

In Vitro Study of Dentin Hypersensitivity Treated by 980-nm Diode Laser

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Abstract:

Introduction: To investigate the ultrastructural changes of dentin irradiated with 980-nm diode laser under different parameters and to observe the morphological alterations of odontoblasts and pulp tissue to determine the safety parameters of 980-nm diode laser in the treatment of dentin hypersensitivity (DH).

Methods: Twenty extracted human third molars were selected to prepare dentin discs. Each dentin disc was divided into four areas and was irradiated by 980-nm diode laser under different parameters: Group A: control group, 0 J/cm²; Group B: 2 W/CW (continuous mode), 166 J/cm²; Group C: 3W/CW, 250 J/cm²; and Group D: 4W/CW, 333 J/cm². Ten additional extracted human third molars were selected to prepare dentin discs. Each dentin disc was divided into two areas and was irradiated by 980-nm diode laser: Group E: control group, 0 J/cm²; and Group F: 2.0 W/CW, 166 J/cm². The morphological alterations of the dentin surfaces and odontoblasts were examined with scanning electron microscopy (SEM), and the morphological alterations of the dental pulp tissue irradiated by laser were observed with an upright microscope.

Results: The study demonstrated that dentinal tubules can be entirely blocked after irradiation by 980-nm diode laser, regardless of the parameter setting. Diode laser with settings of 2.0 W and 980-nm sealed exposed dentin tubules effectively, and no significant morphological alterations of the pulp and odontoblasts were observed after irradiation.

Conclusions: Irradiation with 980-nm diode laser could be effective for routine clinical treatment of DH, and 2.0W/CW (166 J/cm²) was a suitable energy parameter due to its rapid sealing of the exposed dentin tubules and its safety to the odontoblasts and pulp tissue.

Keywords: dentin sensitivity; lasers; scanning electron microscopy

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Introduction

Dentin hypersensitivity (DH) is a type of common disease in cariology and endodontics, which is characterized by acute, non-spontaneous, short- or

long-lasting pain originating from exposure of the dentin to thermal, chemical, mechanical, or osmotic stimuli and which cannot be attributed to any other dental pathology¹⁻⁷. Abrasion, wedge-shaped defects, odontogama and periodontal atrophy can all

result in dentin hypersensitivity. Several theories have been proposed to explain the mechanism of dentin hypersensitivity, including the transducer theory, the gate-control and vibration theory, and the hydrodynamic theory. Currently, the hydrodynamic theory has been widely accepted, and it states that external stimuli cause movement of fluid inside dentinal tubules inwardly or outwardly, promoting mechanical deformation of nerve endings at the pulp/dentin interface, which is transmitted as a painful sensation^{6,7}. Under normal conditions, the dentin is covered by enamel or cementum and is not sensitive to direct stimulation. DH occurs only with the exposure of the peripheral terminations of the dentinal tubules^{3,6,8}. Therefore, blocking the exposed dentinal tubules is critical to the treatment of dentin hypersensitivity^{3,9}.

Laser systems have been used widely in the endodontic field, including Neodymium-Doped Yttrium Aluminium Garnet (Nd:YAG), Erbium-Doped Yttrium Aluminum Garnet (Er:YAG), Carbon Dioxide Laser (CO₂) and diode lasers^{7,10-19}. Recently, an innovative 980-nm diode wavelength laser was introduced, which was first reported on 2004²⁰. It is a high-energy laser, with low purchase and maintenance costs, as well as greater versatility because of its compact size^{20,21}. Currently, several studies have reported that the application of 980-nm diode laser could be used safely in endodontic treatment and in root canal disinfection^{22,23}. However, few studies have reported on the interaction of 980-nm diode laser energy with the dentin surface and the ensuing structural alterations. Whether the 980-nm diode laser can treat dentine hypersensitivity effectively, like other types of lasers, requires more studies to determine.

However, laser energy should be carefully applied due to the adverse thermal effects on the pulp and adjacent structures. Pulpal space pressure and temperature increase with increases in the energy density of laser²⁴⁻²⁷. However, most laser systems produce an increase in pulpal temperature that is dependent on the power setting²⁶⁻²⁹. Odontoblasts, which are located at the dentin-pulp interface, like a densely packed layer sending long cytoplasmic processes into the dentinal tubules, have been recognized as playing important roles in the defense reaction of the dental pulp^{30,31}. Thus, odontoblasts might be the first cells to be damaged by contact with irradiation energy. Few studies have reported on the morphological alterations of dental pulp tissue, especially the odontoblasts, after irradiation with 980-nm diode laser. Furthermore, the

safety parameters of 980-nm diode laser are unknown.

Considering the aforementioned issues, the aims of this *in vitro* study were to investigate, by scanning electron microscopy (SEM), the ultrastructural changes in dentin irradiated with 980-nm diode laser under different parameters and to observe the morphological alterations in odontoblasts and pulp tissue to determine the safety parameters of 980-nm diode laser in the treatment of DH.

Methods

Morphological alterations of dentin irradiated by different parameters of 980-nm diode laser

Twenty extracted human third molars were used for this study. Crowns with caries, restorations, or fractures were discarded. The enamel was removed with plain-cut tungsten carbide fissure burs (Densply; Maillefer Instruments Holding Co., Ballaigues, Switzerland) at high speed under a continuous water spray, and crown dentin discs, with a thickness of 2 mm, were cut perpendicular to the long axis of tooth. The dentin discs were polished with a600-grit carborundum paper (600-grit carborundum paper; Weiyi Metallographic Test Instrument Co., Guangzhou, China) and were washed with distilled water and ultrasound. Each specimen was etched with 35% phosphoric acid (Gluma Etch 35Gel; Heraeus Kulzer, Hanau, Germany) for 1 min to expose the dentinal tubules and then was rinsed with copious distilled water, as well as ultrasound once more. All of the discs were stored in 4°C distilled water containing 0.2% thymol to inhibit microbial growth until use.

Each dentin disc was divided into four areas (3 mm×4 mm), with one area chosen randomly for Group A and then Group B, Group C, and Group D assigned in a counterclockwise direction. According to the parameters of a 980-nm diode laser system (SIROlaser 2.2; Sirona Dental Systems GmbH, Bensheim, Germany), the group characteristics were as follows: Group A: control group, 0 J/cm²; Group B: 2 W/CW (continuous mode), 166 J/cm²; Group C: 3 W/CW, 250 J/cm²; and Group D: 4 W/CW, 333 J/cm². Each area was irradiated twice for 5 seconds. The laser was delivered through a 320-μm optic fiber with a straight handpiece, and the laser tip was held perpendicular to the irradiated surface at a distance of 1 mm to prevent contamination from dentin. The discs were dehydrated at 70°C for 24 h and then were fixed

on stubs with double-faced carbon tape, covered with a 30-nm gold-platinum layer in a vacuum apparatus and examined with SEM (HITACHI S-3000N; Hitachi Ltd., Tokyo, Japan).

Morphological alterations of dentin and pulp irradiated by 2.0-W 980-nm diode laser

According to the results of the above study, we chose 2.0 W/CW (166 J/cm²) as the energy parameter of 980-nm diode laser to be tested.

Ten extracted human third molars were selected. Crowns with caries, restorations, or fractures were discarded. Immediately after extraction, 2 mm of enamel and dentin were removed perpendicular to the long axis of the tooth with plain-cut tungsten carbide fissure burs at high speed under a continuous water spray. The dentin did not receive etching to avoid stimulation of the dental pulp tissue and was washed with distilled water.

Each dentin surface was divided into two areas (3 mm×6 mm), with one area chosen randomly as Group E: control group, 0 J/cm²; with the other becoming part of Group F: 2.0W/CW, 166 J/cm². Each area was irradiated twice for 5 seconds. A plain-cut tungsten carbide fissure bur, at high speed under a continuous water spray, was used to create a sulcus along the cemento-enamel junction of the tooth. The teeth were fractured with a bitapered chisel and a surgical hammer, exposing the entire pulp tissue. The pulp tissues from under the dentin, with or without 980-nm diode laser treatment, were removed from the tooth. The pulp tissues were fixed in 4% formaldehyde (Formaldehyde; Shanghai Hanguang Chemical Reagent Factory,

Shanghai, China) for 2 h, and the crowns were fixed in 2.5% dilute glutaral (Glutaral; Shanghai Hanguang Chemical Reagent Factory, Shanghai, China) solution for 2 h. Otherwise, a plain-cut tungsten carbide fissure bur, at high speed under a continuous water spray, was used to create a longitudinal sulcus along the buccal and palatal surfaces of the crown. The teeth were fractured longitudinally with a bitapered chisel and a surgical hammer, exposing the entire dentinal tubule to observe the morphological alterations in the tubules. Fixed crowns were dehydrated by a series of graded ethanol solutions and then were coated with a gold layer after drying. The morphological alterations of the odontoblasts and dentin surface were examined with an SEM.

Dental pulp tissue from Group E and Group F was fixed in 4% formaldehyde and then was dehydrated in a series of graded ethanol solutions; it was then paraffin embedded and cut sagittally at a thickness of 4 μm and stained with hematoxylin (Hematoxylin H9627; Sigma-Aldrich Corporation, St. Louis, USA) and eosin (Eosin Y E4009; Sigma-Aldrich Corporation, St. Louis, USA). The morphological alterations in the dental pulp tissue were observed by light microscopy (Olympus BX51; Olympus Optical Co., Ltd., Tokyo, Japan).

Results

Morphological alterations of dentin irradiated by different parameters of 980-nm diode laser

SEM analysis showed that the morphological aspects of the specimens treated with 980-nm diode

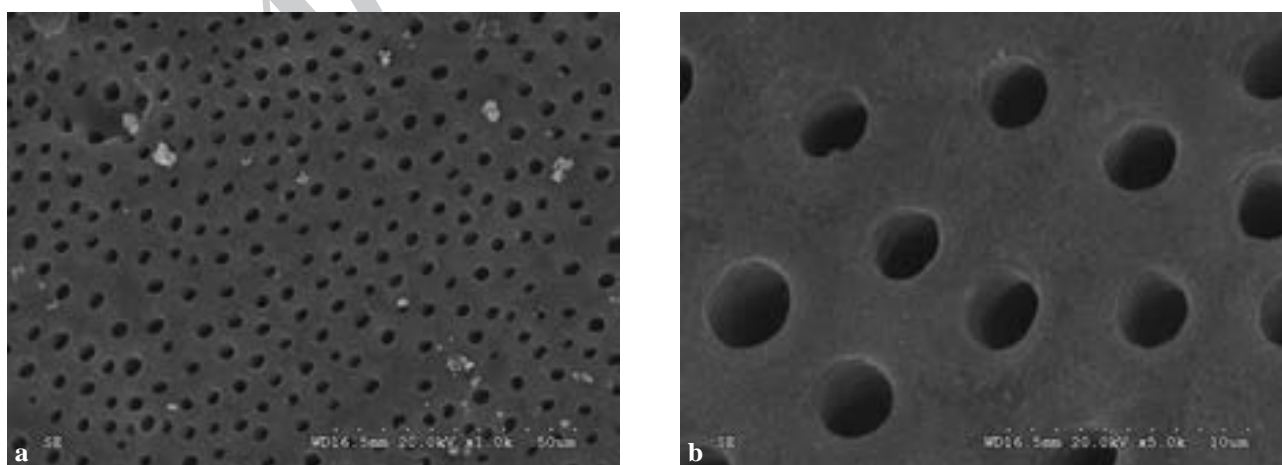


Figure 1. SEM micrograph of dentin surface without laser treatment. (a) A homogeneous surface with visible dentine tubules (×1000). (b) The diameters of the dentin tubules were 2 μm to 5 μm, and the intervals were 3 μm to 8 μm (×5000).

laser were sealed by melting dentin, regardless of the parameter settings.

In Group A (control group), photomicrographs of the specimens showed that the dentin surfaces were smooth, and dentinal tubules were visible and well distributed. The dentin tubules were round or oval, and their diameters were 2 μm to 5 μm , with intervals of 3 μm to 8 μm (Figure 1).

In Group B, the specimens were treated with 2 W/CW (166 J/cm²) 980-nm diode laser. Little or no open tubules were observed, the dentinal tubules were sealed or partially sealed, and the diameters ranged from 0 μm to 2 μm . Melting areas were present on the specimens' surfaces, without fissures and craters (Figure 2).

In Group C, the specimens were treated with 3 W/CW (250 J/cm²) 980-nm diode laser. There were

many more open tubules in this group than in Group B, and the melting degree of the dentin surface was also strengthened. Double-layer structures of tubules were observed (Figure 3).

In Group D, the specimens were treated with 4 W/CW (333 J/cm²) 980-nm diode laser. The overwhelming majority of the dentinal tubules were sealed by melting dentin. The peritubular dentin was deeply colored like scales because of the increased degree of dentin melting (Figure 4).

Morphological alterations of dentin and pulp irradiated by 2.0-W 980-nm diode laser

In Group E (control group), open dentinal tubules were found. The longitudinal section revealed that the dentinal tubule orifices were not occluded, and the

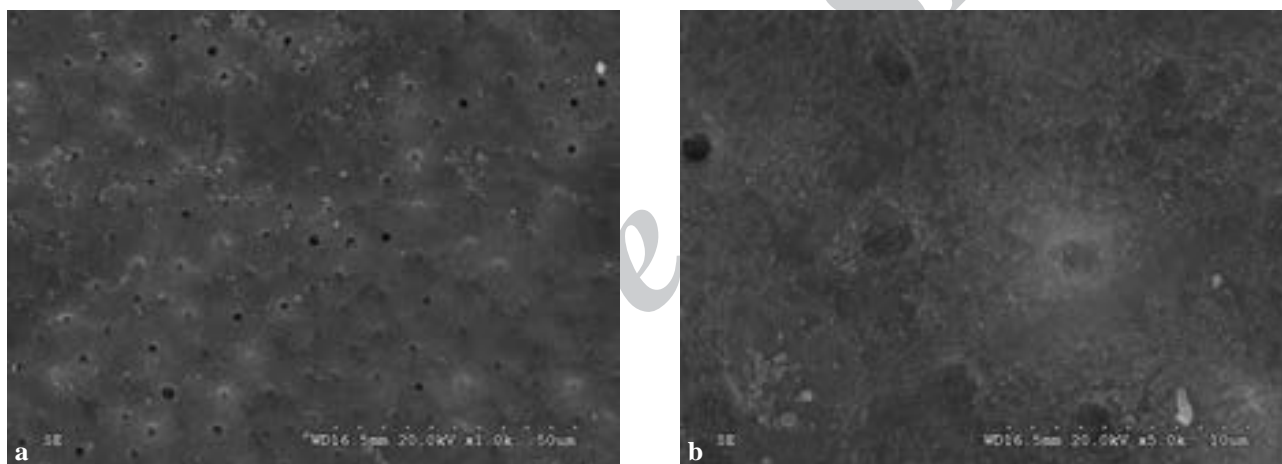


Figure 2. SEM micrograph of dentin surface after 2 W/CW (166 J/cm²) of 980-nm diode laser treatment. (a) Little or no open tubules were observed ($\times 1000$). (b) Melting areas were present on the specimens' surfaces, without fissures or craters ($\times 5000$).

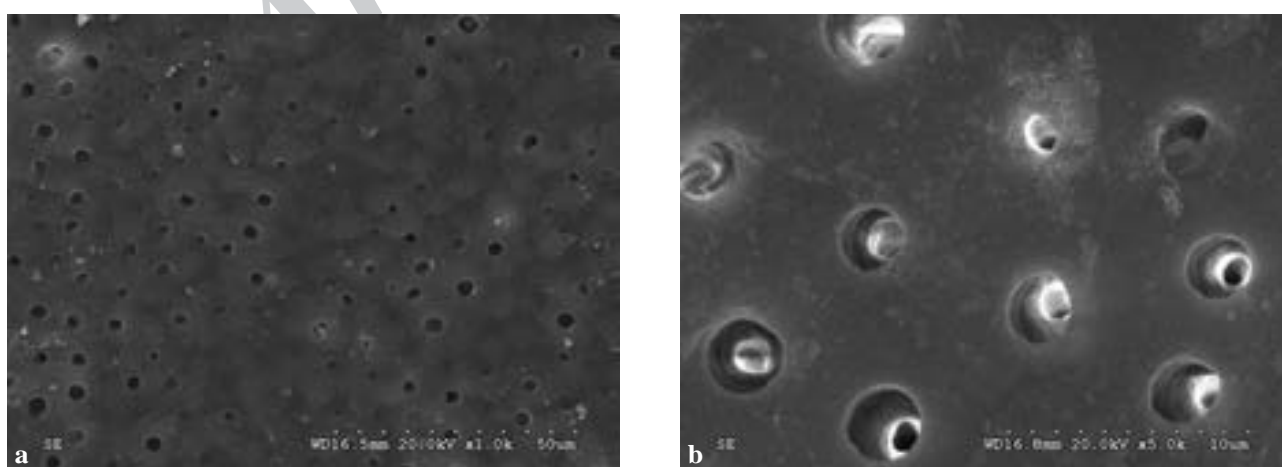


Figure 3. SEM micrograph of dentin surface after 3 W/CW (250 J/cm²) of 980-nm diode laser treatment. (a) The melting degree of the dentin surface was strengthened ($\times 1000$). (b) Double layer structures of tubules were observed ($\times 5000$).

diameters measured from 2 μm to 4 μm (Figure 5). Conversely, Group F (treated with 2.0W/CW, 166 J/cm² of 980-nm diode laser) exhibited homogeneous aspects of the surfaces, totally covered by the melting dentin and without exposure of dentinal tubules (Figure 6). From the longitudinal section parallel to the direction of the dentinal tubules' alignment, the sealing depth of the melted dentin was approximately 8 mm into dentinal tubules. No gap between the melted dentin and tubular walls was noted (Figure 7).

Odontoblasts on the inner surface of the pulpal chamber were observed under SEM, after the removal of the pulp. In Group E, SEM micrography revealed the integrity of the odontoblast cell layer and the well-preserved morphology of individual odontoblast cells. Odontoblasts located at the dentin surface

appeared like a densely packed layer. Potato-like cell bodies were observed, approximately 4 μm to 7 μm long, with collagenic and neural fibers. The cytoplasmic processes of the odontoblasts were connected to each other, and they extended into the dentinal tubules. The opened dentinal tubules were visible, and their diameter ranged from 4 μm to 8 μm (Figure 8). Similar results were observed in Group F. There was no significant difference between the two groups regarding the morphology of the odontoblasts (Figure 9).

The morphology of the pulp was observed by light microscopy after HE staining. No significant differences were observed between the two groups. The morphology of the odontoblast cells and vessels, as well as of the collagenic and neural fibers, was clear and healthy (Figure 10).

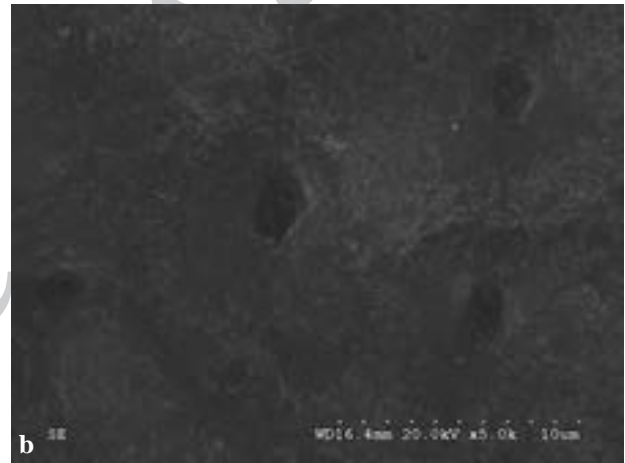
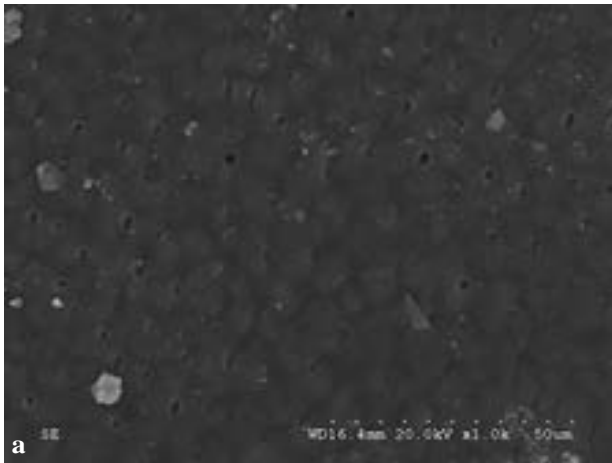


Figure 4. SEM micrograph of dentin surface after 4 W/CW (333 J/cm²) of 980-nm diode laser treatment. (a) The overwhelming majority of dentin tubules were sealed by melting dentin ($\times 1000$). (b) The dentin surfaces were covered by homogeneous and melted dentin, without exposure of dentinal tubules ($\times 5000$).

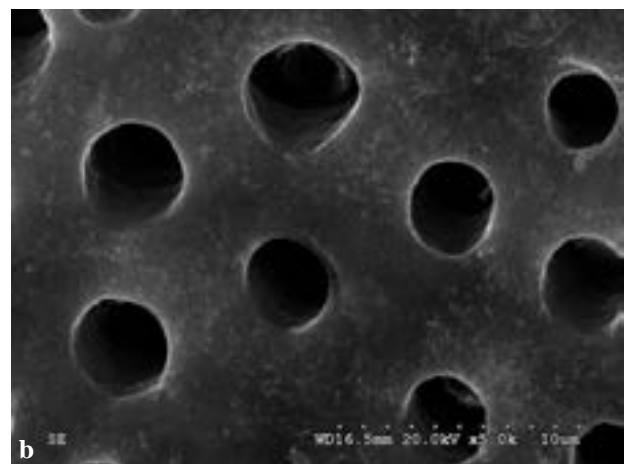
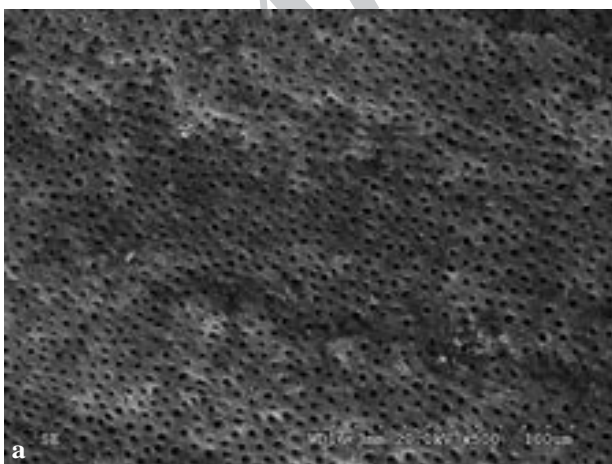


Figure 5. SEM micrograph of dentin surface without laser treatment. (a) Homogeneous surface with visible dentin tubules ($\times 500$). (b) Dentin tubules were round or oval, and the diameters were 2 μm to 5 μm , with intervals of 3 μm to 8 μm ($\times 5000$).

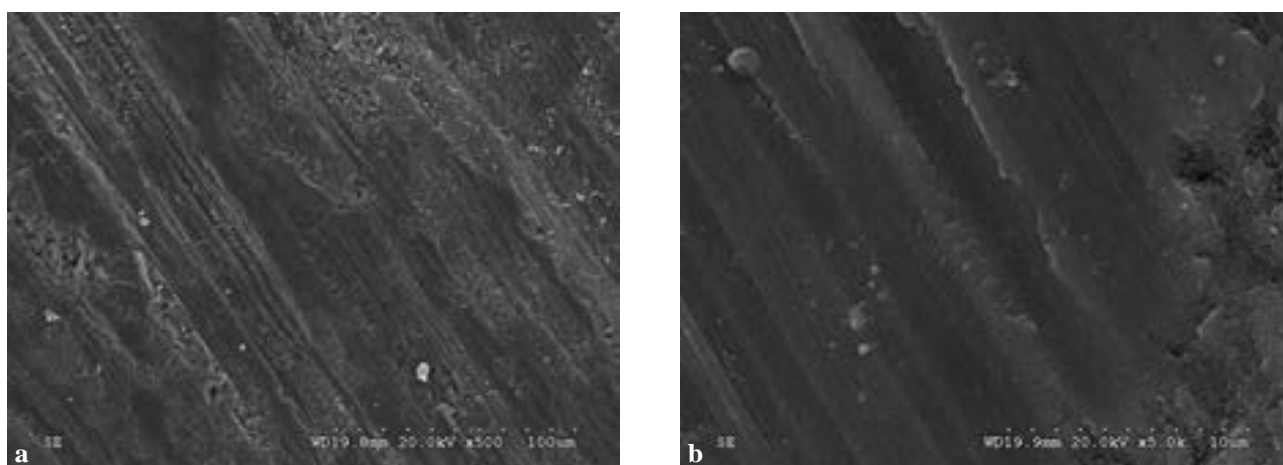


Figure 6. SEM micrograph of dentin surface after 2.0 W/CW (166 J/cm²) of 980-nm diode laser treatment. (a) A homogeneous surface covered by melting dentin, without exposure of dentinal tubules ($\times 500$). (b) No opened dentin tubules were found. ($\times 5000$).

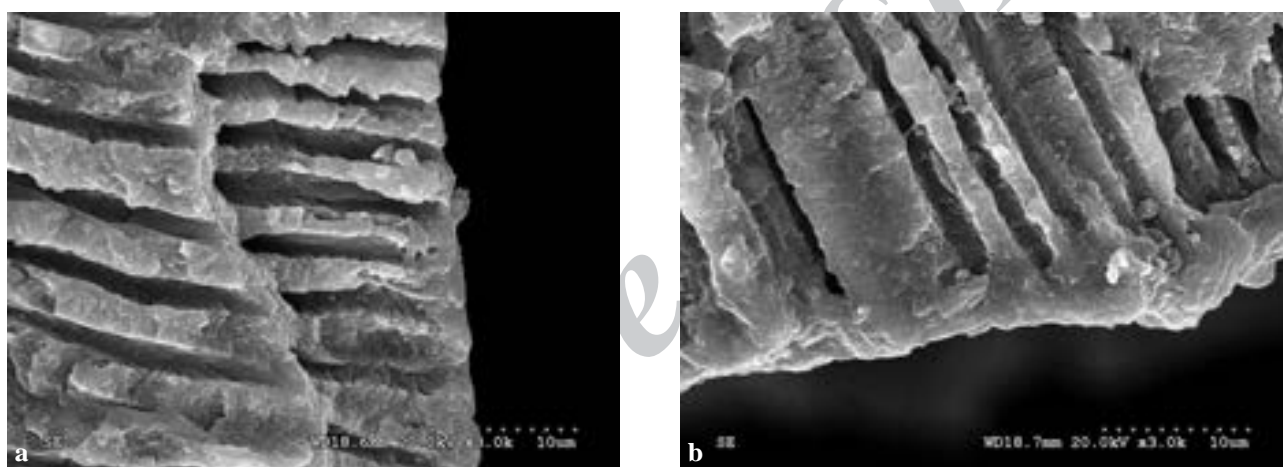


Figure 7. SEM micrograph of longitudinal section parallel to the direction of the dentinal tubules' alignment. (a) SEM micrograph of control group; the dentinal tubules were open ($\times 3000$). (b) SEM micrograph of dentin after 2.0 W/CW (166 J/cm²) of 980-nm diode laser treatment. The sealing depth of the melted dentin reached 8 mm ($\times 3000$).

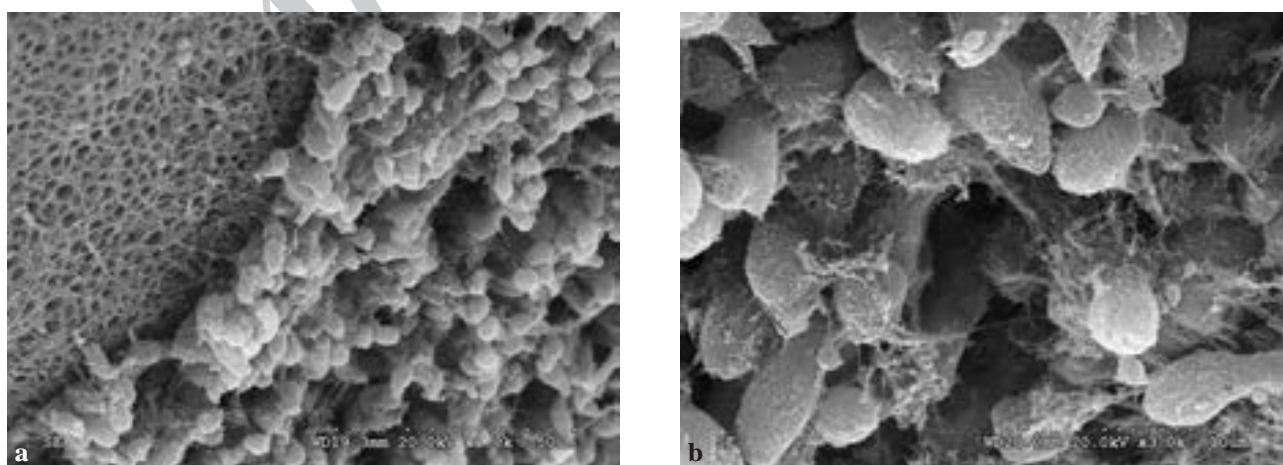


Figure 8. SEM micrograph of odontoblasts in the control group. (a) SEM micrograph revealed the integrity of the odontoblast cell-layer and opened dentinal tubules ($\times 1000$). (b) The well-preserved morphology of the odontoblast cells ($\times 3000$).

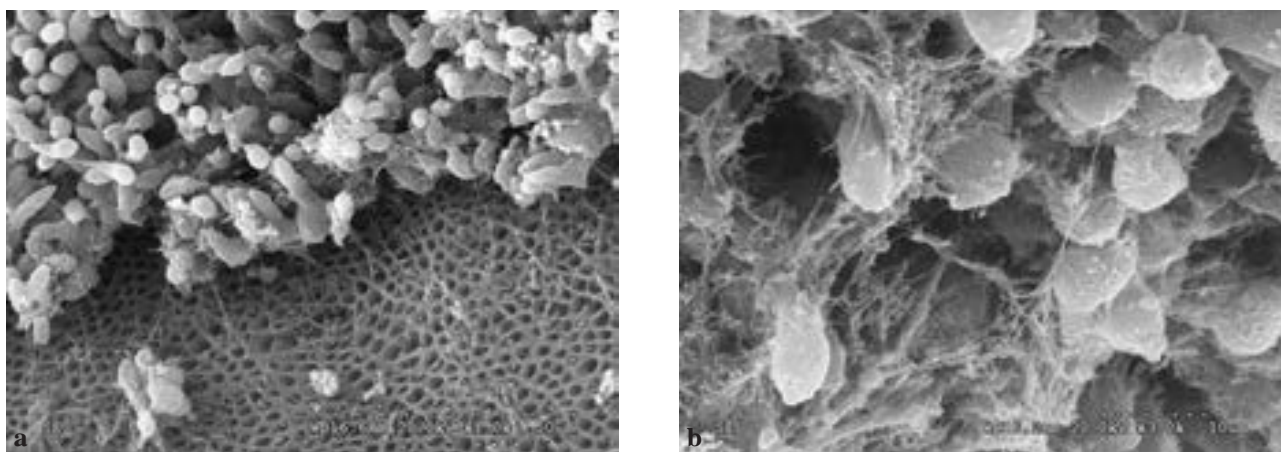


Figure 9. SEM micrograph of odontoblasts after 2.0 W/CW (166 J/cm²) of 980-nm diode laser treatment.

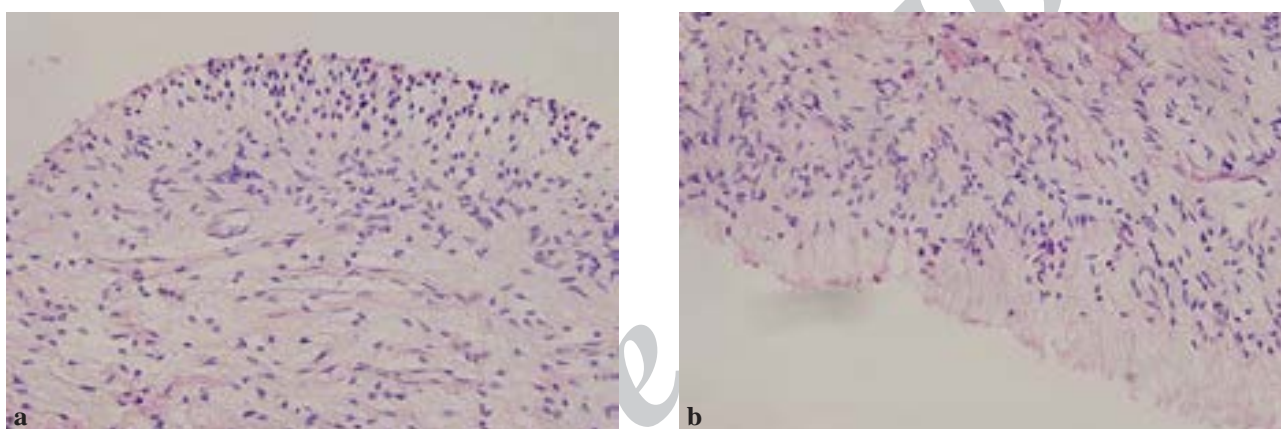


Figure 10. Micrograph of pulp tissue after HE staining. (a) Micrograph of pulp tissue in the control group (×200). (b) Micrograph of pulp tissue after 2.0 W/CW (166 J/cm²) of 980-nm diode laser treatment (×200).

Discussion

Hard-tissue modification by means of laser irradiation is becoming popular in dentistry. The role of Nd:YAG laser, Er:YAG laser and 830-nm wavelength diode laser in DH has been proved. However, the effect of 980-nm wavelength diode laser on DH is not clear. The results of this study show that different parameters of 980-nm wavelength diode laser can seal dentinal tubules. The dentin structure was changed because of the thermal effects caused by laser energy^{23,32-35}. A crystalline arrangement and melting can be attributed to some of the energy being absorbed by the dentin's mineral content, which includes carbonate and phosphate³². The mechanism of action of 980-nm diode laser is likely to be similar to that of 1064-nm Nd:YAG laser because both are located in the near-infrared region of the electromagnetic spectrum³⁶.

Morphological alterations in dentin irradiated with 980-nm diode laser could be observed by SEM, and

they depended mainly on the parameters of the laser, including the output power, frequency, and application mode, because these parameters are directly related to the energy transmitted to the dentin. According to the manufacturer (Sirona Dental Systems), the power of this device ranges from 0.5 to 7.0 W and from 1 to 10 kHz, with a continuous wave operating mode, which consists of an uninterrupted laser beam. In this study, 2 W/CW, 3 W/CW, and 4 W/CW 980-nm diode laser was used, and the energy density was 166 J/cm², 250 J/cm², and 333 J/cm², respectively. The laser's effect on the melting degree of the dentin surface was intensified when the parameters were increased from 2 W to 4 W in the continuous mode. The reason for this result was that the energy of 980-nm diode laser absorbed by the dentin's mineral content was increased, resulting in crystalline arrangement and melting^{32,37,38}.

We found that the number of sealed dentin tubules in Group C was less than that in Group B. The differences could be attributed to the double-layer structure of

the tubules. We speculate that, at first, the superficial layer melted and the crystalline arrangement occurred because low energy was absorbed by the superficial layer of the dentin's mineral content. With increasing energy, the superficial layer of dentin melted again and collapsed into the dentinal tubules, so the double-layer structure formed. In Group D, the surface and deep layer of dentin were both melted by the high energy of laser to form a homogeneous and regular layer that covered the surface of the dentinal tubules.

This study demonstrated that 2 W/CW (166 J/cm²) was suitable parameter for a 980-nm diode laser to seal dentinal tubules without excess melting of the dentin.

The energy of laser can cause adverse thermal effects to the pulp, so lasers should be carefully operated. Studies have shown that pulp responds to externally applied heat^{25,39}, with 15% of teeth failing to recover from an intrapulpal temperature increase of 5.5 °C. If the temperature increase was 11 °C, 60% of teeth could not recover, so energy has a close relationship with the health of pulp tissue. Based on the above studies, 2.0 W/CW (166 J/cm²) was chosen to evaluate the role of 980-nm diode laser in the sealing of dentin tubules, as well as its safety in dental pulp tissue. Sealed dentin tubules were observed after irradiation by 980-nm diode laser (2.0 W/CW, 166 J/cm²). Compared with the control group, the dentinal surfaces in the laser groups were melted, and the overwhelming majority of dentinal tubules were sealed. From the longitudinal sections parallel to the direction of dentinal tubules' alignment, melted dentin entered the dentinal tubules to as deep as 8 µm. No gap between the melted dentin and tubular walls was noted, most likely due to the same components in the dentin and pipe plug. Sealing exposed dentinal tubules, with melted and recrystallized dentin, were caused by the thermal and occlusive effects of laser, resulting in a prolonged role.

From SEM micrographs, no significant morphological alterations of the odontoblasts were found after treatment with 2.0 W/CW (166 J/cm²) 980-nm diode laser and a similar result was observed in the scan of pulp tissue stained by HE. Parameters of 2.0 W/CW (166 J/cm²) could be safe for 980-nm diode laser treatment for DH.

Irradiation with 980-nm diode laser seems to be effective for routine clinical treatment of DH, and 2.0 W/CW (166 J/cm²) is suitable energy parameter due to the rapid sealing of exposed dentinal tubules and its safety to odontoblasts and pulp tissue. Further studies are necessary to evaluate the rapid and prospective

clinical effectiveness, as well as the adverse reactions, after 980-nm diode laser application for DH treatment.

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