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Laboratory Research

Occluding Effect of Nd:YAG Laser and Different Dentin Desensitizing Agents on Human Dentinal Tubules *In Vitro*: A Scanning Electron Microscopy Investigation

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Clinical Relevance

Varying degrees of dentinal tubule occlusion can be achieved with a Nd:YAG laser and the different desensitizing agents. The efficacy of these treatment modalities needs to be determined through clinical investigation.

SUMMARY

Objectives: This *in vitro* study aimed to microscopically evaluate and compare the occluding effect of the Nd:YAG laser and different dentin desensitizing agents on human dentinal tubules.

Materials and Methods: The Nd:YAG laser (SunLase™ 800) and four commercially available and professionally applied dentin desensitizers (Gluma® desensitizer, Tenure Quick®, Quell™ desensitizer, and VivaSens®) were investigated in this study. Sixty-four extracted intact human molars were used. Each dentin surface was divided by shallow indentation into two halves, one of which was used for treatment and the other of which served as a control. The dentin surfaces were etched to remove any smear plugs and to mimic the open dentinal tubules of sensitive dentin using 0.5 M ethylenediaminetetraacetic acid (pH 7.4) for two minutes (applied with a microbrush) and then rinsed with an air-water syringe for 30 seconds. The laser samples (n=16) were ran-

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domly divided into four groups of four samples each. These groups were the step-up technique group, the 14-day group, the one-minute group, and the two-minute group. Forty-eight samples were treated with the four tested desensitizing agents and were randomly divided into four groups (n=12/group). Each group was further subdivided into three subgroups (n=4). Samples of the first subgroup were treated for 14 days, while those of the second subgroup were treated once. Samples of the last subgroup were fractured longitudinally after a single treatment. All of the samples were then examined under a scanning electron microscope.

Results: The Nd:YAG laser–irradiated dentin showed reduction or complete obliteration of the dentinal tubule lumen; thus, the treatment modified the original dentinal structure. The lased dentin surface in the two-minute group showed bubble-like changes in the area of the dentinal tubules' orifices. Statistically, the two-minute group was found to have a significantly higher percentage of partially or fully occluded tubules than did the one-minute group. All of the studied desensitizing agents produced occlusion of the dentinal tubules; however, the appearance of the precipitates, the level of coverage, and the degree of dentinal occlusion varied among the tested products.

Conclusion: Throughout the specified period of this study, occlusion and/or narrowing of the open dentinal tubules have been successfully achieved with both treatment approaches.

INTRODUCTION

Dentinal hypersensitivity is one of the most painful and least predictably treated chronic conditions in dentistry.[1] It is defined as pain arising from exposed dentin, typically in response to chemical, thermal, or osmotic stimuli, that cannot be explained as arising from any other form of dental defect or pathology.[2]

The nature of the exposed dentin is of relevance, as not all patients exhibiting dentin exposure will develop sensitivity. The number and diameter of dentinal tubules at the tooth surface have been shown to be significantly increased in hypersensitive dentin.[3,4] While the exact mechanism of dentinal hypersensitivity is still controversial, the hydrodynamic theory is the most accepted hypothesis.[5] The dentinal tubules, which are open and wide, contain a fluid. According to this theory, this fluid expands when it is exposed to heat and contracts when it is exposed to cold or touch. The contraction and expansion change the pressure in the fluid phase, which in turn activates mechanoreceptor nerves close to the pulp. When nerve receptors are activated, sodium ions enter the dentin and potassium exits. This ion exchange polarizes the nerves and causes pain.[4] The concept of tubule occlusion as a method of dentin desensitization is a logical conclusion based on the hydrodynamic hypothesis.[6] The most commonly used agents in the treatment of dentin hypersensitivity can be broadly classified into the following groups: anti-inflammatory agents (corticosteroids), protein precipitants (formaldehyde, silver nitrate, strontium chloride hexa-hydrate), tubule occluding agents (calcium hydroxide, potassium nitrate, sodium fluoride), tubule sealants (resins and adhesives), and miscellaneous (laser).[1] Lasers may play a prominent role in treating hypersensitive dentin and providing reliable and reproducible results.[7,8] Usually a sequence of treatment methods are used, starting with the most conservative and switching to more aggressive treatment options if the original methods were not effective.

In clinical trials, several resin adhesives have demonstrated significant reductions in cervical dentin hypersensitivity. Gluma desensitizer is a resin material that was originally developed as the primer for a dentin-enamel adhesive system. This material's mode of action is based on resin and protein precipitation. It is believed that Gluma internally occludes the dentinal tubules, probably through coagulation of plasma proteins in the dentinal fluid.[9] According to the manufacturer, it achieves its effect by precipitation of plasma proteins, which reduces dentinal permeability and occludes the peripheral dentinal tubules. These inhibit the flow of fluid through the tubules that causes sensitivity.

Formulations containing potassium salts (eg, chloride, nitrate, citrate, and oxalate) are widely used for treating dentin hypersensitivity. In 2001, Gillam *and others*[10] evaluated four commercially available oxalate-containing products. Tenure Quick® (aluminum oxalate), Sensodyne Sealant® (ferric oxalate), and MS Coat® (oxalic acid) covered the dentin surface and occluded the tubules. However, ButlerProtect® (potassium oxalate) did not cover the surface to any great extent but provided some dentinal occlusion. The authors concluded that professionally applied in-office products containing oxalate are capable of covering the dentin surface and/or occluding the tubules to varying degrees.

Combining solutions of calcium chloride and potassium phosphate can result in the precipitation of amorphous calcium phosphate. Applied to exposed dentin, such a system could occlude dentinal tubules and reduce sensitivity.[11] Quell Desensitizer, which was used in this study, forms a gel on the surface of the tooth that deposits amorphous calcium phosphate (ACP) on the dentin surface and into the open dentinal tubules, according to the manufacturer.

The laser, by interacting with the tissue, causes different tissue reactions, according to its active medium, wavelength, and power density and based on the optical properties of the target tissue.[12] The lasers used for the treatment of dentin hypersensitivity are divided into two groups: low output power (low-level) lasers (helium-neon [He-Ne] and gallium/aluminum/arsenide [GaAlAs] (diode) lasers) and middle output power lasers (Nd:YAG and CO_2 lasers).[7] The laser interaction with the dental pulp causes a photobiomodulating effect, increasing the cellular metabolic activity of the odontoblasts and obliterating the dentinal tubules with the intensification of tertiary dentin production.[12]

It has been suggested[13] from experiments with lasers on hypersensitive teeth that the laser likely results in a melted dentin surface, with occlusion of open dentinal tubules. Cox and others[14] observed melted dentin, crazing on the surface, slight debris formation, and modification of dentin tubule structure where the tubule periphery had melted. The sealing of exposed dentinal tubules with melted and recrystallized dentin is caused by the thermal and occlusive effects of laser, which result in prolonged relief.[14] Dentin desiccation after laser irradiation is another possible mechanism, which could result in temporary relief of dentin hypersensitivity.[15] According to Sun and Tunér,[16] more difficult cases of dentin hypersensitivity can be treated by the use of lasers. A hypersensitive tooth that does not respond to 4 to 6 J per root in two or three sessions is indicated for endodontic treatment. They further recommend that the occlusal scheme is evaluated as part of the treatment protocol.[16]

The use of lasers may open up new dimensions in the treatment of dentinal hypersensitivity. Pashley and others[17] reported that CO_2 laser irradiation can fuse dentin and reduce dentinal permeability. Zhang and others[18] investigated the efficacy of the CO_2 laser in the management of dentinal hypersensitivity and found that the CO_2 laser (1 W in a continuous wave mode and irradiation time ranging from five seconds to 10 seconds) is useful in the treatment of cervical dentinal hypersensitivity without thermal damage to pulp.

The effectiveness of two types of lasers, the Nd:YAG laser (1 W and 10 Hz for 60 seconds at 1064 nm) and the 685-nm diode laser (25 mW and 9 Hz for 100 seconds), were evaluated in terms of dentin desensitization as well as both the immediate and late therapeutic effects on teeth with gingival recession. Both lasers were found to be effective in treating dentin hypersensitivity without adverse effect. However, the Nd:YAG laser was more effective than the diode laser for desensitization of teeth with gingival recession.[19] In another study,[20] the effectiveness of three types of lasers, Er:YAG (2940 nm, 60 mJ/pulse, 2 Hz, 20 seconds), Nd:YAG (1064 nm, 100 mJ/pulse, 15 Hz, 100 seconds), and GaAlAs (Diode; 808 nm, 100 mW, 20 seconds), as dentin desensitizers was evaluated. It has been found that Nd:YAG laser irradiation is more effective in the treatment of dentin hypersensitivity than are the Er:YAG laser and the diode laser.

The Nd:YAG is a near-infrared laser with a primary wavelength of 1064 nm. Other wavelengths exist with Nd:YAG but are not typically used in dentistry. It is very important to note that the Nd:YAG laser is non-ionizing in character and is therefore nonmutagenic. This wavelength is invisible, and, therefore, an additional laser beam or white light aiming beam is always added as an integral part of the system.[21] The ability to perform procedures at very low power settings is a unique and positive feature of the free-running Nd:YAG laser, since the aim is to use the smallest amount of energy to achieve therapeutic goals. The first use of Nd:YAG laser for the treatment of dentin hypersensitivity was reported by Matsumoto and others[25] in 1985.

The laser used in this study, SunLase™ 800 (Dental Laser System, SUNRISE Technologies), provides a constant beam of coherent, bundled, continuous monochromatic light with an emission wavelength of 1064 nm, which was delivered through a 320-μm optic fiber tip with a straight hand piece. The Nd:YAG laser was chosen in this study because it has been investigated in numerous studies and has been reported to be effective in treating dentin hypersensitivity *in vitro* and *in vivo*.[1,8,22,23] In addition, since this laser is one of the most widely studied lasers, it would be a good choice for comparison with other treatment modalities.

Since dentin permeability and hypersensitivity are both reduced when the dentinal tubules are obturated or occluded, techniques and/or agents that effectively occlude the dentinal tubules are extremely important tools in managing hypersensitive dentin. Many desensitizing treatments have been investigated both *in vitro* and *in vivo*.[24-32] However, no study to date has compared the effect of several different desensitizing agents with the effect that the Nd:YAG laser has on the dentinal tubule's morphology. The aim of this study was to quantitatively and qualitatively investigate and compare the occluding effect of the Nd:YAG laser and some desensitizing agents on human dentinal tubules using scanning electron microscopy (SEM).

MATERIALS AND METHODS

Sixty-four freshly extracted caries-free human first and second molars were used in this study. Immediately after extraction, they were stored in a dark glass container of 0.025% thymol solution at 4°C to inhibit microbial growth until use. The roots of the teeth were cut just below the root furcation area using a diamond disc (Rotary Dental Instrument, Kahla, Germany) on a straight hand piece (KAVO EWL type 4415, KAVO, West Germany), then the pulp chamber was closed with sticky wax to prevent seepage of pulpal tissues. Facial or lingual surfaces (depending on which of them is more flat) were ground flat at the cervical one-third of the crown to remove enamel and to expose superficial dentin using 240-grit and 320-grit aluminum oxide paper (HANDIMET II Roll Grinder, BUEHLER, USA).

Each dentin surface was divided into two halves. One half was marked by a dimple on the enamel surface and was exposed to the treatment, and then the other half was used as a control (Figure 1). Fractured specimens were prepared so that the entire surface received the treatment after a minimal groove had been made on the enamel surface vertically and parallel to the long axis of the tooth to aid the fracture procedure. Prior to treatment, the exposed dentin surfaces were polished with wet 400-grit and 600-grit aluminum oxide abrasive paper; an average grinding time of 30 seconds was used for each specimen. Then specimens were placed in an ultrasonicator (SONICER, Yoshida Dental Mfg Co Ltd, Osaka, Japan) filled with distilled water for 30 minutes to remove any debris. The dentin surfaces were etched to remove any smear plugs and to mimic the open dentinal tubules of sensitive dentin using 0.5 M ethylenediaminetetraacetic acid (EDTA) (pH 7.4) for two minutes (applied with a microbrush); samples were then rinsed with an air-water syringe for 30 seconds[28] (Figure 2).

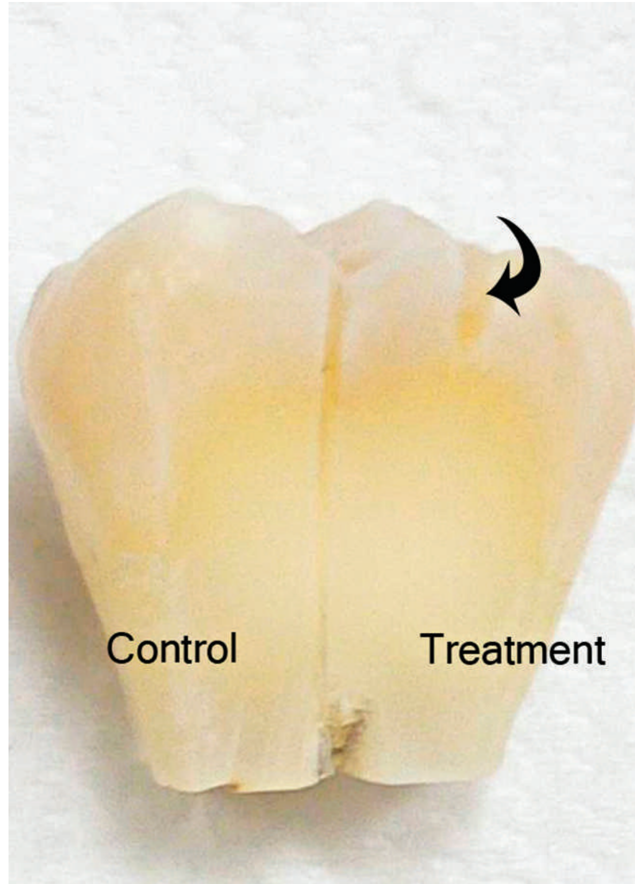

Figure 1. *Prepared tooth specimen with the dentin surface divided into two halves by shallow indentation. Note the dimple that indicates the treatment side (arrow).*

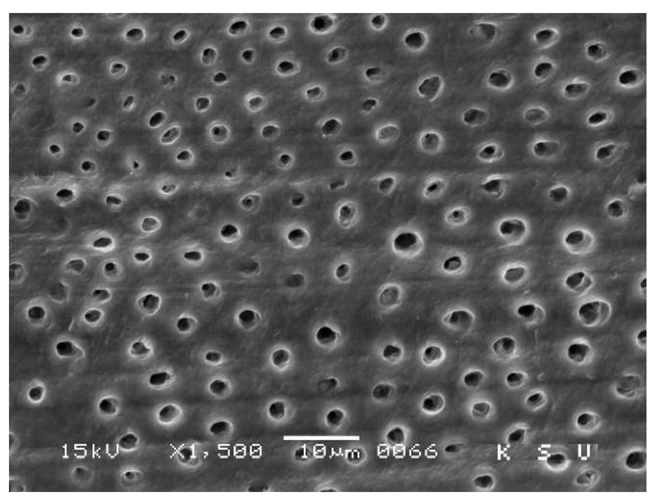

Figure 2. *Scanning electron micrograph (1500×) of dentin surface that has been etched with 0.5 M EDTA (pH 7.4) for two minutes to remove smear layer and to mimic the open dentinal tubules of sensitive dentin.*

Table 1: *Application Time, Techniques, and Power Densities for the Pulsed Nd:YAG Laser Group*

Fiber Type	PPS (pulses per second)	Application Technique	Power, W	Application Time
320 noncontact mode	10	Step-up technique	0.4	30 s for each degree of intensity, with 15-s lag between irradiations
			0.5	
			0.6	
			0.7	
			0.8	
		Single power	0.8	One min
		Single power	0.8	Two min

The samples were randomly divided into the following treatment categories. 1) Pulsed Nd:YAG Laser SunLase™ 800 (Dental Laser System, SUNRISE Technologies) was used to treat the dentin samples. The laser was applied in a "step-up" procedure (30 seconds for each degree of intensity), with a 15-second lag between irradiations for thermal relaxation of the tissue. The machine is supplied with a 40° radius cannula for 320-μm fiber. The laser tip was held perpendicular to the irradiated surface in noncontact mode 2–3 mm away from the tooth in a continuous flowing motion. The laser tip was swept in a zigzag pattern and in circular fashion, respectively; a distance of approximately 1 mm was maintained between consecutive lines to ensure complete surface coverage. According to the manufacturer, the maximum intensity for desensitization must not exceed 0.8 W per application. Application time, techniques, and power densities for the pulsed Nd:YAG laser group are shown in Table 1.

A total of 16 samples were randomly distributed into four groups, as follows: Group 1: Step-up group; four samples were lased with the Nd:YAG laser using the step-up technique mentioned above and were observed immediately; Group 2: Step-up 14 group; four samples were lased twice (days 1 and 14) with the Nd:YAG laser using the step-up technique mentioned above. In between laser applications, the samples were stored in artificial saliva solution until day 14, when the treatment was repeated;

Group 3: One-minute group; four samples were lased with the Nd:YAG laser using only 0.8 W for one minute. Two of the samples were frozen using liquid nitrogen and were fractured using a sharp chisel and a mallet, and the internal tubule morphology was observed immediately under SEM; and Group 4: Two-minute group; four samples were lased with the Nd:YAG laser using only 0.8 W for two minutes. Two of them were frozen using liquid nitrogen and fractured using a sharp chisel and a mallet, and the internal tubule morphology was observed immediately under SEM.

2) The desensitizing agents: The desensitizing materials used and their composition and method of use are summarized in Table 2. The treatment details were determined according to the manufacturer instructions supplied with each material. Group 1: Gluma® desensitizer (Heraeus Kulzer, Dormagen, Germany); Group 2: Tenure Quick® (Den-Mat Corporation, USA); Group 3: Quell™ desensitizer (Pentron Clinical Technologies, LLC, Wallingford, CT, USA); and Group 4: VivaSens® desensitizing varnish (Ivoclar Vivadent AG, FL-9494 Schaan/Liechtenstein).

For each of the four previously mentioned groups, a total of 12 samples were randomly distributed into three subgroups, as follows: i) Multiple applications: four samples received 14 days of treatment. The material was applied every fourth day (days 1, 5, 9, and 13). For the duration of the experiment, the samples were stored in artificial saliva at 37°C until day 14 and were then evaluated. The artificial saliva solution was changed after each treatment application; ii) Single application: four samples received a

Table 2: *The Desensitizing Materials Used in this Study, Their Composition and Method of Use*

Material/Manufacturer	Composition	Method of Use
Gluma® desensitizer	5% Glutaraldehyde, 2-hydroxyethyl-methacrylate (HEMA) purified water	Applied with a microbrush and left for 60 s, dried, then rinsed with water
Tenure Quick®	Aluminum oxalate–based agent	Six coats were applied with a microbrush, dried, then light-cured for 30 s; procedure was repeated once
Quell™ desensitizer	Amorphous calcium phosphate (ACP)–based agent	Using a microbrush, dentin surface was rubbed with part A solution for 10 s (repeated twice) and the surface was left wet. Then part B solution was applied in the same manner and repeated two times as well
VivaSens® desensitizing varnish	Liquid varnish (ethanol, water, and hydroxypropylcellulose) containing potassium fluoride, polyethyleneglycol dimethacrylate, and other methacrylates	Three drops (mixed with a brush precoated with organic acid) were applied by gentle surface rubbing, then gently air-dried for 10 se

single treatment with Gluma and were then observed immediately under SEM; and iii) Fracture samples: four samples received a single treatment application, and then they were frozen using liquid nitrogen and fractured using a sharp chisel and a mallet for observing the internal tubule morphology. Care was taken to avoid potential dissolution of the desensitizing agents on the dentinal surface, which may occur with conventional specimen preparation.

SEM Preparation and Evaluation

The specimens were washed and air-dried and then prepared for SEM examination. The specimens were mounted on SEM stubs and sputter-coated with approximately 300 Å of gold (Fine Coat JFC 1100, Teol Ltd, Tokyo, Japan) and examined under low-vacuum SEM (Jeol JSM-T330 A, Jeol Ltd). The treated dentinal surfaces were scanned and their micromorphology was evaluated. Additionally, the presence of any dentinal surface alteration, precipitation, or debris was noted and described. Representative scanning electron micrographs were taken at different magnifications. They were chosen based on the frequently observed appearance of the treated dentin surface, and they were judged by two evaluators to represent the treatment effect.

Quantitative Assessment

Following the pilot study, it was observed that only the Nd:YAG laser and the Gluma desensitizer groups were amenable to quantitative assessment (percentage of tubule occlusion), because all the other treatment surfaces in other groups were totally covered by the desensitizing materials and, thus, there were no dentinal tubules evident for counting.

The percentage of partially and/or fully occluded tubules was calculated for each representative micrograph from groups 1 and 2; four micrographs were evaluated for each specimen using the following simple formula:

$$Percentage\ of\ partially\ or\ fully\ occluded\ tubules = \frac{Number\ of\ partially\ or\ fully\ occluded\ tubules \times 100}{Total\ number\ of\ tubules}$$

The fully and partially occluded tubules in each micrograph were marked using a computer program (Adobe® Photoshop® Elements 2.0, version 2.0.2, Adobe Systems Incorporated, Adobe, Tokyo, Japan), and the mean percentages were then calculated for both groups. All the micrographs calculated were at magnification 2000×, and the dimension of the picture side was 10 μm.

Using SPSS 12 for windows (SPSS Inc, Chicago, IL), statistical analysis was done using one-way analysis of variance (ANOVA) and the Tukey post hoc test for multiple comparisons at the 5% level of significance (95% confidence level) for the six subgroups (laser step-up , laser twice step-up, Gluma immediate, Gluma 14 days, laser one minute, and laser two minutes).

Qualitative Assessment

The micrographs were evaluated for their surface characteristics and for the patency or occlusion of the dentinal tubules. Ranking criteria for surface characteristics of the micrographs (Figure 3) was conducted using the following descriptive categories (modified and adapted from Kumar and Metha[1]):

A. The dentinal tubules are partially occluded and dentinal orifices are slightly smaller, with little or no debris.
B. The dentinal tubules are mostly occluded; the dentinal surface is devoid of film or precipitate.
C. The dentinal tubules were mostly occluded; the dentinal surface is partially covered with film or precipitation or shows some surface alterations.
D. All the dentinal tubules are totally occluded and the surface is totally covered with precipitate and/or a resin film.

RESULTS

Quantitative assessment for the Nd:YAG laser and Gluma groups showed that the one-minute laser group had the smallest mean percentage of partially or fully occluded tubules, while the two-minute laser group had the highest mean percentage. One-way ANOVA revealed statistically significant differences (in the mean percentages of partially and fully occluded tubules) between at least two of the six groups (laser step-up immediate, laser step-up 14 days, Gluma immediate, Gluma 14 days, laser one minute, and laser two minutes) ($p<0.05$). The Tukey post hoc test for multiple comparison revealed a statistically significant difference ($p<0.05$) between the laser one-minute and the laser two-minute groups; however, for the other groups, no statistically significant differences were found ($p>0.05$).

Qualitative assessment of micrographs of dentin surfaces treated by the tested materials revealed different patterns of material deposition onto the dentin surfaces. The results demonstrated that all of the applied desensitizing agents as well as the laser produced occlusion of the dentinal tubules, although the level of coverage and occlusion varied between the products.

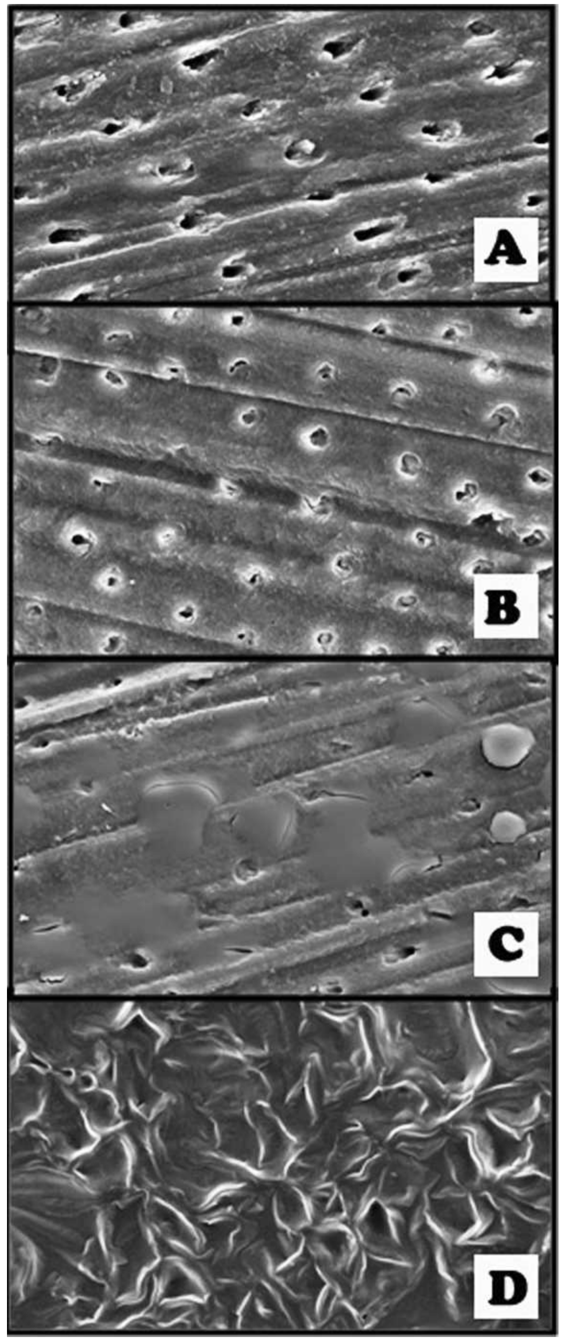

Figure 3. *Representative micrographs of the descriptive categories of the qualitative assessment (picture index).*

Nd:YAG Laser

The lased dentin surface in all treatment subgroups showed occlusion of open dentinal tubules in most areas, with evident narrowing and reduction in the diameter of the affected tubules. The dentin surface showed little or no alteration in the samples treated using the step-up technique; however, the majority of dentinal tubules were fully occluded, and some showed only a reduction in their diameter (Figure 4a). The same micromorphology was observed in the samples treated twice (during 14 days) with the step-up technique, although their dentinal surfaces showed some precipitations of surface debris (Figure 4b). In some irradiated samples, there appeared to be some banding or zigzag patterning at the dentinal surface in the areas where the laser beam had passed, and it reflected the method that was used in

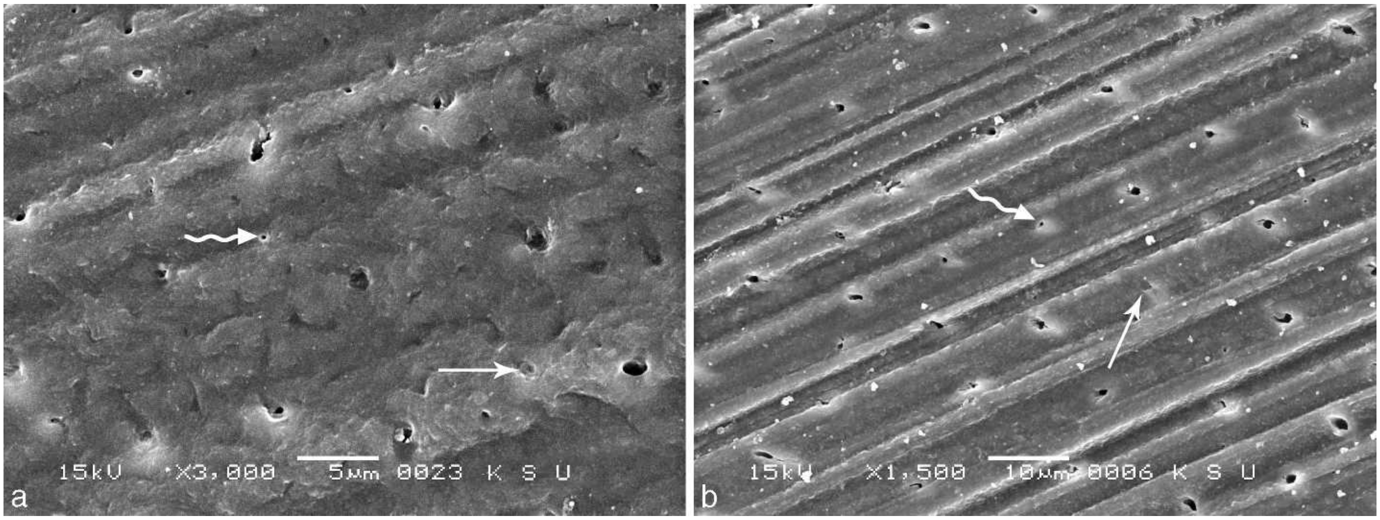

Figure 4. *Scanning electron micrographs showing (A) dentin surface treated once with Nd:YAG laser using the step-up technique(3000×) and (B) after multiple applications and storage in artificial saliva solution for 14 days (1500×). Note the fully occluded tubules (arrow) and the tubules with reduced diameter (curved arrow).*

the application of the laser beam. The samples lased for one minute using the power of 0.8 W showed many occluded tubules as well as some tubules with reduced diameter, and there were no precipitations or surface alterations on the treated dentinal surfaces (Figure 5a). The fractured samples of this subgroup showed some sealed dentinal tubule orifices (Figure 5b). The samples lased for two minutes using the power of 0.8 W showed not only dentinal tubules that were closed and sealed but also peritubular dentin that appeared to be melted. It was smooth and glossy compared to the surrounding dentin surface (Figure 6a), with round-elliptical, bubble-like changes at (and around) the area of the dentinal tubule orifices. The fractured samples of the laser two-minute group showed occluded tubules in some areas at the surface, and in some micrographs the melting of the dentin subsurface could be observed (Figure 6b).

Gluma®

Generally the samples treated with Gluma desensitizer showed some occluded dentinal tubules with a dentin surface that was devoid of debris but showed some intratubular material precipitations. No mi-

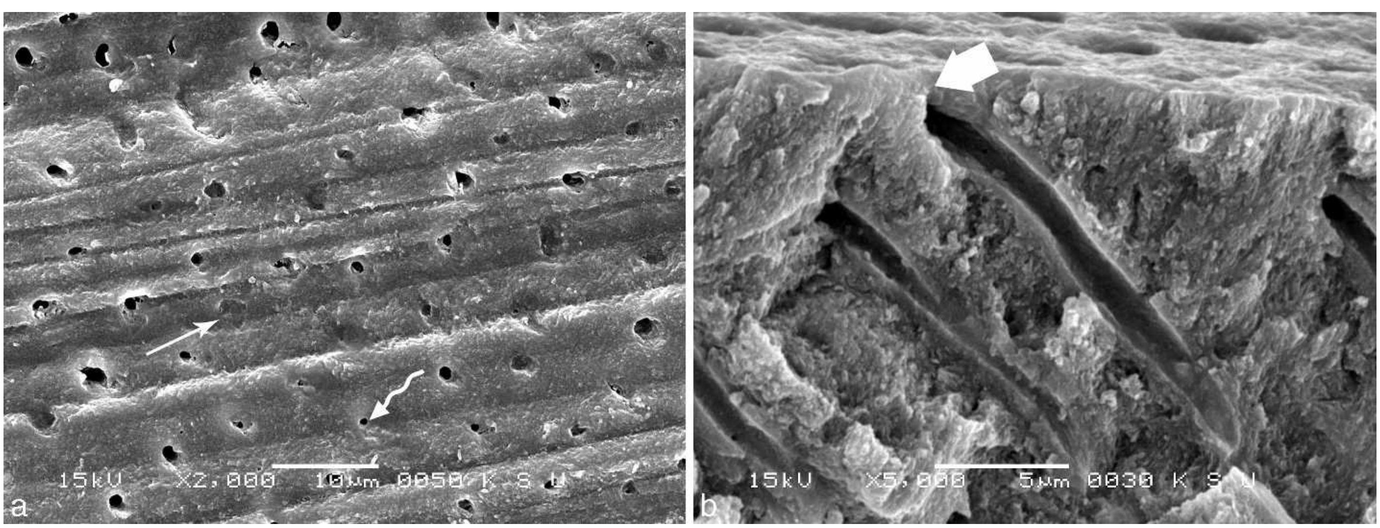

Figure 5. *Scanning electron micrographs showing dentin surface treated with Nd:YAG laser (0. 8W for one minute). (A) Treated dentin surface (2000×). (B) Fractured dentin surface (5000×). Note the fully occluded tubules (arrow) and the tubules with reduced diameter (curved arrow) and the occluded tubule orifice (thick arrow).*

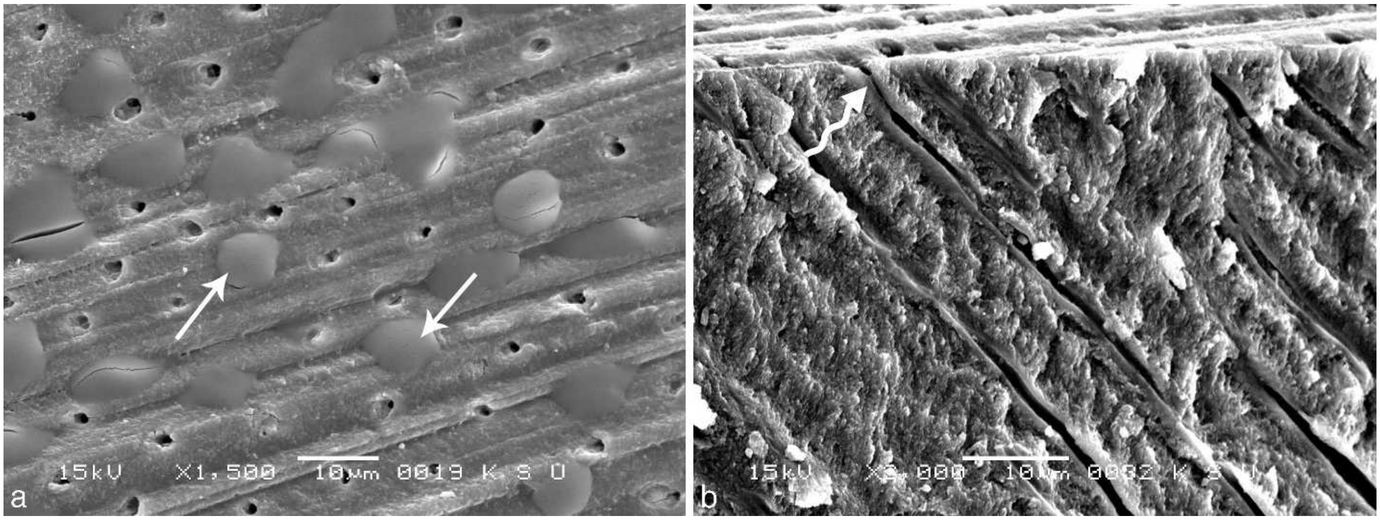

Figure 6. *Scanning electron micrographs showing dentin surface treated with Nd:YAG laser (0.8 W for two minutes). (A) Treated dentin surface (1500×). (B) Fractured dentin surface (3000×). Note the laser-induced surface changes, occluded tubules as well as melting of the area around the tubules (arrows), and the tubule orifices closure or (melting) (curved arrows).*

cromorphological differences were found between the single-application samples and the multiple-application (14-day) treatment samples. Some dentinal tubules were closed, some appeared open, and others showed reduced lumen diameter (Figure 7a). The fractured samples showed some tubular occlusion and tubule plugs. Frequently the precipitations were observed to be extended into the tubule lumen more than they were in any other treatment group, although the surface deposits were comparatively much smaller. In some areas of the fractured samples, the deposits were found to be extended 7.38 μm inside the tubule lumen (Figure 7b).

Tenure Quick®

Total coverage of the dentin surface was observed in all samples treated with this aluminum oxalate–based material, as the dentinal tubules were no longer visible. The treated dentinal surface appeared rough and irregular with multiple crystal-like structures. In some areas there were clusters of the material (highly charged and appeared intensely white). This micromorphology was observed in the single-application as well as the multiple-application (14-day) treatment samples (Figure 8a). When the treated dentin surfaces were fractured, it was observed that the material formed a thick (up to

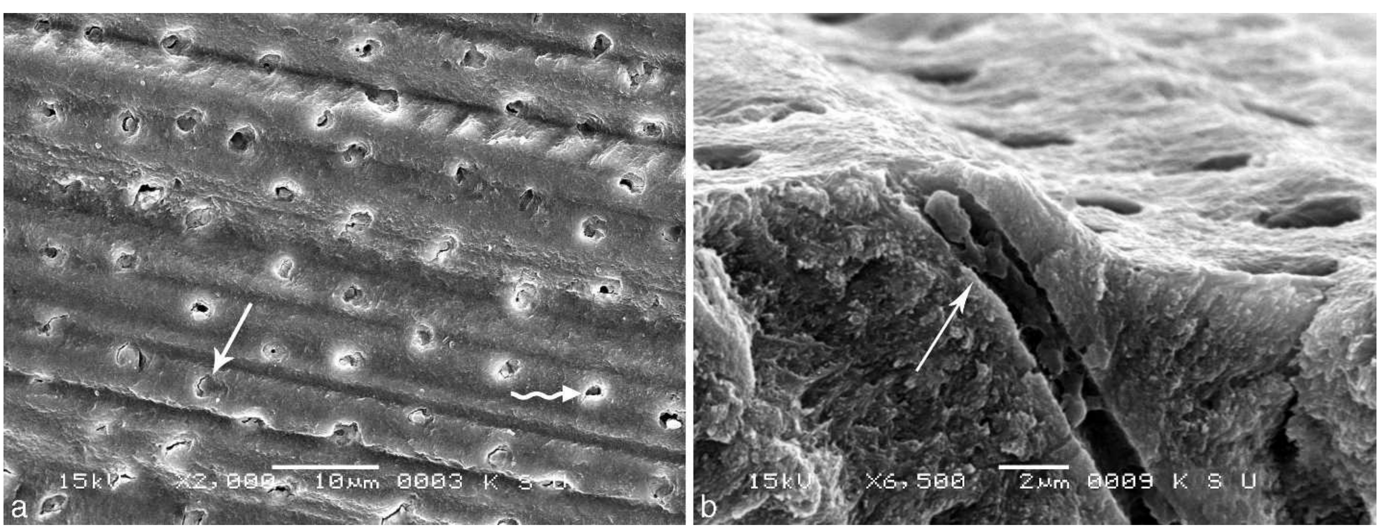

Figure 7. *Scanning electron micrographs showing (A) the dentin surface treated with Gluma (2000×). Note the fully occluded tubules (arrow) and the reduced diameter tubules (curved arrow). (B) The fractured dentin surface (6500×). Note that the material penetrated to a considerable depth into the dentinal tubule lumen (two-way arrow).*

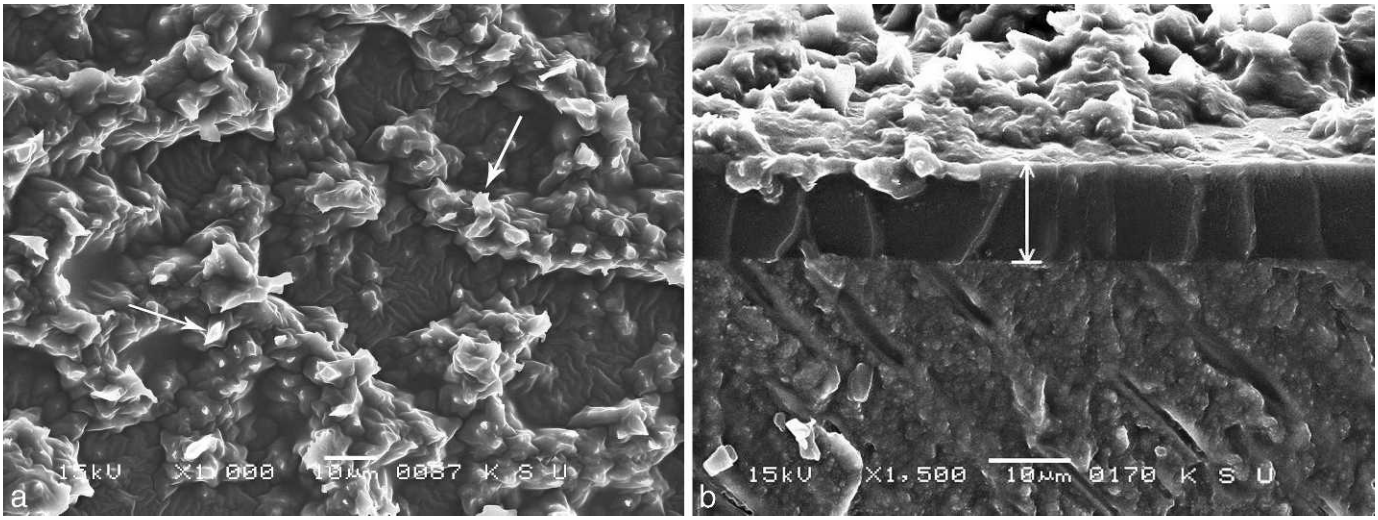

Figure 8. *Scanning electron micrographs showing (A) the surface deposition of Tenure Quick on dentin surface (1000×). Note the complete coverage of the dentinal tubule orifices and the crystal-like structures (arrows). (B) The dentin surface after fracture (1500×). Note the thick material deposit on the dentin surface (two-way arrow) and the completely plugged tubule orifices.*

11.58-µm) solid layer (Figure 8b) that was closely adhered to the dentin surface and that had numerous crystalline surface deposits. In some areas the material penetrated the tubule lumen to some extent.

Quell™

Scanning electron micrographs of dentin samples treated with amorphous calcium phosphate, the active ingredient in Quell desensitizer, showed that the dentin surfaces were totally covered with a thick, rough, porous woven layer, and the dentinal tubules were not visible at all. This micromorphology was observed in the single-application as well as the multiple-application (14-day) treatment samples (Figure 9a). When the treated dentin surfaces were fractured, it was evident that the material occluded the tubule orifice with crystal-like structures, and the rough, thin surface layer (1-1.5 µm) appeared to be closely adhering to the dentin surface. Some material crystals appeared to penetrate the tubule lumen to some extent (Figure 9b).

VivaSens®

All of the samples treated with VivaSens showed total coverage of the dentinal surfaces with an apparently smooth layer of wavy, fiber-like, glossy

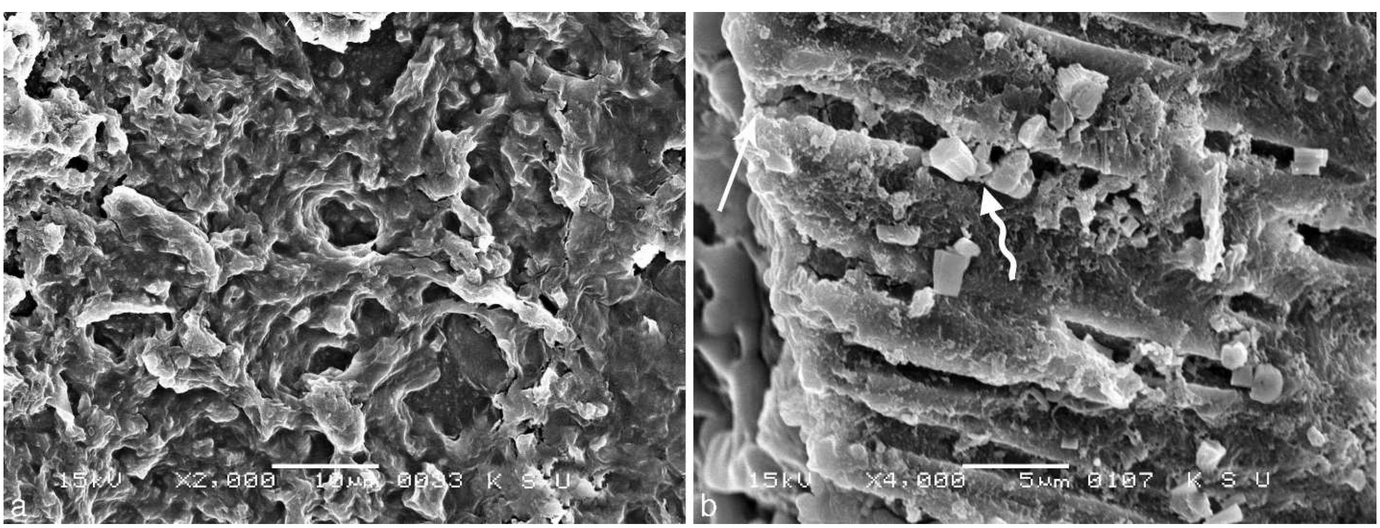

Figure 9. *Scanning electron micrographs showing (A) the surface deposition of Quell desensitizer on dentin surface (2000×). Note the woven porous appearance of the thick deposit that completely masked the dentinal tubule orifices. (B) The fractured dentin surface (4000×). Note the occluded orifices (arrows) and the material extension into the tubule lumen (curved arrows).*

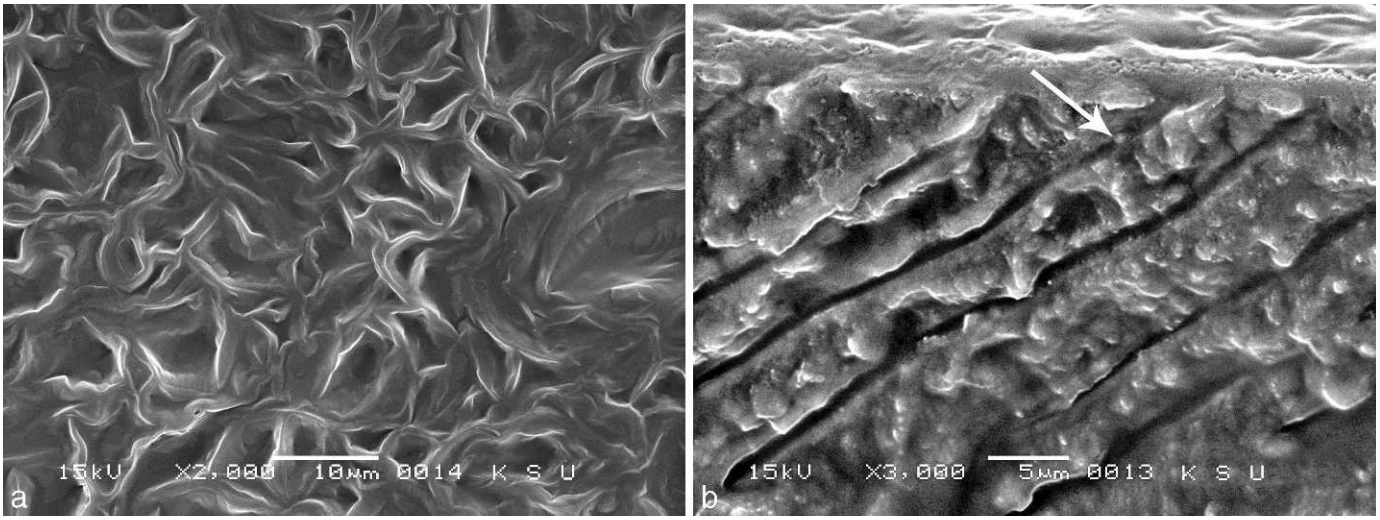

Figure 10. *Scanning electron micrographs showing (A) the dentin surface treated with VivaSens (2000×). Note the complete surface coverage with this resin-based desensitizer. (B) The fractured dentin surface; note the resin-based material extension into the tubules (arrow).*

resin coating that masked the dentinal tubules as well as the whole dentin surface. This micromorphology was observed in the single-application as well as the multiple-application (14-day) treatment samples (Figure 10a). When the treated dentin surfaces were fractured, the surface resin precipitate appeared to be thin (2-5 μm) and intimately coated the dentinal surface. Additionally, this resin material was found to extend into the tubule lumen to some degree (Figure 10b).

For all of the four desensitizing agents, there appeared to be very minor micromorphological differences between the immediate and the 14-day groups. Therefore, the two groups were pooled for the qualitative assessment. The fractured samples were evaluated separately. In the laser-treated groups (Table 3) the majority of the samples fell into categories B and C, while the Gluma samples mainly belonged to category B. For Tenure Quick, Quell, and VivaSens, all of the samples were marked as category D except for the fractured samples, which were marked as category C (Table 4).

DISCUSSION

Interstitial fluid movement within the dentinal tubules is the basis for the transmission of sensations.[27] A possible approach to reducing or eliminating the painful symptoms of dentin hypersensitivity is the interruption of stimuli transmission to the nerve endings of odontoblastic processes by reducing the fluid movement inside the dentinal tubules through the narrowing or occlusion of tubule openings.[30,31] Dentinal tubules can be sealed on the dentin surface, occluded within their orifices or in the subsurface dentin within their tubules. It can be assumed that intradentinal closure or seal is the most promising approach with regard to long-term success.[35]

The dentin surface of all of the samples was prepared by etching with 0.5 M EDTA prior to treatment. This step was done to ensure that the prepared dentin surface was free of any smear layer or smear plugs that might be confused with treatment materials. Furthermore, sensitive dentin has been shown to have wide patent tubules,[3] so the etching process aimed at simulating the open tubules of the sensitive dentin. If etching was not done, the dentin surface would be covered with debris and smear layer from the sample preparation steps; this layer has been shown[30] to reduce the permeability of dentin. Therefore, fluid movement within the dentinal tubules, a prerequisite for sensitivity according to the hydrodynamic theory, would be absent.[33]

The professionally applied desensitizing treatments used in the present study were selected according to their different mechanisms of action, namely the thermal effect of laser energy, the protein precipitation effect of Gluma, the tubule occlusion by ion precipitation produced by Tenure Quik and Quell, and the sealing effect of the resin-based VivaSens desensitizing varnish. These different treatments ultimately produced the desired treatment goal, which was the occlusion of the exposed dentinal tubules. Nd:YAG laser–irradiated samples in all subgroups showed considerable

Table 3: *Micromorphological Ranking of the Nd:YAG Laser Group According to the Qualitative Assessment Categories*[a]

Treatment	Category				
	A	B	C	D	N
Laser step-up	0	3 samples (75)	1 sample (25)	0	4
Laser twice step-up	0		All samples (100)	0	4
Laser one-minute (0.8 W)	0	3 samples (75)	1 sample (25)	0	4
Laser two-minute (0.8 W)	1 sample (25)	0	All samples (75)	0	4

[a] *Parenthetical values represent percent. A = The dentinal tubules are partially occluded and dentinal orifices are slightly smaller, with little or no debris. B = The dentinal tubules are mostly occluded; the dentinal surface is devoid of film or precipitate. C = The dentinal tubules were mostly occluded; the dentinal surface is partially covered with film or precipitation or shows some surface alterations. D = All the dentinal tubules are totally occluded and the surface is totally covered with precipitate and/or a resin film. N = number of specimens.*

occlusion of the open dentinal tubules to varying degrees. It was clear that the laser energy had affected and modified the whole dentin surface and not only the dentinal tubules, where melting and re-solidification of the area surrounding the tubules was seen, particularly in group 4, in which the samples had been lased for two minutes. The lased dentin surface appeared smooth, with round-elliptical, bubble-like changes at (and around) the area of the dentinal tubule orifices. This could be attributed to the photo-thermal effect of the laser energy, whereby the high temperatures caused the tubular orifices to melt and swell, leading to this appearance. Such changes indicate excessive heating of dentin and could result in pulpal damage in vital teeth. Our findings are consistent with those of Cox and

Table 4: *Micromorphological Ranking of the Desensitizer Groups: 1 (Gluma®), 2 (Tenure Quick®), 3 (Quell™), and 4 (VivaSens®), According to the Qualitative Assessment Categories*[a]

Treatment	Category				
	A	B	C	D	N
Gluma® immediate and 14 days	0	All samples (100)	0	0	8
Gluma® fractured	All samples (100)	0	0	0	4
Tenure Quick® immediate and 14 days	0	0	0	All samples (100)	8
Tenure Quick® fractured	0	0	All samples (100)	0	4
Quell™ immediate and 14 days	0	0	0	All samples (100)	8
Quell™ fractured	0	0	All samples (100)	0	4
VivaSens® immediate and 14 days	0	0	0	All samples (100)	8
VivaSens® fractured	0	0	All samples (100)	0	4

[a] *Parenthetical values represent percent. A = The dentinal tubules are partially occluded and dentinal orifices are slightly smaller, with little or no debris. B = The dentinal tubules are mostly occluded; the dentinal surface is devoid of film or precipitate. C = The dentinal tubules were mostly occluded; the dentinal surface is partially covered with film or precipitation or shows some surface alterations. D = All the dentinal tubules are totally occluded and the surface is totally covered with precipitate and/or a resin film. N = number of specimens.*

others,[14] as they observed melting and crazing on the dentin surface, slight debris formation, and modification of dentin tubule structure where the tubule periphery had melted.

The mechanism of the Nd:YAG laser's effect on dentin is thermal energy absorption,[8] leading to laser-induced occlusion or narrowing of dentinal tubules.[7] Whitters and others[34]suggested direct nerve analgesia as a possible mechanism; they conducted a clinical trial using an electric pulp tester to measure the extent and duration of any analgesic effect induced by pulsed Nd:YAG laser treatment. A statistically significant increase in pain thresholds was observed in the mean responses measured five minutes after laser treatment with 113-mJ pulses at 15 pulses (pps) for three minutes. However, the pain thresholds returned to baseline values after 60 minutes. On the other hand, Funato and others[35] suggested thermally mediated effects on microcirculation. They observed some vascular changes shortly after the Nd:YAG laser was applied; these changes included vascular shrinkage, degeneration, coagulation, and stasis associated with irradiation. Their results indicate that the effects of Nd:YAG laser irradiation are primarily thermal, that this laser irradiation has a good hemostatic and coagulation ability, and that some of the changes are nerve-mediated by low-energy irradiation.

Samples treated with Gluma showed some closed tubules, but the majority were open. Similar results were obtained by Kolker and others.[25] Gluma desensitizer contains glutaraldehyde (GA) and 2-hydroxyethyl-methacrylate (HEMA). HEMA, which is well known for its water solubility, may act as a carrier/wetting agent to GA and may thus promote deep penetration of the GA component into the tubules. GA is a biological fixative and effective disinfectant, which upon reacting with the proteins in the dentinal fluid induces a precipitation and thus a partial or total occlusion of dentinal tubules. It kills bacteria and coagulates the plasma proteins within the dentinal fluids, forming a coagulation plug. Bergenholtz and others[36] showed a complete cessation of the outward flux of serum albumin in monkey dentin treated with Gluma *in vivo*. Clinically, Dondi and Melferrari[37] found that Gluma desensitizer showed a statistically significant reduction in sensitivity between baseline and postoperative pain scores and between the postoperative and the one-week responses. The sensitivity scores were not different between one week and six months.

Quantitative assessment of the first two groups (laser and Gluma) revealed some statistically significant differences in terms of tubule occlusion. In general, it could be suggested that the main effect of the two treatments (laser and Gluma) was almost the same, although the mechanism of action was totally different. The laser two-minute group produced a higher percentage of occluded tubules than did the one-minute group, with evident dentin surface changes, such as melting and bubble-like appearance of the dentinal tubules peripheries, which are favorable effects in terms of dentin desensitization. Clearly the time is a key factor in these differences: the longer the exposure of the dentin surface to the Nd:YAG laser energy, the more profound the resulting morphological changes. These morphological changes include closure of a larger number of open dentinal tubules, which is the treatment aim. Therefore, more effective dentin desensitization will presumably take place. However, the thermal effect of the relatively long exposure of the dental pulp to the laser energy must be considered. The minimum energy power that could achieve the desired treatment effect must be the ultimate goal in treating dentin hypersensitivity.

Tenure Quick, one of the tested desensitizers, totally covered the dentin surface with a solid, uniformly thick resin layer that was slightly rough and that had numerous crystal-shaped projections. In addition, the dentinal tubules were found to be plugged below the surface in the fractured samples. Presumably, the oxalate from aluminum oxalate, the active ingredient of this desensitizer, reacts with the calcium ions in the dentinal tubules and forms calcium oxalate crystals that precipitate and block the tubule. It is also possible that the thickness and the density of the precipitate increase with the number of applications. Gillam and others[10] showed very similar SEM results and suggested that the tubules are probably occluded with the methacrylate carrier.

Quell desensitizer, an ACP agent, is based on the idea of the physiologic process of sclerotic dentin. Since the occlusive material is apatitic mineral approximating the chemical formula of the main inorganic mineral dentin content [hydroxyapatite $(Ca_3\,(PO_4)_2)_3\,Ca\,(OH)_2$], it provides natural occlusion of dentinal tubules and thus reduces the permeability and sensitivity. It was suggested that this blocking of the tubules is not merely mechanical but also occurs as a result of a chemical interaction between calcium and phosphate ions, increasing the mineral contents of dentin and forming tri-calcium phosphate crystals, which obliterate the dentinal tubules.

Complete dentinal surface coverage was observed in the samples treated with VivaSens. As the resin liquid varnish sealed the dentinal surface, other constituents may have occluded the dentinal tubules. According to the manufacturer this occurs as a result of precipitation of calcium ions and proteins in the dentinal fluid. Potassium fluoride is one of the constituents of this material, which may form fluorapatite or calcium fluoride, in addition to the depolarizing effect of blocking the nerve conduction of potassium ions, which is another possible mechanism. Preparations containing potassium salts were reported[38-41] to be clinically effective in reducing dentin hypersensitivity. Those different mechanisms were thought to work synergistically to alleviate the dentin sensitivity. However, there are no studies to date regarding the long-term effectiveness of this material.

The relationship between surface and intratubular precipitation with sensitivity is not a simple one. It is not just the amount of precipitation that is important, it is also the quality of deposits, their density, degree of porosity, depth of penetration into tubules, and how well they are bound to the dentin surface. All of the materials tested in this study showed some loss of surface precipitation during preparation for fracturing, which indicates that the surface deposits are not firmly bound to the dentinal surface; given the dynamic nature of the oral environment and the local action of brushing, chewing, and saliva, the longevity of their protection is questionable. Further studies are needed to verify this assumption. The determining factor for the effectiveness of all tested treatments is how long the treatment effect will last, which can be known only through the conducting of long-term *in vivo* studies. As the laser-induced changes altered the dentin surface characteristic and morphology, it could be assumed that the laser may have a longer lasting effect than is noted on similar surfaces treated with the different desensitizing agents. The decision to use one treatment over the other should be based on the severity of individual clinical conditions, as each dentin hypersensitivity case has its own etiology and consequently requires different management. Simple approaches to manage hypersensitivity (such as dietary modifications, occlusal adjustments, the use of over-the-counter desensitizing dentifrice, etc) must not be underestimated. Desensitizing agents are inexpensive and easily applied, with the possibility of multiple reapplications. The initial cost of the laser machine is high, but it is cost effective in the long term.

The qualitative and quantitative results of our study can only form the basis of further research. Primarily, the pulp response to the treatments used should be investigated. Both quantitative and functional studies are required in order to determine the effects of these agents on dentin permeability (fluid flow). Clinical studies are needed as well to determine the effectiveness of these agents over time in terms of reducing the pain arising from dentin hypersensitivity. It is also necessary to simulate intraoral conditions, including brushing, acidic challenges, and other conditions.

CONCLUSIONS

Within the limitations of this *in vitro* study, we conclude the following:

- Based on the principle of tubule occlusion for dentin hypersensitivity treatment, all of the investigated treatments have promising desensitizing potential based on their various mechanisms of action. However, their long-term effectiveness must be determined through future clinical studies.
- Within the parameters used in this study, Nd:YAG laser irradiation on dentin resulted in occlusion or narrowing of the open dentinal tubules to various degrees.
- Thick precipitates were produced by Tenure Quick®, Quell™ desensitizer, and VivaSens®, covering the entire dentin surface and completely masking the dentinal tubule orifices. Gluma® desensitizer produced narrowing and occlusion of tubule lumen to some extent, without evident surface precipitate.

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