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Effects of Three Desensitizing Agents and Erbium-Doped Yttrium Aluminum Garnet Laser on Dentinal Tubule Occlusion: An In-Vitro Study

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Conflicts of Interest: Nil.

Abstract

Objectives: To quantitatively evaluate the effects of two traditional desensitizing agents [5% sodium fluoride (Duraphat fluoride varnish), 5% glutaraldehyde-35% hydroxyethyl methacrylate (Gluma desensitizer)], an effective new agent [8% arginine and calcium carbonate-containing desensitizing paste (Pro-Argin)], and erbium-doped yttrium aluminum garnet (Er:YAG) laser on dentinal tubule occlusion by scanning electron microscopy (SEM).

Materials and Methods: A total of fifty dentin specimens were obtained from freshly extracted, non-caries, sound human third molars and randomly divided into five groups ($n = 10$): **Group 1:** Control (No treatment), **Group 2:** Duraphat Fluoride Varnish, **Group 3:** Pro-Argin Desensitizing Paste, **Group 4:** Gluma Desensitizer, **Group 5:** Er:YAG laser. All specimens evaluated under the SEM and 10 photographs were taken from each sample at 2000× magnification. The number of open, partially occluded, and visible (open + partially occluded) dentinal tubules in each photograph was calculated. SPSS (Statistical package for the social sciences) 21.0 software was used for statistical analysis. The data were analyzed by One-way ANOVA, Dunnett C, and Tukey tests. The significant value for all tests was accepted as 0.05.

Results: No significant difference was observed in the number of open, partially occluded, and visible dentinal tubules between the Pro-Argin Desensitizing Paste and Er:YAG laser groups, but fewer open, partially occluded, and visible dentinal tubules were observed in these groups than in the other groups. That is, Er:YAG laser and Pro-Argin Desensitizing Paste groups showed significantly more tubule occlusion than the other groups ($p < 0.05$).

Conclusions: Er:YAG laser and Pro-Argin Desensitizing Paste groups were found to be more effective than the other groups in occluding the dentinal tubules.

Keywords: Dentin hypersensitivity, scanning electron microscopy, desensitizing agents, Er:YAG laser.

1. Introduction

Dentin hypersensitivity (DH) is a common clinical condition with a wide variation in prevalence, risk factors, and treatment options. It is characterized by a sudden, sharp, and short-term pain that in the exposed dentin in response to thermal, chemical, tactile, osmotic electrical, or evaporative stimuli [1,2].

In many studies, the buccal cervical surfaces of the canine and premolars have been shown to be the areas most affected by DH. Reportedly, DH occurs most frequently in 20- to 40-year-olds, maximizing in young adulthood and decreasing with age. It is also known to occur more frequently in women than in men [3,4].

Primarily, DH occurs because of exposed dentinal tubules. Dentin exposure frequently occurs because of gingival recession and the loss of cervical enamel or cementum through abrasion, attrition, or erosion. Also, parafunctional habits such as bruxism, improper tooth brushing, and coronal fracture or defective restorations can cause DH [5-8].

The hydrodynamic theory, introduced by Brannstrom et al., is the most widely accepted theory for DH. This theory is based on the movement of fluid in the dentinal tubules after thermal, physical, or osmotic stimulation. The movement of fluid leads to the activation of nerve receptors at the pulp–dentin demarcation and results in pain. According to this theory, the ideal treatment of DH decreases the fluid flow in dentinal tubules. Consequently, two approaches have been developed to treat DH. The first blocks the neural response, and the second occludes the exposed dentinal tubules [1,8-16].

Many materials and methods have been developed for treating DH that can be applied at home or by the clinician. These materials are classified in Table 1 according to their mechanisms of action.

The optimal treatment of DH simulates natural desensitizing and provides permanent and rapid tubule occlusion. Grossman has defined the characteristics of ideal desensitizing agents as non-irritating to the pulp, painless application, easy to apply, fast acting, and long lasting [17-18].

An aqueous solution containing 5% glutaraldehyde and 35% hydroxyethyl methacrylate (HEMA) has been reported to be effective in reducing DH (Gluma Desensitizer, Heraeus Kulzer, Hanau, Germany) [19]. Glutaraldehyde decreases permeability by coagulating plasma proteins in the dentinal fluid, whereas HEMA physically blocks the dentinal tubules [20].

Fluoride-containing varnishes are frequently preferred for treating DH [21,22].

Topical fluoride applications create a barrier by precipitating calcium fluoride (CaF_2) on the tooth surface, thus occluding dentinal tubules and consequently reducing permeability and hypersensitivity [23-26].

Laser therapy is currently used for treating DH, particularly because of its rapid, reproducible, and reliable analgesic effect. Although several in vivo and in vitro studies have reported its effectiveness in treating DH, the primary mechanisms involved in the reduction of DH are not completely known [27-31].

Several theories have been proposed regarding DH treatment with laser therapy. It is hypothesized that the Er:YAG (erbium-doped yttrium aluminum garnet) laser forms insoluble salts in the exposed dentinal tubules by causing evaporation of the dentinal fluid and that these salts then eliminate DH by blocking the dentinal tubules.

It is thought that the Er:YAG laser will be used effectively and widely in dentistry and medicine because of its thermomechanical ablation mechanism and high absorption in water [32].

Arginine, a natural amino acid, and calcium carbonate adhere to the negatively charged surface of the dentin at physiological pH and then form a calcium-rich layer that occludes the tubules. It is thought that the mechanism of these agents, called Pro-Argin Technology, is remarkable compared with that of other agents because it blocks the exposed tubules by forming a mineral structure that resembles dentin. Moreover, it develops spontaneously in the oral environment and thus can produce long-term effects [33-38].

This study aimed to quantitatively evaluate the effects of three desensitizing agents (Duraphat Fluoride Varnish, Gluma Desensitizer, and Pro-Argin Desensitizing Paste) and the Er:YAG laser on dentinal tubule occlusion by scanning electron microscopy (SEM).

2. Materials and Methods

2.1 Preparation of Specimens

Freshly extracted, non-caries, sound human third molars were collected for this study and stored in physiological saline at room temperature. The molars were sectioned with a diamond separa at low speed and under water irrigation using a sensitive cutting machine (IsoMetTM 1000, Buehler An ITW Company, Lake Bluff, IL, U.S.A.). First, buccal and lingual enamel layers were removed to expose the crown dentin. The teeth were attached to the acrylic block from the root surfaces, and the occlusal surface was placed on the machine perpendicular to the separa. Next, cuts were made from buccal and lingual surfaces to pulp at 1-mm intervals. The tooth was rotated 90° on the machine so that the occlusal surface was perpendicular to the separa. Another cutting was made in the middle. As a result, four dentin specimens were obtained from one tooth.

2.2 Application of Desensitizing Agents and Er:YAG Laser

A total of fifty dentin specimens were obtained and placed in 18% ethylenediamine tetraacetic acid (EDTA) for 10 min to remove the smear layer on the dentin surface, thus exposing the tubules. The specimens were then washed with distilled water and randomly divided into five groups (n = 10). The desensitizers were applied as follows:

- **Group 1:** Control (No treatment)
- **Group 2:** Duraphat Fluoride Varnish
- **Group 3:** Pro-Argin Desensitizing Paste
- **Group 4:** Gluma Desensitizer
- **Group 5:** Er:YAG laser

Compositions and procedures of application for all agents and the Er:YAG laser are shown in Table 2.

All samples were incubated in 0.9% sodium chloride isotonic solution for 2 days, removed 12 h before obtaining SEM photographs, and dried.

2.3 SEM Analysis

An SEM study was conducted to evaluate the extent to which the applied treatment methods blocked the tubules. First, the specimens were numbered and attached to the aluminum carrier with carbon bands using the sputter technique. The specimens were then covered with a thin layer of Au-Pd alloy with a sputter coating device. They were placed on a SEM device, and 10 photographs were taken from each sample at 2000× magnification. The number of open, partially occluded, and visible (open + partially occluded) dentinal tubules in each photograph was calculated. Tubules that were not completely visible in the photographs were not included in the calculation.

2.4 Statistical Analysis

SPSS (Statistical package for the social sciences) 21.0 software was used for statistical analysis. One-way ANOVA was used to determine the difference in the numbers of open, partially occluded, and visible dentinal tubules between the study groups. On finding differences, binary comparisons were made using Dunnett C and Tukey tests. The significant value for all tests was accepted as 0.05.

Figure 1 summarizes the study design.

3. Results

SEM photographs revealed the dentinal tubules in each group (Figure 2, A–E) and the number of all visible dentinal tubules was calculated from SEM photographs. The numbers of partially occluded and open dentinal tubules were also calculated separately.

The proportion of open dentinal tubules to the visible dentinal tubules and that of partially occluded dentinal tubules to the visible dentinal tubules were calculated for each group. According to the proportion of open dentinal tubules to the visible dentinal tubules, the mean value was lowest for the Er:YAG laser and Pro-Argin Desensitizing Paste groups; thus, the number of completely occluded dentinal tubules was higher in these groups than in the other groups. According to the proportion of partially occluded dentinal tubules to the visible dentinal tubules, the mean value was highest for the Er:YAG laser and Pro-Argin Desensitizing Paste groups; thus, the number of completely occluded dentinal tubules was higher in these groups than in the other groups (Figures 3 and 4).

A statistically significant difference was observed in the numbers of open, partially occluded, and visible dentinal tubules between the study groups (one-way ANOVA, $p < 0.05$; Table 3).

No difference was observed in the number of open and visible dentinal tubules between the Pro-Argin Desensitizing Paste and Er:YAG laser groups, but fewer open and visible dentinal tubules were observed in these groups than in the other groups. A statistically significant difference was observed in the number of open and visible dentinal tubules among the other groups. The number of exposed and visible dentinal tubules from the highest to the lowest in the groups was as follows: the control, Duraphat Fluoride Varnish, and Gluma Desensitizer groups (Dunnett C test, $p < .05$; Table 3).

Similarly, no difference was observed between the Pro-Argin Desensitizing Paste group and the Er:YAG laser group in the number of partially occluded dentinal tubules, but fewer partially occluded dentinal tubules were observed in these groups than in the other groups. Although no statistical difference was observed among the Gluma Desensitizer group with Duraphat Fluoride Varnish and control groups, the number of partially occluded dentinal tubules in the Duraphat Fluoride Varnish group was higher than in the control group (Tukey test, $p < 0.05$; Table 3).

4. Discussion

It is known that the number and diameter of dentinal tubules exposed to the oral environment are directly related to DH. As the number and diameter of the dentinal tubules increase, the intensity of stimulation to the pulp also increases [6,39].

SEM studies showed that the number of dentinal tubules per unit area is eight times greater in hypersensitive dentin than in non-sensitive dentin. It also has been reported that tubule diameters are two times wider [40]. The primary criterion of success in DH treatment is the occluding or narrowing of exposed dentinal tubules.

According to clinical studies, many patient-related psychological and emotional factors can affect DH treatment. Therefore, the mechanism of action of desensitizing agents and the ability to block the dentinal tubules may not be quantitatively determined. Furthermore, the placebo effect in the diagnosis and treatment of DH is significant because diagnosis and successful treatment depend on the subjective response of the patient. The trust and positive relationship between the patient and the physician as well as the patient’s desire for relief contribute to the placebo effect. As a result, the mechanisms of action of the desensitizing agents cannot be quantitatively assessed solely through clinical studies. Morris et al. emphasized that factors not easily addressed in clinical DH studies are the placebo effect and the subjective perception of pain [41].

On the other hand, in vitro studies alone are not adequate to determine the ideal treatment option for DH. It is known that long-term success cannot be achieved if individual habits and diet-related factors that cause DH are not eliminated. Thus, both clinical and in vitro studies should be conducted, and their results should be compared. Based on this information, the current study was planned in vitro to understand the potential occlusion and desensitization mechanisms of desensitizing agents.

Although studies on DH treatment showed a reduction in hypersensitivity, they have not reported treatment methods for eliminating DH in the long term [42-51]. Because the agents applied in DH treatment can move from the dentinal tubules over time, reapplication may be required. Although lasers are a promising solution to this issue, hypersensitivity can reoccur with current treatments.

Duraphat Fluoride Varnish, Gluma Desensitizer, and Pro-Argin Desensitizing Paste were used as active treatment agents in this study. The efficacy of the Er:YAG laser in DH treatment was evaluated in another group.

In this study, dentin specimens were incubated in 18% EDTA for 10 min to mimic dentinal tubules that were exposed to the oral environment. In a study by Briang et al., specimens were first incubated for 5 min in 17% EDTA, followed by another 5 min in hypochlorite solution [52]. In an in vitro study conducted by Gholami et al., dentinal tubules were exposed by keeping dentin specimens in 14% EDTA [53].

The deposition of the calcium fluoride compound on exposed dentin surfaces reduces the diameter of the dentinal tubules, thereby reducing the potential for stimulating the pulp by the hydrodynamic mechanism [54,55]. Tal et al. have reported that fluoride ions mechanically occlude the dentinal tubules and thus prevent stimulus transmission. Because of the small size of the CaF_2 crystal, it has been reported that a single NaF application may not be effective in occluding the diameter of the dentinal tubules and may require numerous applications [26,57-59].

The application of fluoride varnishes can reduce pain in patients; however, most studies have indicated that fluoride varnishes reduce DH only for a short time.

This study also found that the proportion at which Duraphat Fluoride Varnish blocks the dentinal tubules was the lowest (Figures 3 and 4).

Gluma Desensitizer used in this study included 5% glutaraldehyde and 35% HEMA. Glutaraldehyde is a biological fixative. Reportedly, it reacts with proteins in the dentinal fluid to cause precipitation and thus ensures partial or complete occlusion of the dentinal tubules. Furthermore, studies have shown that HEMA is an effective hydrophilic monomer for dentin bonding [20, 60-62]. Successful results have generally been reported in studies conducted using Gluma Desensitizer [62-65]. Fluid passages from dentin samples were evaluated in an in vitro study. Gluma Desensitizer-treated samples have shown minimal fluid passage from dentin even after 3 weeks [63]. In another study in which Gluma Desensitizer-treated specimens were examined under an electron microscope, it was shown that Gluma Desensitizer blocked the dentinal tubules up to 50 µm deep [62].

A few studies have shown that DH cannot be treated after Gluma Desensitizer application. Reportedly, Gluma Desensitizer does not completely occlude the dentinal tubules and may cause bacterial contamination [66]. SEM photographs of dentin specimens from this study showed that Gluma Desensitizer completely occluded some of the dentinal tubules, whereas others were open and most were partially occluded.

Pro-Argin Desensitizing Paste (8% arginine), Gluma, and NovaMin (5% calcium phosphosilicate) agents were evaluated for efficacy in treating DH. Visual analog scale scores were recorded at baseline, immediately after application, and 15 and 30 days later. A significant decrease was observed in control sessions according to baseline in all three groups. However, in one study, Pro-Argin provided a significantly higher reduction in hypersensitivity than Gluma and NovaMin at the end of 30 days (p<0.016) [67]. In the current study, Gluma Desensitizer showed dentin tubular occlusion rates similar to those shown by Duraphat Fluoride Varnish but lower than those shown by Pro-Argin Desensitizing Paste and Er:YAG laser.

Another agent used in this study was the 8% arginine and calcium carbonate-containing desensitizing paste developed by Kleinberg et al. Clinical trials have reported that Pro-Argin Desensitizing Paste is highly effective in reducing DH. In vitro studies of the mechanism of action have shown that this new approach works by occluding the dentinal tubules [36].

In an in vitro study conducted in 2009, 8% arginine and calcium carbonate-containing desensitizing paste (Colgate Sensitive Pro-Relief) was applied to the exposed dentin surface and, consequently, arginine, calcium, phosphate, and carbonate-containing plugs were formed. SEM photographs of dentin specimens from this study showed that Pro-Argin Desensitizing Paste occluded almost all dentinal tubules, whereas a small number of dentinal tubules were partially occluded [36].

Pro-Argin Technology has provided clinically proven benefits and advantages over many traditional desensitizing agents, relative to the limited number of studies performed. In a clinical study, 5% NovaMin-containing toothpaste, 8% arginine-containing toothpaste, and herbal toothpaste were evaluated for their effects on DH for 4 weeks. In contrast to other studies on Pro-Argin Desensitizing Paste, NovaMin showed a greater decline in sensitivity scores than Pro-Argin Desensitizing Paste [68].

Currently, different laser types with various settings and conditions are used for treating DH. The Er:YAG laser (non-contact, SP mode, 80-90 mJ/pulse, 2 Hz) was used in this study because it can be used safely on hard tissue, can be absorbed at high levels by water and hydroxyapatite crystals, does not generate thermal damage, and does not carbonize [69].

The Er:YAG laser causes evaporation of the dentinal fluid and precipitation of the organic elements and insoluble salts on the exposed dentinal tubules. Thus, it plays an important role in preventing DH. It has also been shown that the Er:YAG laser reduces inflammatory mediators through its high bactericidal effect and thus increases the pain threshold [70]. Some in vitro studies have shown that the Er:YAG laser significantly blocked the dentinal tubules that were exposed [52,71].

SEM photographs in this study showed that almost all exposed dentinal tubules in the Er:YAG laser-treated specimens were completely occluded and that a small number of the dentinal tubules were partially occluded, similar to the results observed in Pro-Argin-treated specimens. Furthermore, it appears that there was an irregular surface structure in the SEM photographs because it is believed that the open dentinal tubules were occluded by melted intertubular dentin.

In a clinical study, the Er:YAG laser (energy level: 60 mJ/pulse, repetition rate: 2 Hz) was applied to the hypersensitive dentin for 2 min. Visual analog scale scores measured after 4 weeks were significantly lower than the baseline scores ($p < 0.05$) [72]. Another study investigated the in vitro effects of the Er:YAG laser (30 Hz, 60 mJ/pulse, 10 sec) and a desensitizing paste containing 8% arginine and calcium carbonate alone or in combination by SEM. According to the results, occluding or narrowing of dentinal tubules was observed in all groups. The highest occlusion was observed in the combination group. No statistically significant difference was found between the Er:YAG laser and desensitizing paste containing 8% arginine and calcium carbonate groups [73].

In this study, only physical changes in open dentinal tubules were quantitatively assessed. Such in vivo differences as etiologic factors and placebo effects that may alter the efficacy of desensitizing agents have not been evaluated. Also, it is thought that the differences between this study and previous studies are related to methods of obtaining dentin specimens and the manner and timing of administration of the agents.

5. Conclusions

The Er:YAG laser, which causes evaporation of dentinal fluid, and Pro-Argin Desensitizing Paste, a revolutionary technology based on arginine and calcium carbonate, effectively blocked the dentinal tubules. They have been found to be more effective than Gluma Desensitizer and Duraphat Fluoride Varnish in eliminating DH. Gluma Desensitizer and Duraphat Fluoride Varnish were also found to be effective in occluding the dentinal tubules compared with control group, but they were not found to be as successful as the Er:YAG laser and Pro-Argin Desensitizing Paste in blocking the dentinal tubules. However, if etiological factors in the oral environment are not eliminated, the effectiveness of these agents and Er:YAG laser will change. Therefore, in vitro results should be supported by long-term clinical trials.

Table 1: Classification of materials, for treating DH, according to their mechanisms of action.

Inhibitors of Nerve Stimulation	Anti-inflammatory Agents	Materials That Block The Dentin Tubules	Dentin Covering Materials	Homeopathic Medication	Lasers
-Potassium nitrate	-Corticosteroids	**I. Ions / Salts** -Calcium hydroxide -Calcium phosphate -Calcium carbonate -Ferroz oxide -Potassium oxalate -Stannous fluoride -Sodium fluoride -Strontium fluoride -Kalay floride -Sodium monofluorophosphate -Stannous fluoride-Sodium fluoride combination -Bioactive glasses (SiO_2-P_2O_5-CaO-Na_2O) **II. Protein precipitators** -Strontium chloride hexahydrate -Silver nitrate -Pro-Argin and calcium carbonate -Gluteraldehit -Zinc chloride **III. Casein phosphopeptides**	-Fluoride varnishes -Sealant on -Adhesive resins -Cam ionomer cements -Composite on -Methyl methacrylate	-Propolis	**I. Low-Output Lasers** -He-Ne (Helium-Neon) Laser -Ga-Al-As (Gallium-Aluminum-Arsenite) (Diode) Laser **II. Middle-Output Lasers** -Nd:YAG (Neodymium: Iridium Aluminum Garnet) Laser -CO2 (Carbon Dioxide) Laser -Erbium Lasers (Er:YAG (erbium-doped yttrium aluminium garnet) and Er,Cr:YSGG(erbium, chromium:yttrium-scandium-gallium-garnet)

Table 2: Compositions and procedures of application for all agents and the Er: YAG laser

	Manufacturer	Composition	Procedures of Application
Duraphat Fluoride Varnish	Colgate-Palmolive Company, ABD	5% Sodium fluoride	Applied with a microbrush in a thin layer, then removed with a cotton pellet
Gluma Desensitizer	Heraeus Kulzer, Hanau, Germany	5% gluteraldehyde, 35% HEMA (2-hydroxyethyl methacrylate), water	Applied with a microbrush and left for 60 s, dried, then rinsed with water
Pro-Argin Desensitizing Paste	Colgate Sensitive Pro-relief Colgate-Palmolive Company, ABD	8% arginine and calcium carbonate	Applied with a polishing tire for 3 seconds, rinsed with water, then dried
Er/YAG Laser	Fotona Light Walker DT, US	Non-contact, SP mode, 80-90 mJ/pulse, 2 Hz	Applied 6 cm distance from left to right, repeated 3 times, then soaked with saliva

Table 3: Number of open, partially occluded and visible dentinal tubules in each groups (Mean±SD)

	Number of Open Dentinal Tubules	Number of Partially Occluded Dentinal Tubules	Number of Visible Dentinal Tubules
Control	35,9 ± 7,60^{a}	9,9 ± 2,28^{a}	45,8 ± 9,48^{a}
Duraphat Fluoride Varnish	13,9 ± 2,84^{b}	14,4 ± 3,09^{b}	28,3 ± 5,55^{b}
Gluma Desensitizer	7,9 ± 1,66^{c}	12 ± 2,49^{ab}	19,9 ± 3,78^{c}
Pro-Argin	1,3 ± 1,15^{d}	3,3 ± 1,7^{d}	4,6 ± 2,71^{d}
Er:YAG Laser	1,10 ± 1,10^{d}	3,1 ± 2,02^{d}	4,2 ± 2,97^{d}

There is a statistical difference between the values indicated by the different letters in the same column. (One-way ANOVA, Dunnet C Test, Tukey Test, p <0.05). There is no statistical difference between the values indicated by the same letters.

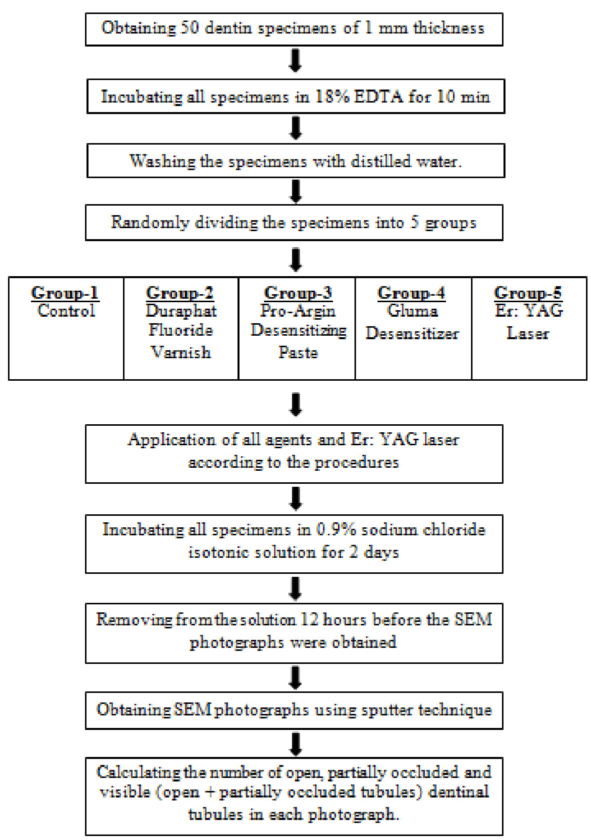

Figure 1: Summary of Study Design

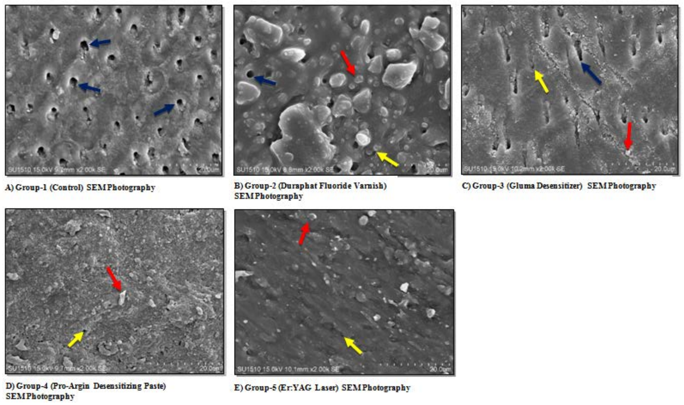

Figure 2: A) Open dentin tubules (blue arrows) were observed on the surface of the dentin samples suspended in 18% EDTA for 10 minutes. B) Some of the dentin tubules were completely occluded (red arrow), some of them were partially occluded (yellow arrow), others were open (blue arrow). C) Most of the dentin tubules were partially occluded (yellow arrow), some of them were open (blue arrow), others were completely occluded (red arrow). D) Almost all of the dentin tubules were completely occluded (red arrow), and a small number of exposed dentin tubules were seen to have a partially occluding (yellow arrow). E) Almost all of the dentin tubules were completely occluded (red arrow), and a small number of exposed dentin tubules were seen to have a partially occluding (yellow arrow).

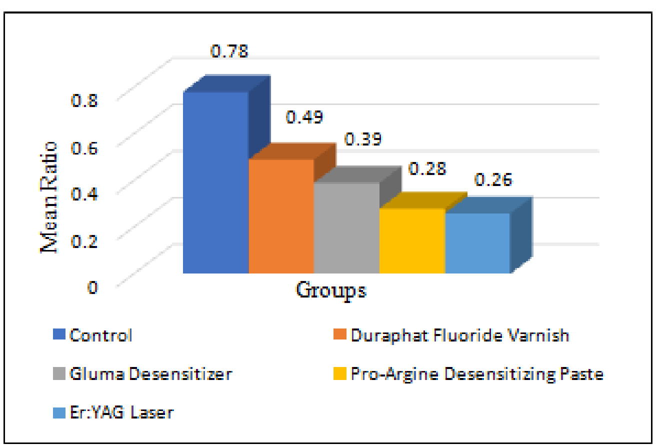

Figure 3: Proportion of open dentinal tubules to visible dentinal tubules. The mean value was lowest for the Er:YAG Laser and Pro-Argin Desensitizing Paste groups, thus the number of completely occluded dentinal tubules in these groups was higher than in the other groups.

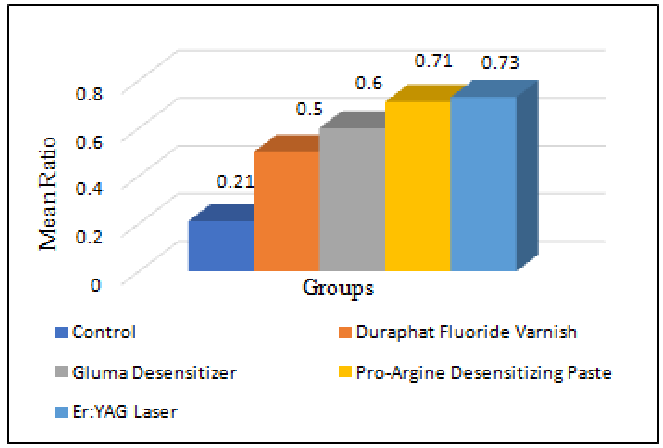

Figure 4: Proportion of partially occluded tubules to visible tubules. the mean value was highest for the Er:YAG laser and Pro-Argin Desensitizing Paste groups; thus, the number of completely occluded dentinal tubules was higher in these groups than in the other groups.

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