In vitro study of dentin hypersensitivity treated by Nd:YAP laser and bioglass

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Summary
Objectives. An ideal material has yet to be discovered that can completely treat dentin hypersensitivity. However, if a highly biocompatible material such as bioglass, could be melted by laser irradiation to achieve better sealing depth for dentinal tubules, it may subsequently bond to dentin structures under a physiological environment and offer a prolonged therapeutic effect.

Methods. The authors used four types of energy parameters to melt the composition-modified bioglass. These four types were 30 Hz, 330 mJ/pulse (G\textsubscript{C} mode), 30 Hz, 160 mJ/pulse (G\textsubscript{K} mode), 10 Hz, 400 mJ/pulse (D\textsubscript{C} mode), and 10 Hz, 200 mJ/pulse (D\textsubscript{K} mode). The temperature elevation, occlusive depth of bioglass, and phase changes in the bioglass after laser irradiation were evaluated by means of scanning electron microscope (SEM), thermometer, and X-ray diffractometer (XRD).

Results. The occlusive depths of 2 and 10 $\mu$m in the dentinal tubules were achieved when the bioglass underwent 30 Hz, 160 mJ/pulse (G\textsubscript{C} mode) and 30 Hz, 330 mJ/pulse (G\textsubscript{K} mode) of laser treatments, respectively. The bioglass experienced a temperature increase of less than 600°C, and no phase transformation was observed after Nd:YAP laser irradiation.

Significance. The melting point of a composition-modified bioglass could be reduced and its use plus Nd:YAP laser have the potential in clinical use to treat dentin hypersensitivity.

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Introduction

Dentin hypersensitivity has long been a troublesome symptom afflicting many patients with a higher prevalence in the 20-29.9 year (34.9%) and 30-39.9 year age groups (33.3%) [1]. The intraoral
distribution was such that the teeth predominantly affected were premolars and molars, while the incisors were least affected [2]. The major symptom of dentin hypersensitivity is characterized by brief, sharp pain that occurs in response to thermal, tactile, osmotic, chemical, and evaporative stimuli [3]. Occasionally, the pain may persist as a dull sensation although the stimuli have been removed [4]. Sensitivity to cold stimuli is the strongest [5] probably because fluid flow away from the pulp produces greater pulp nerve responses than stimuli that cause an inward flow such as heat [6].

Dentin hypersensitivity is closely related to the exposure and patency of dentinal tubules [3]. Previous studies have demonstrated that sensitive teeth have an increased number of patent dentinal tubules (25.6% compared to 9.3%) [7] and are wider in diameter (0.83 μm compared to 0.43 μm) than the dentinal tubules of nonsensitive dentin [8]. Many factors may contribute to the exposure of dentinal tubules, such as occlusal wear, abrasion due to brushing, dietary erosion, parafunctional habits, gingival recession, aging, chronic periodontal disease, tooth abnormally positioned in the arch, periodontal surgery, incorrect toothbrushing habits, root preparation [9], and abfraction [10].

Four theories have been proposed to explain the mechanism of dentin hypersensitivity: the transducer theory, modulation theory, gate-control and vibration theory, and the hydrodynamic theory [11]. The most widely accepted theory is the hydrodynamic theory, which states that stimulus application induces pressure changes across dentin. As a result of the pressure changes, rapid shifts of fluids take place within the dentinal tubules, followed by the excitation of sensory nerves in the pulp dentin border [12]. Therefore, either physically blocking the exposed dentinal tubules or reducing the excitability of the relevant sensory nerves would effectively treat dentin hypersensitivity. According to the Poiseuille-Hagen equation, which states that the movement of the dentinal fluid in a tubule is proportional to the fourth power of tubular radius and the pressure difference between two ends of a tubule [13], permanent blockage of exposed dentinal tubules and subsequent reduction of fluid flow should be an efficient strategy to treat dentin hypersensitivity.

Among currently employed physical and chemical agents, laser and bioglass used separately have shown potentials as methods for treating dentin hypersensitivity. Many types of lasers have been evaluated in the past. For example, the application of Nd:YAG laser at an energy output of 30 mJ melts the dentin surface and occludes the open dentinal tubules [14] and has been suggested as a satisfactory treatment tool [15]. CO2 laser with 1 W for 5–10 s duration of irradiation could treat cervical dentin hypersensitivity without adversely affecting the pulp [16]. Er:YAG laser has also been useful with the desensitizing effects attributed to the deposition of insoluble salts in the exposed dentinal tubules [17]. Despite the fact that lasers can achieve the aforementioned promising results in treating dentin hypersensitivity, the occlusive depth into dentinal tubules was not examined. In this in vitro study, Nd:YAP laser was chosen because it was claimed by the manufacturer to exhibit the capability to treat dentin hypersensitivity. On the other hand, bioglass has also been demonstrated to be a suitable material in treating dentin hypersensitivity [18], periodontal intrabony defects [19] as well as pulp capping [20]. However, the bioglass used alone could not homogeneously cover the orifices of dentinal tubules, leaving many open tubules. Therefore, there appears to be no ideal material which can fulfill the requirements proposed by Grossman [21]: non-irritant to the pulp, relatively painless on application, easily applied, rapid in action, and permanently effective without staining tooth structure. As a result, the authors applied Nd:YAP laser in combination with components-modified bioglass to help the melting process of bioglass and to produce a better sealing depth of dentinal tubules. The thermal effect of Nd:YAP laser irradiation was measured and the crystalline structures of bioglass were also investigated using X-ray diffractometer (XRD).

Materials and methods

Preparation of the bioglass paste

The bioglass used in this study was based on a Na2O-CaO-SiO2 system. Powder mixtures of the nominal composition of Na2O 36.6%, CaO 2%, SiO2 61.4%, MnO2 3%, FeO2 3% in weight ratio were mixed in a ball mill pot and 100 ml of ethanol was added to wet-mill the powder together for 30 min. The powder was dried overnight in an oven at 80 °C to remove the ethanol. This homogeneous powder was placed in a platinum crucible and heated in a SiC furnace to 1400 °C for 1.5 h. The melted glass was then removed from the furnace and poured into 0 °C ice water to quench as glass frit. All glass frit was pulverized by a Spex 8000 alumina ball mill and sieved to a powder less than 53 μm. The bioglass powder was mixed with 64% phosphoric acid in
Specimen preparation

Twenty extracted human molars were used for this study. Crowns with caries, restoration, or fracture were discarded. Any remaining soft tissue was thoroughly removed from the tooth surface with a dental scaler (Sonicflex 2000, KaVo Co., Biberbach, Germany). All teeth were then stored in 4°C distilled water containing 0.2% thymol to inhibit microbial growth until use.

While hydrated, crown dentin discs with a thickness of 3 mm were cut perpendicular to the long axis of the tooth by means of a low-speed diamond wafering blade (Isomet; 10.2 cm × 0.3 mm, arbor size 1/2 in., series 15HC diamond; Buehler Ltd, Lake Bluff, IL). Each dentin disc was divided into two halves to obtain 40 specimens and the enamel was removed with a plain-cut tungsten carbide fissure bur at high speed under a continuous water spray (Fig. 1(a)). One half received 30 Hz, 330 mJ/pulse (G+ mode) and 10 Hz, 400 mJ/pulse (D+ mode) of laser treatments with 10 specimens for each energy setting. The other half received 30 Hz, 160 mJ/pulse (G− mode) and 10 Hz, 200 mJ/pulse (D− mode) of laser treatments with 10 specimens for each energy setting. Each specimen was etched with 37% phosphoric acid solution to remove the smear layer and then rinsed with copious distilled water and dried with clean air.

Laser treatment

An Nd:YAP laser (Lokki, Vienne, France) that provided a constant beam of coherent, continuous monochromatic light with an emission wavelength of 1.34 µm and a pulse duration of 150 µs was used in this study. 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 vaporized bioglass and dentin. After application of a thin film of bioglass paste, the laser tip was swept in a mesiodistal fashion with an irradiation area about 3 mm × 5 mm and a speed of about 3 mm/s with a total irradiation time of 5 s to simulate clinical manipulation. Energy densities of target surface following G+, D+, G−, D− modes irradiation were 330, 133, 160, 67 J/cm², respectively.

Scanning electron microscopy (SEM) examination

The morphology, microstructure, and sealing depth of dentinal tubules by bioglass paste after laser irradiation were observed under a scanning electron microscope. The specimens were immersed in 2.5% cold glutaraldehyde in 0.1 mol/L cacodylate buffer at pH 7.4 for 8 h. All specimens were then serially dehydrated in graded ethanol solutions (50, 60, 70, 80, 90, 95, and 100% ethanol) at 45-min intervals, critical point-dried by CO₂, mounted on aluminum stubs, sputter-coated with ~20 nm of gold/palladium, and finally examined by a Hitachi SEM (Model S-800, Tokyo, Japan) at an accelerating voltage of 15 kV. From SEM observation, the energy parameters that could melt bioglass and seal dentinal tubules were selected for undergoing temperature elevation measurement.

Temperature elevation measurement

Twenty extracted human upper premolars were used in measuring temperature increase during laser treatment. Teeth were sectioned longitudinally from the central groove of each occlusal surface with a low-speed diamond wafering blade to obtain 40 specimens. The buccal or lingual cervical regions with dimensions of about 3 mm × 5 mm were wet-polished with 600 grit silicon carbide paper to remove the enamel and cementum (Fig. 1(b)), etched with 37% phosphoric acid solution to remove the smear layer and then rinsed with copious distilled water and dried with clean air.
solution, rinsed with copious distilled water, and dried with clean air to expose the dentinal tubules and prepare them for subsequent laser treatment. The site of the pulp cavity corresponding to the outer lased area was slightly polished with 600 grit silicon carbide paper until the thickness was 2.5 mm. Then, the site of the pulp cavity was applied with a silicone heat transfer compound (Unick, Unick Chemical Co., Taipei, Taiwan) to promote heat conduction in the thermocouple (Philips, type K025, diameter 0.25 mm, Tokyo, Japan). Paraffin wax was used to isolate the thermocouple and prevent the influences caused by environment temperature. The thermocouple was connected to a digital oscilloscope (LeCroy 9310M, Dual 300 MHz, oscilloscopes; LeCroy Corp., Geneva, Switzerland), plotter (X-Y plotter, DXY-880; Roland Digital Group Co., Tokyo, Japan), and digital thermometer (YF-160, type K; Yu Hong Co., Taipei, Taiwan) to record the mean temperature elevation and standard deviation. One-way analysis of variance followed by Student’s t-test was used to examine the statistical significant difference of temperature rise.

Forty specimens were randomly divided into four groups. Groups A and B received 30 Hz, 330 mJ/pulse (G+C mode) of laser treatment with and without use of bioglass paste, respectively. Groups C and D received 30 Hz, 160 mJ/pulse (G+K mode) of laser treatment with and without use of bioglass paste, respectively. The operation mode of laser irradiation was the same as that of specimens for SEM observation.

**X-ray diffraction analysis**

The crystalline phases of the bioglass paste before and after laser irradiation were determined by a Rigaku X-ray powder diffractometer (Rigaku Denki Co., Ltd, Tokyo, Japan) with Cu Kα radiation and Ni filter. The scanning range was 10–60°, with a scanning speed of 4°/min. To determine the contents of different phases, relative intensities of the characteristic peaks of each phase were used.

**Results**

**Scanning electron microscopy (SEM) examination**

Fig. 2(a) shows the bioglass paste covering dentin surfaces after 30 Hz, 330 mJ/pulse (G+ mode) of laser treatment. The dentin surfaces were covered with a melted and glassy bioglass without exposed dentinal tubules. From the longitudinal section parallel to the direction of dentinal tubules alignment, we found that the melted bioglass entered the dentinal tubules as deep as 10 μm (Fig. 2(b)). A gap between melted bioglass and tubular walls, probably due to contraction of cooled bioglass, was noted. After 30 Hz, 160 mJ/pulse (G− mode) of laser treatment, the bioglass covering the dentin surfaces also appeared as homogeneous, smooth, and melted surfaces without exposure of dentinal tubule orifices (Fig. 3(a)). Compared with G+ mode of laser irradiation, the sealing depth of G− mode was only about 2 μm into the dentinal tubules (Fig. 3(b)). When the bioglass...
paste was subjected to 10 Hz, 400 mJ/pulse (D+ mode) of laser treatment, the laser energy was insufficient to melt all bioglass paste (Fig. 4(a)). Many open dentinal tubule orifices were found. The longitudinal section revealed that some dentinal tubule orifices were not occluded (Fig. 4(b)). The 10 Hz, 200 mJ/pulse (D- mode) of laser treatment could not melt bioglass paste and did not exhibit any occlusive effect (not shown).

Temperature elevation measurement

The table demonstrates mean temperature rise and standard deviation after different treatments.

X-ray diffraction analysis

Fig. 5(a) and (b) shows the X-ray diffraction patterns of the bioglass paste before and after...
30 Hz, 330 mJ/pulse of Nd:YAP laser irradiation. Both patterns exhibited amorphous patterns without any crystalline peak. Because it was very difficult to determine exactly the temperature elevation and phase transformation of bioglass after laser irradiation, the authors heated bioglass in a SiC furnace at ascending temperatures to 900 °C to explore the phase transformation of bioglass.

**Discussion**

Exposed dentinal tubules have been considered as the leading cause of dentin hypersensitivity, and numerous treatment methods have been suggested based on occlusion of exposed dentinal tubules. These treatment methods could be classified into chemical and physical agents [9]. Chemical agents included strontium chloride, sodium fluoride, formalin solution [22], ferric oxalate [23], calcium hydroxide, stannous hydroxide, calcium oxalate, ferric phosphate [24], potassium nitrate [25], while physical agents contained fluoride-releasing resin [26]. Although these materials yielded promising results, their therapeutic effects were relatively short-lived or decreased with time because agents that blocked the dentinal tubules could not bind to the tubular walls. Thus, they were kept in place merely by mechanical retention. After daily tooth brushing, mastication, or immersion in oral fluids over time, these agents were gradually abraded or solubilized and eventually lost their occlusive action.

Laser and bioglass are two novel agents used for treating dentin hypersensitivity. Although the exact mechanism of laser action was debated, two hypotheses have been assumed. One assumption is that the sealing of exposed dentinal tubules with melted and recrystallized dentin is caused by the thermal and occlusive effects of laser [27]; the other involved dentin desiccation after laser irradiation [28]. The former effect resulted in prolonged relief, while the latter effect was only a temporary relief of dentin hypersensitivity. Although laser treatment was conducive to alleviating symptoms of dentin hypersensitivity [15,17], the longevity of the effects is still questionable.

Bioglass is a biocompatible material and has a high surface reactivity that can induce osteogenesis in physiological systems. Its basic components are silicon dioxide, sodium oxide, calcium oxide, and phosphorus oxide. In a previous study it was demonstrated that bioglass in combination with CO₂ laser might afford an alternative method in
treated tooth crack or fracture [29–31]. In the pilot study, when only the commercial bioglass, Abmindent I (Abmin Technologies Ltd, Turku, Finland) was used, the sealing effect of dentinal tubules was less than 1 μm. For that reason, in this study the thermal effect of Nd:YAP laser was used to help melting the bioglass deeper into the dentinal tubules. Nd:YAP laser was preferred as it has a wavelength of 1340 nm and its light energy was easily absorbed by black pigment. However, the result was not satisfactory as a homogenous melting of the commercial bioglass could not be reached, leaving many open dentinal tubules. Therefore, the bioglass was modified to a material composed of Na₂O 36.6%, CaO 2%, SiO₂ 61.4%, MnO₂ 3%, FeO₂ 3% in weight ratio. In contrast to the commercial product, Bioglass®, whose composition is 45% silica, 24.5% calcium oxide, 24.5% sodium oxide, and 6% phosphorus pentoxide in weight ratio [19], the components were altered by removing phosphorus oxide to lower the melting point of the bioglass and some transitional metal oxides (MnO₂ and FeO₂) added to increase Nd:YAP laser absorption. The results showed that the modified bioglass could be completely melted by either 30 Hz, 330 mJ/pulse (G+ mode) or 30 Hz, 160 mJ/pulse (G− mode) of laser treatment, and the exposed dentinal tubules were covered by melted bioglass (Figs. 2(a) and 3(a)). The sealing depths of bioglass were 10 and 2 μm, respectively (Figs. 2(b) and 3(b)). The energy settings of 10 Hz, 400 mJ/pulse (D+ mode; Fig. 4(a) and (b)) and 10 Hz, 200 mJ/pulse (D− mode) were not suitable to treat dentin hypersensitivity because they were not sufficient to completely melt the bioglass paste.

The mechanism of bioglass bonding to bone begins from the contact of bioglass with water. Sodium ions of bioglass exchange with hydrogen ions of water, causing pH elevation of the local environment. Then Si-O-Si network dissolution occurs at high pH followed by repolymerization of Si-O-Si bond, forming a silica rich layer. The calcium and phosphate ions migrate to the surface of the silica rich layer, and both ions play a significant role in bone/graft bonding [32]. Since both the dentin and bone share a similar chemical composition of 35% organic matter composed of principally collagen and 65% almost exclusively hydroxyapatite crystals [33], the bioglass/dentin bond is also likely to take place in a physiological environment. This result would help to preserve the bioglass in the dentinal tubules and prolong treatment efficiency. Because the therapeutic efficacy of the commercial bioglass has never been evaluated, the retentive duration of the modified bioglass after Nd:YAP laser treatment could not be compared in this study. Schwarz et al. [17] showed that the desensitizing effects of Er:YAG laser alone could persist for 6 months, therefore it is expected that the possible efficacy of the method used in this study can extend over 6 months. Nevertheless, the exact outcome needs to be further investigated.

However, laser energy should be carefully operated. Otherwise, it may cause adverse thermal effects to the pulp. According to the studies of Zach and Cohen regarding pulp response to externally applied heat [34], 15% of teeth failed to recover from an intrapulpal temperature increase of 5.5 °C. If the temperature increase was 11 °C, 60% of teeth could not recover. Temperature increase below 5.5 °C produced only minimal intrapulpal changes. In this study, 30 Hz, 160 mJ/pulse (G− mode) of laser treatment plus use of bioglass produced a temperature rise below 5.5 °C (Table 1). Although 30 Hz, 330 mJ/pulse (G+ mode) of laser treatment plus use of bioglass produced a temperature rise of 12.06 ± 3.58 °C, the in vivo use of laser treatment in this mode should be much lower than this value because blood flow, blood perfusion, conduction through gingiva and bone in the clinical situation can act as a heat reservoir of teeth [35]. The temperature rise of teeth placed in a 37 °C water bath after laser irradiation was reported to be 5 °C lower than the temperature rise of isolated teeth [35]. The in vivo condition in 30 Hz, 330 mJ/pulse (G+ mode) of laser treatment plus use of bioglass should be further investigated.

Table 1  Mean temperature rise and standard deviation of different treatments.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Temperature rise (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: G+ mode with bioglass</td>
<td>12.06 (3.58) (10)*</td>
</tr>
<tr>
<td>B: G+ mode without bioglass</td>
<td>24.28 (7.70) (10)*</td>
</tr>
<tr>
<td>C: G− mode with bioglass</td>
<td>3.19 (0.63) (10)*</td>
</tr>
<tr>
<td>D: G− mode without bioglass</td>
<td>17.14 (3.42) (10)*</td>
</tr>
</tbody>
</table>

Significant (P<0.05) by Student’s t-test with one-way analysis of variance.

a Values are means (standard deviation) (number of specimens).
paste after heat treatment from 100 to 900 °C demonstrated that calcium phosphate and silicon dioxide were the major crystalline phases when the temperature exceeded 700 °C (Fig. 6). No crystalline peaks could be identified when the heated temperature was below 600 °C. It is suspected that Nd:YAP laser irradiation on the bioglass produces a temperature rise of no more than 600 °C.

In this in vitro study, dentin discs were used to evaluate the occlusive effect of Nd:YAP laser plus bioglass. This method was proved to be an effective way to examine the occlusion of materials in complex phenomenon of dentin hypersensitivity [36]. However, the diameters of dentinal tubule orifices become wider when they are near the pulp. The cross-sections in different planes presented various densities and diameters of dentinal tubules. The cross-sections in different planes demonstrated that calcium phosphate and silicon dioxide were the major crystalline phases when the temperature exceeded 700 °C [8].

References

Dentin hypersensitivity treated by laser and bioglass


