Effect of bioactive glasses and neodymium:yttrium-aluminum-garnet laser on dentin permeability

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Abstract

Context: Dental hypersensitivity and loss of dental tissues are commonly observed in patients, and most of the problems are caused due to total or partial exposure of dentinal tubules.

Aims: The purpose of this study is to evaluate the performance of 45S5 bioactive glass and niobophosphate (NbG) associated with neodymium: yttrium-aluminum-garnet (Nd:YAG) laser for the reduction of dentin permeability.

Materials and Methods: Fifty bovine dentin discs were made and distributed randomly into five groups (n = 10). The Nd:YAG laser was applied with the bioactive glasses using the energy parameters (60 and 80 mJ), forming the groups; NbG_60: NbG + Nd:YAG (60 mJ); NbG_80: NbG + Nd:YAG (80 mJ), 45S5_60: 45S5 + Nd:YAG (60 mJ); 45S5_80: 45S5 + Nd:YAG (80 mJ) and C: control (untreated dentin). The permeability was measured with a split chamber device. The samples were subjected to the erosive challenge and a new permeability measurement was done. Furthermore, the dentin was analyzed by scanning electron microscopy/energy-dispersive X-ray spectroscopy (SEM/EDS).

Statistical Analysis Used: The data were analyzed using Kruskal–Wallis and Dunn’s tests (α = 0.05).

Results: Greater reduction in dentinal permeability was observed for 45S5 bioactive glasses (45S5_60 and 45S5_80) followed by NbG_80 and NbG_60 (P < 0.05). The SEM/EDS analysis showed the formation of a barrier after the dentin treatment.

Conclusions: Bioactive glasses with Nd:YAG laser on the dentin surface may be a promising alternative for the reduction of dentin permeability.

Keywords: Bioglass; dentin permeability; lasers

INTRODUCTION

The dentin hypersensitivity (HSD) has become increasingly frequent in the dental clinic, affecting about 30% of the adult population.[1,2] One of the reasons for HSD may be the dentinal exposure and movement of the fluid inside the dentinal tubules, as proposed by Brännström.[3]

The painful symptoms caused by dentinal exposure can be treated by desensitization through neural depolarization,[1] and chemical or physical obliteration of the dentinal tubules.[4] However, the majority of the studies bring transient alternatives for the treatment of HSD,[4,5] reflecting the lack of long-lasting protocols.

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To help the formation of a mineralized layer on the dentin, studies have shown that the bioactive materials are promising for the treatment of HSD. Especially bioactive glasses, may reduce the sensitivity more permanently. The 45S5 bioactive glass has been widely diffused in the dental environment due to its structural proximity to the mineralized part of the bone and dental tissues, may be capable of stimulating the deposition of hydroxyapatite on the dentin surface by the precipitation of Ca\(^{+2}\) and PO\(^{3-}\) ions. However, a disadvantage of this biomaterial is the formation of a more permeable bioactive layer.

The literature has shown that the niobium-based materials promote better chemical stability of the glasses, optimizing their biocompatibility to the dentin, resulting in the “experimental NbG bioglass.” This biomaterial assists in the formation of apatite by the deposition of an amorphous calcium compound transforming into hydroxyapatite, allowing the crystallization and mineralization of the exposed dentin.

High-power neodymium:yttrium-aluminum-garnet (Nd:YAG) laser is used to obliterate the dentinal tubules due to the formation of a melting layer and recrystallization of the exposed dentin. However, side effects, such as the formation of craters, depressions, and microcracks on the irradiated surface have been reported in the literature. The biochemical effect of the bioglass could be optimized by using the association of this material with high-power Nd:YAG laser.

The formation of a bioglass layer or incorporation of these glass particles into the dentin could minimize the occurrence of these micro defects in the dentin. Another expected influence is on pH control, remineralization of dentin and obliteration of dentin tubules by the constant deposition of calcium and phosphate ions on this substrate.

The influence of Nd:YAG associated with bioglass is scarce in the literature and the NbG is an experimental bioglass with expressive chemical stability, biocompatibility with great potential for interaction with dentin.

Thus, the objective of this study was to evaluate the dentin permeability after the incorporation of bioglass to the dentin, associated with Nd:YAG laser at 60 and 80 mJ. The null hypotheses tested are: (1) the different irradiation parameters associated with the bioglass do not change the permeability and (2) the erosive challenge does not promote changes in dentin permeability.

**MATERIALS AND METHODS**

**Preparation of specimens**

Fifty freshly extracted bovine incisors were cleaned and stored in distilled water until the preparation moment of the dentin specimens, which period did not exceed 6 months after extraction.

The teeth were sectioned transversely, below the cementoenamel junction, the crowns were brought to the cutting machine for circular specimens (Micro Mill, Washington, USA). After obtaining the 6 mm × 1 mm thick dentin disks, they were taken to the circular polishing machine for surface smoothness (DP-10, Panambra, São Paulo, SP, BR).

**Permeability readings**

The permeability readings were performed in four moments with TDH 03 (ODEME Medical and Dental Equipment Ltd., Joaçaba, SC, BR). 1 - Minimum permeability (Pmin) (presence of the smear layer), 2 - Maximum permeability (Pmax) (after the maximum opening of the tubules), 3 - Permeability treatment (Ptrat): (after the treatments) and 4 - Erosive permeability (Pde) (after the erosive challenge).

For the reading of the Pmin, the smear layer was maintained on the outer face of the dentin disc and citric acid was applied at 0.3% for the 30s on the pulp face, removing the smear layer of this surface, as to subsequently obtain the Pmax values the same solution was applied to the external face of the dentin disc.

**Bioglass application and treatment of experimental groups**

**Pilot experimental study**

For the definition of the experimental groups and sample calculation, tests were carried out in pilot groups. The isolated use of laser or bioactive glasses (45S5/NbG) did not allow the formation of suitable groups for measurement of the permeability. Nd:YAG laser irradiation in the small thickness of the dentin promoted extensive microcracks and cracks in the dentin, as observed in the scanning electron microscopy (SEM). These dentin failures negatively influenced the evaluation of the permeability of the treated groups. Moreover, in the groups treated only with bioactive glasses, there was no adhesion of these materials on the dentin surface, detaching easily during the evaluation of the permeability.

Thus, the difficulty in maintaining the particles of the bioglass on the surface of dentin justified the association of this material with the laser.

**Bioactive experimental groups**

The specimens were randomly distributed in five groups, each with n = 10. The Nd:YAG laser was applied with the bioglasses in two energy parameters, forming the groups: Table 1 NbG-60, NbG-8, 45S5-60, 45S5-80: and C: control (untreated dentin).
Table 1: Materials, composition, and method of application on the dentin surface

| Materials   | Composition       | Groups     | Method of application                                                                 |
|-------------|-------------------|------------|---------------------------------------------------------------------------------------|
| NbG*        | NbO₃: 41.8%       | NbG-60     | Surface drying with absorbent paper                                                   |
|             | P₂O₅: 32.5%       |            | Suspension: 0.02 g of powder+10 μL of distilled H₂O                                    |
| CaO: 18.8%  | Application with microbrush: On the buccal face of the dentin disc form active for the 20 s |
| Al₂O₃: 2.7% | 60 mJ, 10 Hz, 0.6 W in contact with surface sweep treated with the suspension for the 40 s |
| Na₂O: 1.2%  | NbG-80            |            | Surface drying with absorbent paper                                                   |
|             |                   |            | Suspension: 0.02 g of powder+10 μL of distilled H₂O                                    |
| SiO₂: 45%   | Application of the suspension with microbrush: on the buccal face of the dentin disc form active for the 20 s |
| CaO: 24.4%  | 60 mJ, 10 Hz, 0.6 W in contact with surface sweep treated with the suspension for the 40 s |
| Na₂O: 24.6% | 45S5-60           |            | Surface drying with absorbent paper                                                   |
| P₂O₅: 6%    |                   |            | Suspension: 0.02 g of powder+10 μL of distilled H₂O                                    |
| SiO₂: 45%   | 45S5-80           |            | Application of the suspension with microbrush: on the buccal face of the dentin disc form active for the 20 s |
| CaO: 24.4%  | 80 mJ, 10 Hz, 0.8 W in contact with surface sweep treated with the suspension for the 40 s |

*Carbonari, 2003; Kokubo et al. NbO₃: Niobium oxide, P₂O₅: Phosphate pentoxide, CaO: Calcium oxide, Al₂O₃: Aluminum oxide, Na₂O: Sodium oxide, SiO₂: Silicon dioxide, H₂O: Water 1990

After bioglass application to the dentin, the specimens were irradiated with Nd:YAG (Pulse Master 600 IQ, American Dental Technologies, Austin, Texas, USA), with a wavelength of 1064 nm and 400-μm fiber. The surface was homogeneously scanned for the 40s, with the fiber positioned in contact perpendicularly to the dentin surface. For the treatment of the experimental groups, the parameters of 60 mJ, 10 Hz and 0.6W (density = 315 mJ/cm²) and 80 mJ, 10 Hz and 0.8W (density = 421 mJ/cm²). [3]

**Erosive challenge**
After 24 h of treatment, the erosive challenge was performed. Four cycles were implemented daily for 5 days, being each cycle consisted of immersion of the specimens in 0.3% citric acid (pH 2.3) for 2 min and immersing them in artificial saliva (pH = 7) for 1 h. In the end, the specimens were stored in eppendorfs containing ultrapure water and kept in an oven at 37°C ± 1°C for 24 h.

**Scanning Electron Microscopy and Dispersive Energy Spectrometry X-ray**
Specimens were metalized (Desk II, Denton Vacuum, Moorestown, New Jersey, USA) and prepared for SEM (INSECT S50-FEI, Brno, Czech Republic) and energy-dispersive X-ray spectroscopy (EDS) (Esprit 1.9 Bruker, Schwarzschildstr, Berlin, Germany). The SEM (1000x) reading was held after the treatments and to the erosive challenge, and EDS analysis was performed after the treatment of the dentin.

**Statistical analysis**
The hydraulic conductance data were converted in percentage by the formula P% = (P/PMax) × 100, where P represents the Pmin, Ptrat, or Pde. The mean values and the standard deviations of the (P) variation were obtained for each group analyzed. The Shapiro–Wilk test showed no normal distribution of data. The Kruskal–Wallis and Dunn post hoc statistical tests were used to identifying the statistical differences and compare the treatments performed, using was SPSS 23.0 (IBM, Armonk, NY, USA) and α = 5%.

**RESULTS**

**Dentin permeability assessment**
A statistically significant difference was observed between the in mean values of Ptrl and Pde the groups (P < 0.05) [Figure 1]. Regarding the untreated group (C), there was a remarkable decrease in permeability.

45S5-60 and 45S5-80 were found to be more effective in reducing permeability than the NbG group, and the different Nd:YAG energies did not promote statistical differences for 45S5-60 and 45S5-80 groups.

No statistically significant difference was observed after the erosive challenge in the different experimental groups.

**Scanning electron microscopy and energy-dispersive X-ray spectroscopy analysis**
SEM images showed a fused layer of bioglass on the dentin after irradiation of Nd:YAG and this layer was preserved after erosive challenge when compared to the untreated group [Figure 2].

Regardless of the energy applied, an irregular layer of bioglass particles fused to the dentin in the 45S5 bioglass and NbG groups. However, for the NbG group, a more uniform layer was observed for NbG-80.

EDS allowed observing the presence of Ca²⁺ and PO₄⁻³ on the dentin after treatments with 45S5 bioglass or NbG associated with Nd:YAG. The untreated samples presented a higher amount of carbon and a lower amount of calcium and phosphorus.
Several materials have been developed for the treatment of HSD, but most of them remain unstable against the acid challenges that occur in the mouth. The present study demonstrated that the use of the 45S5 and NbG when associated with Nd:YAG laser (60 and 80 mJ) reduced dentinal permeability, rejecting the first hypothesis. The second hypothesis of the study was not rejected because no difference in dentin permeability was found after the erosive challenge.

The permeability readings were performed in the presence of smear layer for comparing the change of the permeability after the total opening of the tubules and after the treatments, according to the studies that evaluate the dentin permeability by hydraulic conductance for the treatment of HSD. To analyze the degree of permeability reduction, a control group was admitted, decreasing the occurrence of false positives.

The 45S5 is being widely studied material in dentistry due to its ability to stimulate the deposition of hydroxyapatite. The NbG has the niobium pentoxide capable of forming a more chemically stable bioactive layer on the dentin. Joining the bioactivity of 45S5/NbG, with the capacity of the Nd:YAG laser in promoting the dentin melting, an effective obliteration of the dentinal tubules could be reached.

However, the different irradiation parameters could influence the degree of absorption and transmission of energy of the glass particles into the dentin substrate as well as the aggregation of ions to the exposed tubule. Hoopo et al. showed that at the wavelength (around 1064 nm), similar to the one used in the present study, was able to absorb approximately 20% of the energy in the phosphate bioglass transmitting 80%. However, the Nd:YAG laser is not absorbed well by dentin, but can produce a melted, recrystallized, and glazed surface. These structural changes may occur in the dentin when 50 mJ x 10Hz x 40s are used, and energy levels >100 to 150 mJ already characterize the formation of craters.

The results of this study indicated that the 45S5 associated with Nd:YAG showed a greater reduction of permeability when compared to NbG in the same parameters, findings ratified in SEM and EDS. These data allow suggesting that after 24h, the layer formed by 45S5 allows greater ionic exchanges with the medium, and due to its solubility, provides the deposition of Calcium and Phosphate immediately. The layer formed by the NbG for being more stable releases slowly Ca$^{++}$ and PO$_4^{-3}$ confirming the lower reduction of the permeability found in this study. This trend is observed by Bakry et al. that verified, by SEM, a larger tubular seal when 45S5 is associated with the CO$_2$ laser and by Farmakis et al. that combined Novamin® with Nd:YAG.

The energy parameters appeared to influence the degree of obliteration of the dentin treated by NbG, resulting in a greater reduction in permeability reduction when associated with 80 mJ, compared to 60 mJ, also illustrated in SEM. Arise the hypothesis that higher power densities could favor the incorporation of the glass particles, as suggested by Farmakis et al. Furthermore, the modification of the dentine by using the Nd:YAG, could be proportional to the increase of the irradiation, resulting in a layer of extensive melting on the dentin surface. It is speculated that the NbG associated with Nd:YAG, provided a greater reduction of the permeability, compared to 45S5, due to its greater chemical stability. However, it is likely that NbG, which is a more inert material, requires higher energy densities to form the dentin melting layer with the fused particles. The NbG may require a longer time for the formation of the bioactive layer, unlike 45S5, which produces quick and rich ionic exchanges with the medium.

Therefore, 45S5 bioglass does not appear to be influenced by irradiation on the dentin permeability. It is known that for a decrease of pain in cases of HSD, not all the tubules need to be completely obliterated; thus, a significant reduction of the tubule opening may be relevant for the treatment of HSD, as observed in the SEMs.

The erosive challenge was used to clinically simulate the continuous action of intrinsic and extrinsic acids. It was observed that the permeability of the untreated group (C) did not present differences after the erosive challenge because, for the development of the proposed methodology, the tubules were already in the maximum opening. The erosive challenge did not promote changes in the treated dentin, probably due to the interaction of the laser with the dentin, modifying the structure of the crystals, reducing their solubility. Less obliteration of the dentinal tubules
in Nd:YAG treated groups is observed after erosion. However, the laser with bioglass can form a resistant layer to demineralization due to the presence of carbonate free radicals after the morphological changes occurring by irradiation. Such radicals could replace hydroxyl ions and interact with phosphate ions, forming a less soluble phase, and the presence of bioglass would contribute to increasing the availability of calcium and phosphate ions.

**CONCLUSION**

The bioactive glasses (45S5 and NbG) with Nd:YAG may be an alternative capable to reduce dentin permeability. NbG bioactive glass seems to be more sensitive to the Nd:YAG laser parameter variation.

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**Conflicts of interest**

There are no conflicts of interest.
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