Original Article

Micromorphological Evaluation of Dentin Treated with Different Desensitizing Agents

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Abstract:

Introduction: The purpose of a desensitizing agent is a permanent coating or filling of dentin surface. Morphological analysis in vitro of this treated surface is essential to understand the interaction between desensitizing agent and hypersensitive dentin. The aim was to evaluate the morphology of four dentin surface treated with desensitizing agents.
Methods: This was an in vitro laboratory study, where fifteen specimens from extracted human premolars were obtained. The enamel was removed to expose the dentin surface, polished with silicon carbide abrasive papers and etched with 6% citric acid for 2 min. The specimens were randomly divided into 5 groups: G1 - without treatment (control) (C), G2 - fluoride varnish (FV), G3 - potassium oxalate (PO), G4 - 2-step self-etching adhesive system (AS), G5 - diode laser (DL). The specimens were cleaved in the lingual-buccal direction, prepared for analysis by Scanning Electron Microscope and the surface and interior of the dentinal tubules were observed at 1500× magnification.
Results: In the control group, the dentin etching promoted smear layer removal and exposure of dentinal tubules. In the group of fluoride varnish, a film was observed on the surface, with plugs of varnish into tubules. In the group of oxalate, partial obliteration of the tubular entrances was observed. In the group of the adhesive system, the tubules were obstructed through the formation of hybrid layer and a physical barrier on the surface. In the group of the diode laser, dentin melting and solidification with partial occlusion of dentinal tubules were observed.
Conclusions: All desensitizing agents evaluated demonstrated ability to modify the surface of dentin, with partial or total occlusion of dentinal tubules. Thus, it is suggested to do more clinical studies to verify the effectiveness of the findings.
Keywords: agents, dentin desensitizing; dentin; diode lasers.

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Introduction

The essential processes for the development of dentinal hypersensitivity (DH) are concentrated in the exposure of dentin due to loss of enamel or cement, resulting in dentinal tubules being opened to their full extent[1,2].

The action of desensitizing agents recommended for the treatment of DH takes into consideration the hydrodynamic theory[3] that is based on the movement of fluid inside the dentinal tubules in response to iatrogenic or environmental trauma[4].

Thus, the treatment possibilities are concentrated on changing or blocking the pulp nerve response and interruption of transmission of the stimulus, thereby reducing dentinal fluid movement by narrowing or occluding the dentinal tubules[5,6]. This occlusion, both mechanical and chemical leads to a reduction in dentin permeability[7]. This is possible by filling the tubules or by the combination of dentinal tubule elements and the precipitation of proteins.

Covering the surface is also one of the treatments for reducing DH, and can be performed by sealing the dentin with composites, glass ionomer cement, varnishes and dental adhesives. Other alternatives are surgery for coverage with gingival tissue, use of lasers or homeopathic medication, such as propolis, for example[5,6].

The literature describes a large quantity of products, technique and equipment for the treatment of DH; however, there is still no consensus about the best form of treatment and management of this condition that appears so frequently in daily clinical practice.

Among the possible treatments for DH, the purpose of the present study was to verify, by means of scanning electron microscopy, the action of different desensitizing agents, such as fluoridated varnish, potassium oxalate, a self-etching adhesive system and high power diode laser on the surface morphology of dentin, to reach a greater understanding of the mechanisms of action with regard to their clinical application.

The hypothesis of the study was that all the therapies cause morphological alterations in dentin, capable of causing complete or partial occlusion of the dentinal tubules.

Methods

The research protocol was approved by the Research Ethics Committee of the Federal University of Santa Maria (UFSM), Brazil (CAAE: 0171. 0.243.000-10).

Fifteen specimens obtained from extracted human premolars provided by the UFSM Tooth Bank were used, the eligibility criteria being that they should be healthy. Teeth with defects, fractures and restorations were excluded.

The teeth were washed, cleaned and polished with a Robinson brush and pumice stone, stored in a 0.5% *Thymol* solution at room temperature to be disinfect, until they were used in the study.

Afterwards, enamel/dentin blocks were obtained from the cervical third on the vestibular face of the teeth, using a double-faced diamond disc (KG Sorensen, São Paulo, Brazil) fitted to a straight handpiece at low speed, under constant cooling. The enamel from the cervical region of each fragment was removed to expose the dentin, using a cutting machine (Labcut 1010, Extec Co, Enfield, USA) and polished with silicon carbide abrasive papers for 20s, of 400, 600, 1200, 1500, 2000 and 2500 grit (3M do Brasil Ltda, São Paulo, Brazil) to standardize the smear layer. The cuts were made in order to obtain blocks measuring 4 mm long x 4 mm wide, and a channel was made in the lingual region of specimens to guide their cleavage. The specimens were cleaned in an ultrasonic bath for 12 min[8] and stored in deionized water up to the time of dentinal surface treatment.

All specimens were etched with 6% citric acid (Dermapelle Farmácia de Manipulação, Rio Grande do Sul, Brazil) for 2 min and washed with deionized water to remove the smear layer, in order to promote opening of the dentinal tubules.

The specimens were randomly distributed into five groups (n=3), and each specimen received a single application of the product. The studied groups were as follows: G1 – without treatment (control) (C); G2 – fluoridated varnish (FV); G3 - potassium oxalate (PO); G4 – 2-step self-etching adhesive system (AS); G5 – diode laser (DL). The composition, commercial brand and application modes are given in detail in Table 1.

After the treatments, the specimens were cleaved with a sharp blade (dental chisel) perpendicularly to the vestibular surface, in the lingual-vestibular direction, in order to enable analysis of the surface and interior of the dentinal tubules.

The cleaved specimens were dried in an oven at 37°C for 12h[9]. After this the specimens were sputter coated with gold alloy in a Desk II Denton Vacuum sputter coater (Moorestown, NJ, USA) and observed by Scanning Electron Microscopy (SEM) (Model Jeol A110- Jeol Inc. Tec. – Tokyo, Japan) at 1500× magnification. Photomicrographs were obtained for comparative morphological evaluation of the specimens in two directions: on the dentin surface and perpendicular to it (obtained by the method of specimen cleavage).

The images were qualitatively evaluated, considering the dentin surface characteristics, presence of deposits and condition of the dentinal tubules.

Table 1. Composition and application mode of products and equipment used.

Material	Chemical composition	Application mode
Duraphat® Colgate-Palmolive Company (New York, USA)	5% Sodium fluoride, Colophonia, ethyl alcohol, lacquer-gum, mastic gum, saccharine, aroma, white beeswax	Applied on the surface to be treated using a paint brush, and allowed to harden with saliva.
Oxa-Gel Kota Indústria e Comércio Ltda (São Paulo, Brazil)	Monohydrated Potassium Oxalate, carboxymethylcellulose, distilled water, chlorhexidine, fluoride	Applied on the dentin surface using a paint brush, allowed to rest [10, 11] for 2 minutes, and excess gel was removed.
Clearfil™ SE Bond Kuraray (Osaka, Japan)	Primer: 10-MDP, HEMA, hydrophilic dimethacrylate, water, catalyzer Bond: 10-MDP, HEMA, microparticle dimethacrylate monomer, catalyzer	Primer applied with a paint brush for 20 seconds; dried with light jet of air, Bond applied, excess removed and a delicate jet of air applied; light polymerized for 10 seconds.
Thera Lase Surgery DMC Equipamentos Ltda (São Carlos, SP, Brazil)	Light Emitting Diode Laser; Wavelength: 810 – 830 nm; Power between 0.5 – 4.5W, delivery system by fiber optic with diameter of (300 µm).	Irradiation was performed in non contact mode, at a distance of 1mm from the dentin surface, with horizontal scanning movements, for 20s. The protocol used was as follows: Power of 1 W, continuous mode, energy 20J, generating an approximate energy density of $100J/cm^2$ (calculated by the formula $D = J / \pi \times r^2$; that is, $20J / 0.2cm^2 = 100J/cm^2$).

Results

The photomicrographs of the control group and groups treated with desensitizing agents are shown in Figures 1 to 5.

In the control group that received only dentin etching with 6% citric acid for 2 min, it is possible to observe the exposed, dis-occluded dentinal tubules, without the presence of smear layer and smear plugs (Figures 1a and 1b).

In the 5% fluoridated varnish group, in the vestibular view, it is not possible to visualize the dentinal tubules, due to the presence of a film of varnish covering the entire surface (Figure 2a). When figure 2b was analyzed, infiltration of this film into some of the tubules is perceived, forming plugs of varnish.

The potassium oxalate group presented a deposit of material partially occluding the entrances of dentinal tubules, when the surface was analyzed, and no continuous film was observed on the surface (Figure 3a). In Figure 3b, the presence of crystals of various diameters was perceived inside the tubules, formed in a band close to the dentinal surface.

As regards the application of the two-sept self-etching adhesive system, a continuous and uniform film was seen on the dentinal surface, and it was not possible to find visible tubules (Figure 4a). When Figure 4b was analyzed, the formation of an resin-dentin interdiffusion zone (hybrid layer) was observed, as well as the presence of adhesive on the dentin, forming an approximately 15 µm thick film.

The high power diode laser presented interaction with the dentinal substrate, with slight fusion and resolidification of the surface, with dentinal tubules

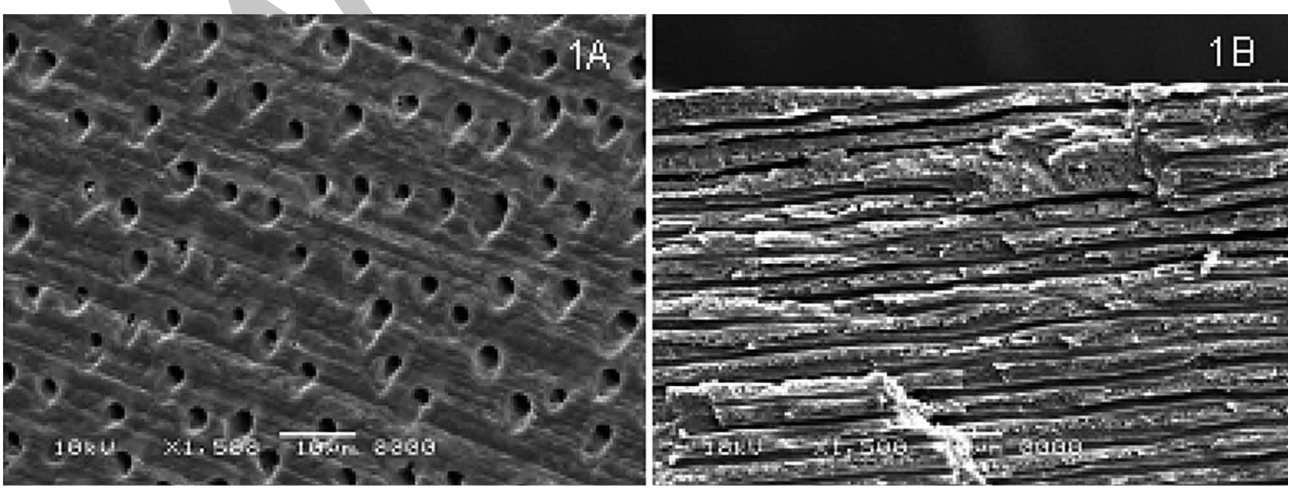

Figure 1. Photomicrographs with reference to Group 1. Note the disoccluded dentinal tubules (1500×).

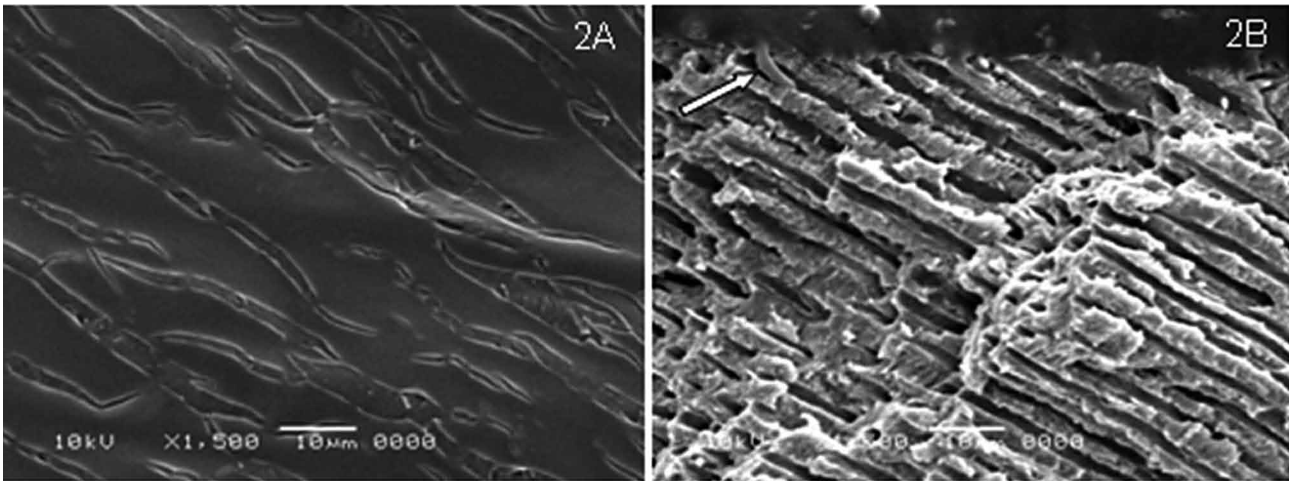

Figure 2. Photomicrographs with reference to Group 2. Note the varnish covering the entire dentin surface (2A) and some tubules with plugs of varnish (arrow) (2B) (1500×).

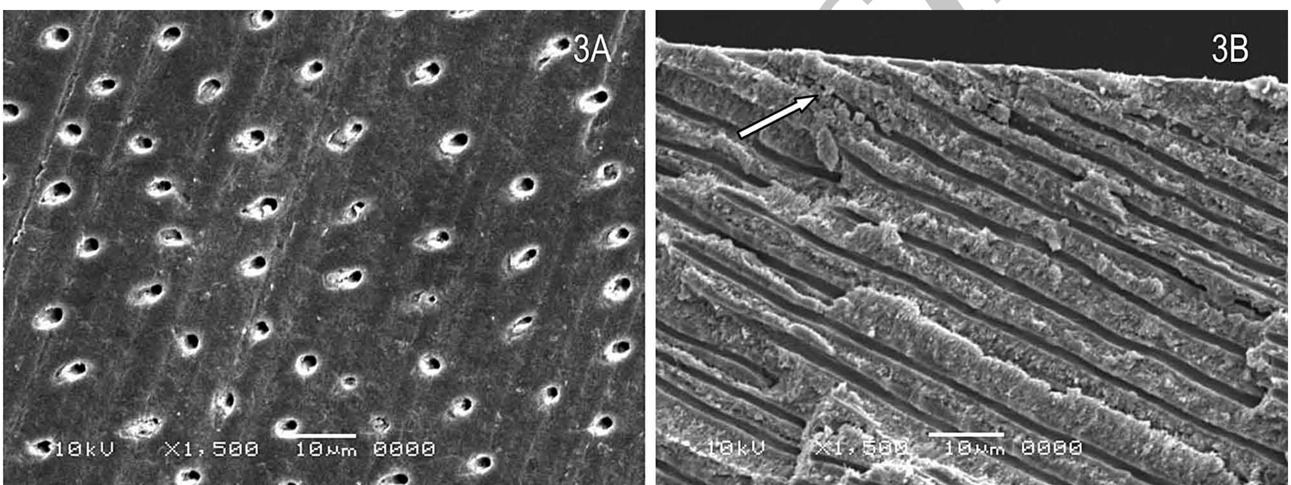

Figure 3. Photomicrographs with reference to Group 3. Note the partially occluded dentinal tubules (3A) and presence of crystals in the tubule (arrow) (3B) (1500×).

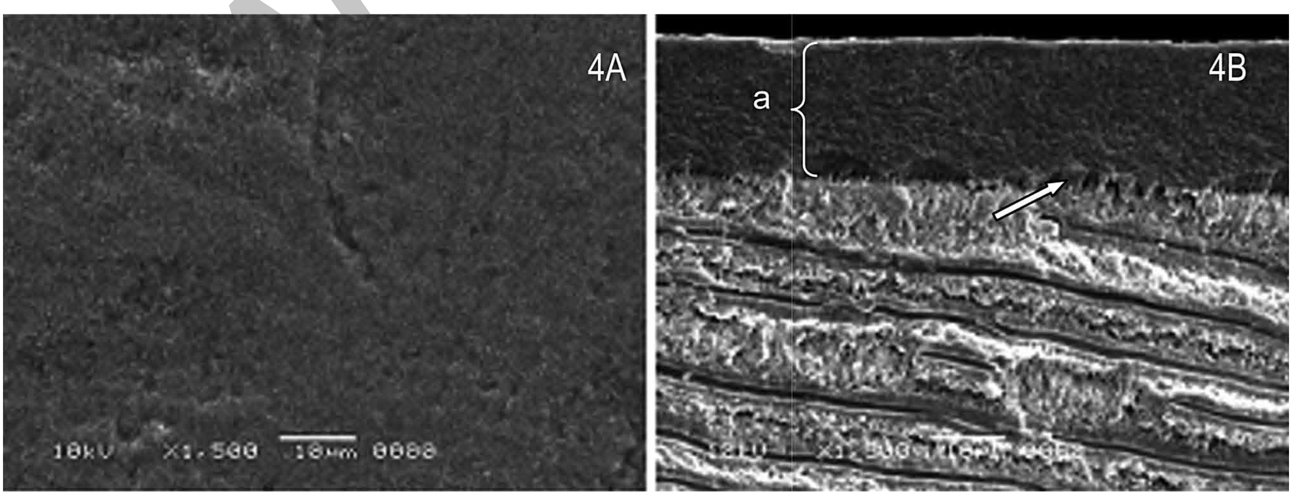

Figure 4. Photomicrographs with reference to Group 4. The dentinal surface was observed to be completely covered by the adhesive (4A), with the formation of a hybrid layer (arrow) and adhesive film (a) (4B) (1500×).

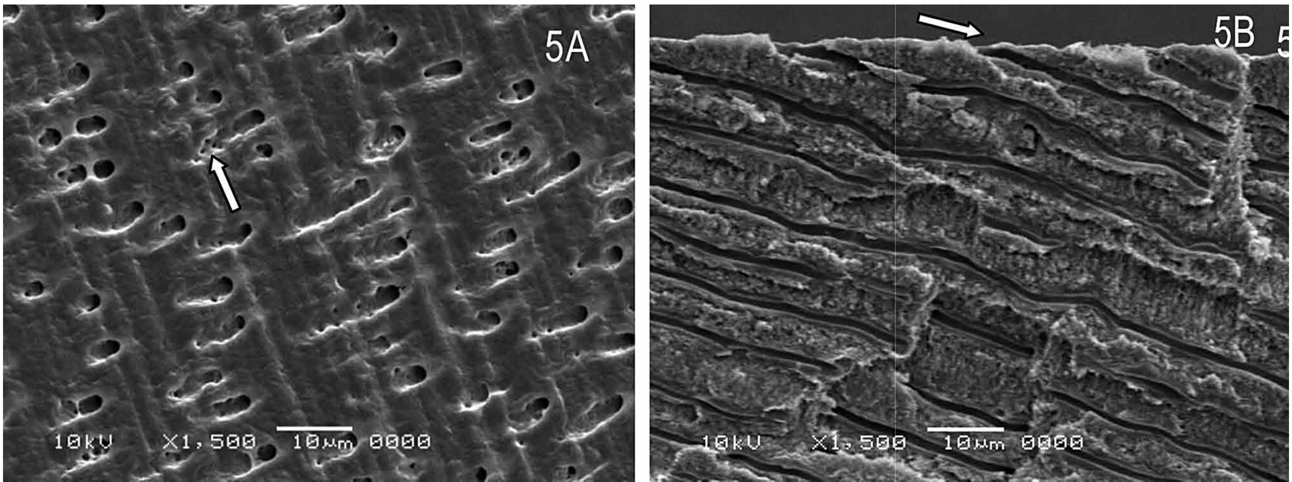

Figure 5. Photomicrographs with reference to Group 5. Dentinal tubules were observed with partially occluded tubule entrances and irregular areas in the intertubular dentin (arrow) (5A). Dentin surface irregularities were also observed (arrow) (5B) (1500×).

with less defined margins and partially obliterated (Figure 5a). When Figure 5b was analyzed, dentin surface irregularity was perceived.

Discussion

Considering the greater prevalence of DH in the cervical regions of premolars[12,13], micromorphological evaluation of this region is fundamental for the understanding of the mechanism of action of desensitizing agents at their clinical application site, taking into account the trajectory of dentinal tubules. Morphological analysis from observation of the occlusion of the tubules is directly related to their permeability[14].

In the present study, for the group in which no desensitizing therapy was applied, it was possible to perceive the removal of the smear layer and smear plugs, according to the pattern expected by etching the dentin with 6% citric acid for 2 min[15,16]. By simulating the opening of dentinal tubules in teeth with DH, the desensitizing action was verified under conditions similar to those found clinically[1], since the presence of the smear layer over the dentinal tubules may contribute significantly to the reduction in DH[17].

As regards the fluoridated varnish, the presence of a regular film over the dentin provides the site with an additional barrier[18]. Nevertheless, this film may easily be removed or displaced by washing the surface, leading to partial occlusion of the tubules and formation of an irregular layer, which may reflect a treatment without any effect in the long term[9]. The mechanism of action expected from fluoridated compounds is explained by the chemical reaction of calcium fluoride with calcium ions from the dentinal fluid, enabling the formation of calcium fluoride crystals, thereby gradually diminishing the flow of fluid due to the reduction in tubule diameter[19]. The crystals may be presented with small diameters inside the tubule, and may not have a significant occlusive effect[20], therefore clinical analysis of these compounds is necessary.

No crystal formation was observed in the specimens of the present study, which were evaluated immediately after application of the product. Thus, the presence of a film of varnish may play an important role in the reduction of pain, with plugs of the material being capable of penetrating into the tubule.

Potassium oxalate is characterized by the formation of crystals resulting from the combination of calcium ions from the dentinal fluid with oxalate[10]. The result of this chemical reaction is the precipitation of calcium oxalate crystals, which are insoluble in the face of acid challenge, at the tubule entrance and within it[16,20]. Arrais, Chan and Giannini[8] when performing SEM analysis of a specimen fractured in the lingual-vestibular direction, observed that potassium oxalate promoted occlusion within the tubule, with the formation of some crystals with the same diameter as that of the dentinal tubule. In this study, there was no formation of a uniform film capable of sealing the dentin surface, as the excess superficial gel is removed on conclusion of the application time[9]. In addition to this occlusive effect, *in vitro* studies have shown that the presence of intratubular potassium ions may be related to desensitization of the intradental nerve, by inactivating the generation of the action potential, however, this effect is still inconclusive[21].

The adhesive system used presented a superficial mechanical barrier capable of sealing the dentinal tubule orifices[21,22]. As regards the use of self-etching adhesive systems that do not remove the smear layer, and produce a smaller opening of dentinal tubules, one expects a reduction in dentin permeability[23]. The hybrid layer formed by these adhesives, as verified in the present study, has been shown to be thin, and due to the fact that the monomers penetrate into the tissue simultaneously to its demineralization, it favors a reduction in demineralized spaces not filled with adhesives[24], which may generate discomfort and sensitivity.

Whereas fluoridated varnish, potassium oxalate and the adhesive system are, in some ways, deposited on the surface and within the tubules, the high power diode laser interacts with the dentinal substrate changing its morphology. This laser has been shown to be highly absorbed by pigmented tissues, it has shown weak interaction with mineralized structures, and is not capable of causing considerable morphological alterations, due to the coefficient of water absorption being low[25,26]. However, in the surfaces irradiated with the parameters proposed in this study, characteristics of fusion and resolidification of dentin were observed, and may contribute to a reduction in permeability in clinical application.

Haypek et al.[27], when irradiating root surfaces with a power of 1.4 W, using the fiber optic in contact mode, found a modified smear layer, showing a surface with a smoother appearance, corresponding to fusion and resolidification, without the presence of open dentinal tubules, and with a temperature increase corresponding to safe limits. Whereas Kreisler et al.[28] verified the morphological alterations with the fiber optic in contact mode, at angles of 90° and 10°, with irradiation time intervals of 10, 20 or 30 s for each specimen, and found no negative effects on the root surface with the use of powers of 0.5 and 1 W.

A morphological analysis comparing the effects of different high power lasers on the occlusion of dentinal tubules was performed by Gholami et al.[25]. The results of mean dentinal tubule diameter after irradiation with Er,Cr:YSGG; diode at 810 nm; CO_2 and Nd:YAG were, 1.73; 3.27; 2.10 and 1.64 µm, respectively, in comparison with 3.52 µm before laser irradiation. The reduction in tubular diameter with the irradiation of all the lasers was considered statistically significant ($p<0.05$), and with the use of diode laser, slight fusion and peritubular and intertubular dentin irregularity was observed. However, it is known that the light-dentin interaction may be influenced by the physical and chemical properties of dentin, which vary from tooth to tooth, and therefore, cannot be controlled experimentally[29].

Therefore, the *in vitro* morphological evaluation of the dentin surface treated with desensitizing agents is the first stage in the understanding of the interaction of these agents with dentin. Further clinical studies are necessary to evaluate the behavior of these materials in the face of the challenges of dietary and toothbrushing habits, to verify their effectiveness over the course of time.

Conclusions

All the desensitizing agents used in this study were shown to be effective in occluding the dentinal tubules, with different mechanisms of action.

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