Qualitative Microanalysis of Ions and Ultrastructural Changes in Dentin Exposed to Laser Irradiation and to Metal Salts Solution

Carlos E. Glauche, MSD, Patricia M. de Freitas, MSD, Nilson D. Vieira Jr., PhD and José Luiz Lage Marques, DDS

1Professional Master Course of Lasers in Dentistry, Institute of Energy and Nuclear Research, IPEN, São Paulo, SP 05508-900, Brazil
2Department of Restorative Dentistry, School of Dentistry, University of São Paulo (USP), São Paulo, SP 05508-900, Brazil
3Center for Laser and Applications, Institute of Energy and Nuclear Research, IPEN, São Paulo, SP 05508-900, Brazil
4Department of Restorative Dentistry, School of Dentistry, University of São Paulo (USP), São Paulo, SP 05508-900, Brazil

Background and Objectives: This study evaluated the ultrastructural changes in dentin after treatment with the Nd:YAG laser and/or metal salt solutions and verified the presence of Sn$^{++}$, Sr$^{++}$, and F$^-$/Cl$^-$ in dentin structure.

Study Designs/Materials and Methods: Sixty dentin disks were randomly divided into groups (n = 10): (I) control (no treatment), (II) Nd:YAG (1.5 W, 100 mJ, 15 Hz, 125 J/cm$^2$), (III) 10% SnF$_2$ aqueous solution for 30 minutes, (IV) Nd:YAG+10% SnF$_2$ aqueous solution for 30 minutes, (V) 10% SrCl$_2$ toothpaste for 30 minutes, (VI) Nd:YAG+10% SrCl$_2$ toothpaste for 30 minutes. Then, all samples were prepared for scanning electron microscopy (SEM) and the samples from Groups I to IV for the energy dispersive X-ray microanalysis (EDX).

Results: SEM evaluation revealed occluded dentinal tubules and a dentin surface altered by the laser irradiation. The EDX microanalysis revealed Sn$^{++}$ at a depth of 250 μm in Group IV and not deeper than 100 μm in Group III. In Group V, Sr$^{++}$ was not deeper than 50 μm, but it could be detected at a depth of 500 μm in Group VI. F$^-$ was found only in Group IV.


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Key words: dentinal tubules; Nd:YAG laser; metal salt

INTRODUCTION

Dentin hypersensitivity is a relatively common pain condition and two theories of how it arises are proposed. One of the theories implicates the nervous structures in the dentinal tubules and at the periphery of the odontoblasts and their processes. The presence of open dentinal tubules may lead to tooth sensitivity due to the exposure of nerve fiber endings [1]. The other, named the hydrodynamic theory, involves a change in the fluid flow in the dentinal tubules [1]. According to Liu et al. [2], the reduction of open tubules or decrease in their diameters is the aim of dentin hypersensitive therapy.

In spite of their inhibitory plaque formation activity [3,4], divalent metal salt solutions, such as stannous fluoride and strontium chloride, have been used as important agents for reducing dentin hypersensitivity [5,6]. Markowitz et al. [7] found that such solutions depress the excitability of intradental nerve cell membranes without altering the resting membrane potentials. When applying fluoride metal salt solutions on a dental surface, the fluoride can possibly be incorporated in the dental structure and provide mechanical and chemical protection for the exposed dentin [8,9].

The laser irradiation was recently introduced as a therapy for dentine hypersensitivity and has also been reported to produce physical and chemical structural changes in dentin [2,10]. After the application of the Nd:YAG laser, morphological dentin changes are characterized by a melted and re-solidified surface and by the presence of craters, cracks, and irradiation globules [11–13]. Some studies have demonstrated that laser irradiation can lead to the occlusion or narrowing of dentinal tubules and possibly reduce dentin sensitivity [2,14,15].

In order to find a more effective technique to reduce dentin sensitivity, some studies have associated laser irradiation with treatment with metal salts. Moritz et al. [16] showed the permanent integration of tin and fluoride with the dentin surface when irradiated by CO$_2$ laser, followed by the application of a stannous fluoride preparation. In 1999, Lan et al. [17] observed that over 90% of
dentinal tubule orifices were occluded after the treatment with NaF varnish and Nd:YAG laser irradiation.

Although a wide variety of treatment methods have been studied, there is little information about the association of laser irradiation and treatment with metal salts. This study aimed to evaluate the ultrastructural changes caused by combined action of Nd:YAG laser radiation and treatments with a SnF$_2$ solution and SrCl$_2$ toothpaste on the dental surface, and to determine the presence of Sn$^{++}$, Sr$^{++}$, and F$^-$/C$^0$ inside the irradiated and non-irradiated dentin.

MATERIALS AND METHODS

The study protocol was reviewed and approved by the local ethical committee. Ten recently extracted third molar teeth were collected and each one had the root surface embedded in a self-curing polyester resin in a polyvinylchloride ring mould. After the resin polymerization, the moulds were removed and the teeth were sectioned labio-lingually on a hard tissue microtome (LABCUT 1010, Extec) to obtain dentin discs of 2 mm each. Six 2 mm dentin discs were obtained from each tooth. They were polished with sandpaper, cleaned, and kept frozen in distilled water until the experiment began [18]. Next, the dentin samples were soaked in an EDTA solution at 14% for 2 minutes [13] and washed in distilled water to expose the dentinal tubules [19]. The dentin specimens were divided into six groups (n = 10), as shown in Table 1.

Pulsed, high power Nd:YAG laser equipment (D-Lase 300, American Dental Technologies, San Carlos, CA, USA) was used to irradiate the dentin surface. The equipment provides an infrared beam operating at 1,064 nm, with pulse duration of 150 microseconds, repetition rates varying from 10 to 80 Hz, and maximum average power of 5 W. The laser application was made by contact, using a 320 $\mu$m diameter optical fiber. The entire dentin surface was irradiated by scanning once in each direction, horizontally and vertically, in order to promote homogeneous irradiation and to cover the entire sample area. A He–Ne laser, coaxial with Nd:YAG beam, was used as a guide light. The laser irradiation parameters were as follows: average power of 1.5 W, 100 mJ pulse energy, repetition rate of 15 Hz, and fluency of 125 J/cm$^2$ for 60 seconds. The energy delivered at the end of the fiber was measured with a power meter and corresponded to the energy depicted on the equipment display.

RESULTS

SEM Evaluation

In Group I (Fig. 1), SEM analysis revealed a smooth and homogeneous dentin surface. The peritubular dentin was removed and the intertubular dentin looked packed and melted, with an appearance similar to that of glazing. The samples from Group II (Fig. 2) showed ultrastructural changes in the dentin. Pits and whitish globules were found among heterogeneous and rough structures due to the melting and re-solidification of the dentinal surface. Although craters were observed, carbonization areas were absent in the irradiated dentin samples. Dentinal tubules

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
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<tbody>
<tr>
<td>I</td>
<td>Control (without treatment)</td>
</tr>
<tr>
<td>II</td>
<td>Nd:YAG laser irradiation</td>
</tr>
<tr>
<td>III</td>
<td>10% SnF$_2$ aqueous solution for 30 minutes</td>
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<tr>
<td>IV</td>
<td>Nd:YAG laser irradiation + 10% SnF$_2$ aqueous solution for 30 minutes</td>
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<tr>
<td>V</td>
<td>SrCl$_2$ toothpaste (SENSODYNE) for 30 minutes</td>
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<tr>
<td>VI</td>
<td>Nd:YAG laser irradiation + SrCl$_2$ toothpaste (SENSODYNE) for 30 minutes</td>
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Considering that Nd:YAG laser wavelength is absorbed to a greater extent by pigmented tissues [20–22], waterproof black ink was applied on the dentin surface of samples from Groups II, IV, and VI to enhance laser absorption. After the laser irradiation, the samples from Group II, IV, and VI were washed with ethanol (95° GL) to remove the black ink from the dentin surface (post-washing process) [21].

To avoid hydrolysis and oxidation [23], both the 10% SnF$_2$ aqueous solution [8] and the 10% SrCl$_2$ toothpaste (SENSODYNETM) were prepared immediately before use. The 10% SrCl$_2$ toothpaste (SENSODYNE) was modified from Penney and Karlsson [24].

After the surface treatment, each sample received one more transversal cut. One part was submitted to scanning electron microscopy (SEM) analysis and the other to the energy dispersive X-ray microanalysis (EDX) test. For the SEM evaluation, dentin samples were dehydrated through a series of increasing alcohol grades (70%–100%) for a total of 24 hours. Then, they were sputter coated using a carbon-coating device. The SEM was performed with PHILIPS XL 30 equipment (Philips LX 30, Eindhoven, Holland). Analyses of the Sn$^{++}$, Sr$^{++}$, or F$^-$/C$^0$ distribution were performed by EDX. Qualitative ion analyses were made at the dentin surface and at depths of 23, 50, 100, 130, 250, and 500 $\mu$m.

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**TABLE 1. Experimental Groups**

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Fig. 1. Group I: Normal dentin (control group). Dentin presents opened dentinal tubules and a homogeneous surface. Magnification ×2,000.
appeared to be occluded. Samples from Group IV presented a modified surface caused by laser irradiation and a superficial whitish layer was evident, as shown in Figure 4. In Group VI, sample evaluations revealed a homogeneous grayish surface with craters and cracks (Fig. 6). Both Groups III and V, revealed a surface with slight morphological changes and little white globules on the dentin surface (Figs. 3 and 5).

**EDX Microanalysis**

The EDX microanalysis data are shown in Table 2. Group III showed a high concentration of Sn²⁺ on the dentin surface. However, at a 130 μm depth, no signs of metal ions were observed. In the samples from Group IV, Sn²⁺ was detected at least as deep as 250 μm into the dentin, and the presence of F⁻ was evident at the surface and down to 50 μm. Fluoride could not be detected in the samples from Group III. In Groups V and VI, the presence of Sr²⁺ was found. These ions could be detected in the irradiated dentin at a depth of at least 500 μm, while in the samples submitted to the toothpaste treatment only, the presence of the ion was detected at a maximum depth of 23 μm.

**DISCUSSION**

With regard to the uptake mechanism of metal elements by dentinal tissues, Sognnaes [25] stated that enamel and dentin are permeable to ions crossing in both directions. In agreement with this observation, Yokoyama et al. [26] observed the great capacity of the metal ions to move microscopically into the dentin, suggesting that tin ions are strongly adsorbed into calcified tissues with a high organic content. Metal salts can act by adsorption on or in the organic connective tissue of dentin and the dentinal tubular process, resulting in the blockage of impulses to nerve pathways [27].
The use of Nd:YAG laser for treating dentin hypersensitivity is based on its capability to alter the structure and chemistry of the dentin surface. When applied to dentin, it induces melting and re-solidification of the surface, leading to a nonporous glazed surface with occluded dentinal tubules [10]. In addition, laser irradiation associated with treatment with divalent metal salt solutions appears to alter the absorption of ions on dentin surface. In this study, a whitish layer of SnF₂ was clearly evident on a heterogeneous and rough structure due to the melting and re-solidification of the dentinal surface (Group IV). The layer could be attributed to the presence of tin-phosphate and calcium fluoride compounds as a result of a substantial tin and fluoride deposition [8]. Also, the effects of irradiation may have enhanced this deposition. The results suggest that irradiated dentin can absorb more ions than an untreated surface and this could be the result of the morphological alteration that occurs after exposure to laser. One believes that micro-spaces caused by irradiation with laser can act as ion precipitation sites [28,29].

In Group VI (laser irradiation +10% SrCl₂ toothpaste) a homogenous gray layer was found on the dentin surface probably due to the deposition of a thick layer of the substance. In spite of the post-washing process of the samples, a substantial quantity of the deposit could not be removed [32]. According to the photomicrographs (Figs. 1 and 2), a great retentive potential, both chemical and mechanical, was possibly produced by the dentin conditioning with laser irradiation. The EDX of Group III revealed the presence of metal ions up to a depth of 100 μm. When applying a SnF₂ solution at 10% for 30 minutes, Penney and Karlsson [24] reported the presence of tin ions just at 10 μm into the dentin. According to these authors, there was no marked tin ion diffusion through the dentin, mainly due to the capacity of Sn²⁺ to form insoluble salts when linked to phosphate. On the other hand, authors reported a greater diffusion of F⁻, probably due to the more soluble byproduct that resulted from its association with divalent calcium ions. In this study, fluoride ions could not be detected in Group III. This can be attributed to the methodology applied, since elements with low atomic weight, like that of fluoride, are hardly detected by EDX analysis. Fluoride detected in Group IV, at 50 μm within the dentin, may be related to the retentive potential of laser irradiation on dentin surface, as mentioned previously. Zhang et al. [12] have also shown that the fluoride concentration within the root surfaces was higher in samples first irradiated and then treated with NaF and Ag(NH₃)₂F solutions. The authors reported an F⁻ ion penetration depth of nearly 20 μm.

Moritz et al. [16,33] irradiated teeth in patients with dental hypersensitivity. A CO₂ continuous laser (with an...
output power of 0.5 W) was used and under an atomic absorption spectroscopy analysis, the presence of Sn$^{++}$ and F$^-$ in the irradiated dentin was detected. Although the method does not show the penetration depth of the searched elements, the authors suggested that combining laser and fluoride solution treatments results in F$^-$ integrating permanently with dentin. In spite of its ability to reduce dentin hypersensitivity, fluoride can also play an important role in maintaining tooth mineral equilibrium, enhancing remineralization, and reducing demineralization [34–36].

Analytical determination of Sr$^{++}$ in irradiated dental tissues has been widely discussed in the literature. In Group V, (10% SrCl$_2$) metal ions were found at a depth of 23 μm. In spite of the shallow penetration, some interference by the chemical element in the inner dentin components may have interfered with the diffusion of the compound. Penney and Karlsson [24], reported the presence of strontium in relatively high concentrations at a depth of 200 μm. A higher penetration was found in Group VI (down to 500 μm) when using the combined laser-metal salt solution treatments. The dentin treated with laser irradiation revealed greater metal adsorption when compared to the metal-salt solution individually. With regard to mechanical and chemical actions, such as traumatic brushing and the erosion process, being able to affect the dentin surface and possibly remove the outer dentin, the penetration of tin ions into inner dentin seems to be important to the long-term effect of the treatment for dentin hypersensitivity.

Finally, it can be concluded that the uptake of elements depends on dentin conditioning before the treatment. The ultrastructural changes in dentin tissue caused by laser radiation, melting, and re-solidification resulted in a greater uptake of Sn$^{++}$, Sr$^{++}$, and F$^-$ by the dentin when compared with the samples that were not irradiated.

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