent in the dentinal tubules.<sup>28</sup>

Different studies have suggested that laser applications in combination with fluoride can increase the effect of fluoride.<sup>57,67,68</sup> In an *in vitro* study conducted to analyze the mechanism of the combined application of laser and fluoride, fluoride penetration in the root dentin was found to be better than that with fluoride alone and to inhibit demineralization.<sup>67</sup> In another *in vitro* study, the combined use of CWV and Nd:YAG lasers was reported to result in a surface structure in which most of the dentinal tubules were occluded.<sup>27</sup>

In this study, after the erosion-abrasion cycle, the surface roughness values of the TMD-L, EN-L, and CWV-L groups were found to be significantly lower than those of the Er,Cr:YSGG laser-only group ( $p < 0.001$ ). The reason for this outcome might be that the layer formed by DDAs before laser application reduced the negative effects of the laser on the dentin surface.

In addition, the tubule plugs formed by the combined-application groups were resistant to the erosion-abrasion cycle, similar to those of the laser group, and there was less surface damage in these groups than in the control groups. These findings suggest that combined DDA-laser treatment could be more effective for DH treatment under different oral conditions than DDA treatment alone. Laser application increased the tubular plugging efficiency of DDAs. In addition, resistant tubule plugs after the erosion-abrasion cycle could serve as a barrier against bacteria, preventing bacteria adhering to the dentin surface from progressing to the dentin tubules.

One of the limitations of this *in vitro* study was that although the early term results of DDAs and laser applications were determined by both SEM examinations and profilometric analyses, long-term effects were not examined. In addition, artificial saliva containing calcium was used in our study. Artificial saliva cannot show the enzymatic and microbiological effects of human saliva. Human saliva can protect tooth surfaces against erosion and abrasion by forming pellicles on tooth surfaces in the oral environment. In addition, the use of artificial saliva could have increased the effects of the tested DDAs because of its calcium content.<sup>26</sup>

However, the different findings of *in vitro* studies are due to differences in the large number of experimental parameters, such as the type of DDA; the type of laser; the laser application parameters, with different effects on tissue; the pH of the acidic solution used for erosion; test cycle time; and other various assessment methods.

In addition, in this study, since test specimens obtained from teeth extracted for orthodontic purposes were used, the detected changes in surface roughness and SEM properties are likely smaller than those that occur in teeth with clinical DH. Dentin surfaces associated with clinical DH complaints and frequent exposure to erosion abrasion could be expected to be more affected and damaged than the teeth observed here, especially in cases in which the buffering effect of saliva is weak. Further damage could be expected on clinically sensitive dentinal surfaces and areas frequently exposed to erosion abrasion, particularly where the buffering effect of saliva is weak. Therefore, more comprehensive *in vitro* and clinical studies are needed to gain insight into this issue.

## CONCLUSIONS

Effects of different DDAs, Er,Cr:YSGG laser, and their combined application on dentin surface roughness, their effectiveness in occluding dentin tubules, and the resistance of these applications to erosion-abrasion

cycles were investigated. After application, DDAs alone did not cause an increase in the surface roughness of dentin, whereas all other treatments led to provoked surface roughness. In addition, none of the applications could prevent an increase in surface roughness after an erosion-abrasion cycle. Despite the increase in surface roughness of all test groups, morphological changes, cracks, and surface irregularities on dentin surfaces were less apparent. In addition, laser and DDA treatment increased the plugging efficiency of DDAs resulting in tubule plugs more resistant to erosion-abrasion cycle. The findings suggest that combined laser and DDA treatments could be more effective than DDAs alone.

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### Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Karadeniz Technical University, School of Medicine. The approval code issued for this study is 2019/60.

### Conflicts of Interest

The authors of this manuscript 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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