In Vitro Study of DP-bioglass Paste for Treatment of Dentin Hypersensitivity

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Sealing of exposed dentinal tubules is generally considered the most effective strategy to treat dentin hypersensitivity. On this account, we fabricated a DP-bioglass paste that created a homogeneous blockage on open dentinal tubules and formed a deep precipitate within dentinal tubules. DP-bioglass paste was prepared by mixing 20% to 60% phosphoric acid and DP-bioglass to treat dentin surfaces. CO₂ laser irradiation was used to melt the DP-bioglass paste. The results demonstrated that 30% phosphoric acid was the optimum concentration to produce homogeneous occlusion on exposed dentinal tubules and 60 μ m of sealing depth. CO₂ laser irradiation could melt the DP-bioglass paste and create about 10 μ m of sealing depth. Moreover, temperature rise during CO₂ laser irradiation was only $4.86 \pm 0.47^\circ\text{C}$. The results presented in this work suggested that DP-bioglass paste could produce considerable sealing depth in dentinal tubules with the potential of prolonging the therapeutic effect efficaciously.

Key words : DP-bioglass, CO₂ laser, Dentin hypersensitivity

INTRODUCTION

Dentin hypersensitivity is an annoying symptom afflicting numerous patients, particularly and prevalently among those in the age groups of 20–29.9 years (34.9%) and 30–39.9 years (33.3%)¹. Dentin hypersensitivity is typically characterized by brief, sharp pain that occurs in response to thermal, tactile, osmotic, chemical, or evaporative stimuli and which cannot be attributed to any form of pathology or dental defect². Sensitivity is strongest with cold stimuli³ probably because fluid flows away from the pulp, thereby producing a greater pulp nerve response, as compared to a stimulus that causes an inward flow such as heat⁴. Intraoral distribution study revealed that premolars and molars were the predominantly affected teeth, while the incisors were the least sensitive ones⁵.

Dentin hypersensitivity is intimately associated with exposed dentinal tubules⁶. When comparisons were made between sensitive and non-sensitive dentin, it was found that the number of patent dentinal tubules was 35.6% in the former and 9.3% in the latter⁷. In addition, the diameter of dentinal tubules was wider (0.83 μ m) in sensitive dentin than in non-sensitive dentin (0.43 μ m)⁸. Exposure of dentinal tubules may be ascribed to many causative factors, such as attrition from occlusal wear, abrasive tooth brushing, dietary erosion, parafunctional habits, gingival recession, aging, chronic periodontal disease, periodontal surgery, root preparation, and abfraction lesions⁹. In these circumstances, the odontoblastic processes inside the open dentinal tubules are sensitive to external irritation.

Among the theories proposed to explain the

mechanism of dentin hypersensitivity, the most broadly 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 excitation of sensory nerves in the pulp dentin border¹⁰. Therefore, using physical agents to reduce the excitability of the relevant sensory nerves or chemical agents to block the exposed dentinal tubules are practicable methods to treat dentin hypersensitivity. The Poiseuille-Hagen equation demonstrates the pressure difference between two ends of a tubule, and it also shows that the movement of the dentinal fluid in a tubule is proportional to the fourth power of tubular radius¹¹. Consequently, dentin hypersensitivity can be successfully treated by permanently blocking the exposed dentinal tubules in order to reduce fluid flow.

Physical agents that have been suggested included Nd:YAG laser¹² and GaAlAs laser¹³, while chemical agents were namely ferric oxalate¹⁴, potassium nitrate¹⁵, visible light-cured materials¹⁶, and fluoride varnishes¹⁷. Although these materials were reported to be effective, their therapeutic effects were generally short-lived or diminished with time because these agents could be easily removed by daily tooth brushing or acidic beverage drinking. Consequently, to produce a long-lasting seal, the material must penetrate the dentinal tubules and occlude the orifices of dentinal tubules.

Suge *et al.*¹⁸ has reported that a calcium phosphate precipitation (CPP) method could achieve 10 to 15 μ m of sealing depth into the dentinal tubules. If a deeper sealing depth could be created, then a longer therapeutic effect could be expected. After treatment

with CPP solution, the dentinal surfaces were further neutralized by 1.0 mol/L NaOH as a post-treatment solution. However, the basic solution could cause atrophy and degeneration of gingival epithelium¹⁹.

Bioglass is a highly biocompatible material. We have used the bioglass combined with laser treatment to achieve 10 μ m of sealing depth for dentinal tubules²⁰. In this *in vitro* study, we fabricated DP-bioglass paste and applied CO₂ laser to melt the bioglass with the intent of creating a deeper seal within the dentinal tubules. The microstructure and crystalline structure of DP-bioglass paste were examined using scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDX) and X-ray diffractometry (XRD) respectively. In addition, the thermal effect of CO₂ laser was also evaluated.

MATERIALS AND METHODS

DP-bioglass paste preparation

The DP-bioglass used in this study was based on a Na₂O-CaO-SiO₂-P₂O₅ system. Powder mixture of the nominal composition of Na₂O 8.4%, CaO 40.6%, SiO₂ 39%, P₂O₅ 12% in weight ratio was mixed in a ball mill pot, and 100 ml of ethanol was added to wet-mill the powder together for eight hours. 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 1410 °C for 1.5 hours. The melted glass was then removed from the furnace and poured into 0°C ice water to be quenched into glass frit. All glass frit was pulverized by a Spex 8000 alumina ball mill and sieved to a powder less than 53 μ m. The DP-bioglass powder was mixed with 60%, 50%, 40%, 30%, or 20% phosphoric acid aqueous solution in a powder/liquid ratio of 0.05 g/0.1 ml to form five types of DP-bioglass paste.

Specimens preparation

Sixty extracted human third molars from 16- to 40-year-old individuals with informed consent at the National Taiwan University Hospital 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 3-mm thickness were cut perpendicular to the long axis of the tooth by means of a low-speed diamond wafering blade (Isomet; Buehler Ltd., Lake Bluff, IL). Each specimen was immersed in 17% EDTA followed by two minutes of ultrasonic vibration to remove the smear layer, then rinsed with copious distilled water and dried with clean air. These dentin discs were

divided into four groups (A to D) with 15 in each group for DP-bioglass paste treatment. Each group was further subdivided into five subgroups to receive five types of DP-bioglass paste. Group A was designated to examine the occlusive effect of DP-bioglass paste on the orifices of dentinal tubules, whereas Groups B and C were to be evaluated in terms of the sealing depth of DP-bioglass paste and Ca/P ratio of precipitation respectively. A groove was prepared using tapered fissure bur on the specimens of Groups B and C to facilitate the sectioning of specimens with a chisel. Group D was designated to receive DP-bioglass paste with CO₂ laser irradiation. We applied the DP-bioglass paste (powder/liquid ratio of 0.05 g/0.1 ml) to the specimens for one minute with an applicator sponge, and then stored all specimens (Groups A to D) in 37°C, 100% humidity environment for three days to simulate the oral cavity and enable the DP-bioglass paste to take occlusive effect. The sealing depth of dentinal tubules from the dentinal surface to the bottom of DP-bioglass was measured in 20 dentinal tubules obtained from three independently treated specimens¹⁸.

Laser treatment

A CO₂ laser (Sharplan 1030, Sharplan Lasers Inc., USA) that provided a constant beam of coherent, continuous monochromatic light with an emission wavelength of 10.6 μ m and a spot size diameter of 0.8 mm was used in this study. The laser was delivered with a straight handpiece, and the laser tip was held perpendicular to the irradiated surface and 1 mm away to prevent contamination from vaporized DP-bioglass and dentin. After application on a thin film of DP-bioglass paste, the laser tip swept in a mesiodistal fashion with an irradiation area of approximately 3 mm $\times$ 2 mm and a speed of about 3 mm/sec – up to a total irradiation time of two seconds – to simulate clinical manipulation. Energy density of target surface following irradiation was 100 J/cm².

Scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDX) examination

The occlusive effect, microstructure, sealing depth, and Ca/P ratio of DP-bioglass paste were observed using SEM-EDX. The specimens were immersed in 2.5% cold glutaraldehyde in 0.1 mol/L cacodylate buffer at pH 7.4 for eight hours. All specimens were then serially dehydrated in graded ethanol solutions (50%, 60%, 70%, 80%, 90%, 95%, and 100% ethanol) at 45-minute intervals, critical point dried in CO₂, mounted on aluminum stubs, sputter-coated with ~20 nm of carbon, and finally examined by a SEM (Model S-800, Hitachi, Tokyo, Japan) at an accelerating voltage of 15 kV.

Temperature elevation measurement

Ten extracted human upper premolars were used in measuring temperature increase during CO₂ laser treatment. Teeth were sectioned longitudinally from the central groove of each occlusal surface with a low-speed diamond wafering blade to obtain 20 specimens. The buccal or lingual cervical regions with dimensions of about 3 mm × 2 mm were wet-polished with 600 grit silicon carbide paper to remove the enamel and cementum, etched with 37% phosphoric acid solution for 30 seconds, 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 a thermocouple (type K025, diameter 0.25 mm, Philips, Tokyo, Japan). Paraffin wax was used to isolate the thermocouple from influences caused by environmental temperature. The thermocouple was connected to a digital oscilloscope (9310M, Dual 300 MHz, LeCroy Corp., Geneva, Switzerland), 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.

Twenty specimens were randomly divided into two groups. One group received DP-bioglass paste with CO₂ laser irradiation, while the other group received CO₂ laser treatment only. The operation mode of laser irradiation was the same as that of specimens for SEM-EDX observation.

X-ray diffraction (XRD) analysis

Crystalline phases of the DP-bioglass paste before and after mixing with phosphoric acid were determined by a X-ray powder diffractometer (Rigaku Denki Co., Ltd., Tokyo, Japan) with CuK α radiation and Ni filter. Scanning range was 10 degrees to 60 degrees, with a scanning speed of 4 degrees/min. To determine the contents of different phases, relative intensities of the characteristic peaks of each phase were used.

RESULTS

Scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDX) examination

The dentin specimen after 17% EDTA and two minutes of ultrasonic vibration exhibited a clean and even surface (Fig. 1(a)). There was no smear layer and exposed dentinal tubules' orifices were noted.

Fig. 1 (a) SEM micrograph of dentin surface treated with EDTA and 2 minutes of ultrasonic vibration. A clear and even surface free of smear layer was found on the dentin specimen. (b) SEM micrograph of DP-bioglass paste application mixed with 30% phosphoric acid. The orifices of dentinal tubules were completely occluded. (c) SEM micrograph of DP-bioglass paste application mixed with 20% phosphoric acid. Incomplete coverings with many exposed dentinal tubules were noted.

Fig. 2 (a) SEM micrograph of a longitudinal section parallel to the direction of dentinal tubules alignment. Sealing depth of DP-bioglass paste could be as deep as $60\mu\text{m}$ when the concentration of phosphoric acid was 30% and above (arrow). (b) No sealing depth could be found when concentration of phosphoric acid was 20%.

Fig. 3 (a) SEM micrograph of DP-bioglass paste after CO_2 laser irradiation. It shows a homogeneous, glassy, and melted surface with no exposed dentinal tubules. (b) Sealing depth was about $10\mu\text{m}$ (between white arrows).

Fig. 1(b) shows that the orifices of dentinal tubules were completely covered by DP-bioglass mixed with 30% phosphoric acid. Application of DP-bioglass mixed with 40%, 50%, and 60% phosphoric acid produced similar occlusive effect on the orifices of dentinal tubules. However, when the concentration of phosphoric acid was decreased to 20%, a reduced sealing effect on dentinal tubules could be observed (Fig. 1(c)). Indeed, it could be seen that many exposed dentinal tubules were not occluded. From the longitudinal section parallel to the direction of dentinal tubules alignment, the sealing depth could be as deep as $60\mu\text{m}$ when the concentration of

phosphoric acid was 30% and above (Fig. 2(a)). In contrast to the deep penetration, no sealing depth could be found when the concentration of phosphoric acid was 20% (Fig. 2(b)).

When DP-bioglass paste was irradiated by CO_2 laser, a homogeneous, glassy, and melted surface with no exposed dentinal tubules was obtained (Fig. 3(a)). This phenomenon could be found irrespective of the concentration of phosphoric acid. The sealing depth was about $10\mu\text{m}$ (Fig. 3(b)).

Table 1 demonstrates the Ca/P ratio of DP-bioglass precipitation inside the dentinal tubules. Examined sites were located 5, 20, $50\mu\text{m}$ from the

Table 1 Ca/P ratio of DP-bioglass precipitation inside dentinal tubules by X-ray quantitative analysis

Concentration of phosphoric acid	Area examined from the orifices of dentinal tubules		
	5 μ m	20 μ m	50 μ m
30%	1.17	1.26	1.42
40%	1.14	1.27	1.41
50%	1.13	1.22	1.34
60%	1.10	1.21	1.36

Table 2 Mean temperature rise and standard deviation after CO₂ laser irradiation

Group	Temperature rise (°C)
No DP-bioglass application	17.75 $\pm$ 1.12 (10)* ^a
DP-bioglass application	4.86 $\pm$ 0.47 (10)*

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

^aValues are means (standard deviation) (number of specimens).

orifices of dentinal tubules. As the depth increased, the Ca/P ratio also became greater. No peculiar relationship could be found among different concentrations of phosphoric acid.

Temperature elevation measurement

Table 2 demonstrates the mean temperature rise and standard deviation with and without DP-bioglass application after CO₂ laser irradiation. Temperature was elevated to 17.75 $\pm$ 1.12°C when DP-bioglass paste was not applied. However, the temperature rise decreased to 4.86 $\pm$ 0.47°C when DP-bioglass paste was used. There was a significant difference between these two groups.

X-ray diffraction (XRD) analysis

Fig. 4 shows the XRD diffraction patterns of DP-bioglass mixed with different concentrations of phosphoric acid. Before mixing with phosphoric acid, DP-bioglass exhibited an amorphous pattern without any characteristic peak (Fig. 4(a)). When the concentration of phosphoric acid was 40% and above, prominent crystalline peaks could be observed at the positions of $2\theta = 23.1^\circ$, 24.2° , 30.6° (Figs. 4(b), (c), (d)). Compared with the standard JCPDs card, the crystalline phase that existed in the DP-bioglass paste was identified as calcium phosphate monohydrate ($\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$). As the concentration of phosphoric acid was decreased to 30% and 20%, the characteristic peaks shifted to the positions of $2\theta = 11.6^\circ$, 21.0° , 29.3° (Figs. 4(e), (f)). These peaks indicated that the major crystalline compound of DP-bioglass paste was dicalcium phosphate dihydrate ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$).

The XRD diffraction patterns of DP-bioglass

Fig. 4 XRD diffraction pattern of (a) DP-bioglass and DP-bioglass mixed with (b) 60%, (c) 50%, (d) 40%, (e) 30%, and (f) 20% phosphoric acid. DP-bioglass exhibited an amorphous pattern without any characteristic peak. When concentration of phosphoric acid was 40% and above, the crystalline phase that existed in the DP-bioglass paste was calcium phosphate monohydrate ($\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$). As the concentration of phosphoric acid was decreased to 30% and 20%, the major crystalline compound of DP-bioglass paste was dicalcium phosphate dihydrate ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$).

Fig. 5 XRD diffraction pattern of DP-bioglass paste mixed with (a) 40% phosphoric acid and (b) 30% phosphoric acid after CO_2 laser irradiation. Compared with those patterns before CO_2 laser irradiation, they were of a similar profile but the magnitude of characteristic peaks was lower.

paste lased by CO_2 laser shared a similar profile with those before CO_2 laser irradiation, except that the characteristic peaks were of a lower magnitude (Figs. 5(a), (b)).

DISCUSSION

Blockage of open dentinal tubules is generally accepted as the most effective strategy to treat dentin hypersensitivity. To resist the friction of daily tooth brushing and food chewing, the blockage should not only cover the exposed dentinal tubules but also penetrate the dentinal tubules as deeply as possible. Otherwise, therapeutic efficacy will be short-lived and repeated application of desensitizer will be indispensable²¹. Be it physical or chemical agents that have been proposed to achieve a considerable sealing depth, both entailed the use of calcium phosphate¹⁸. By dissolving $\text{Ca}(\text{OH})_2$ or $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ in H_3PO_4 or HCl and using NaOH as the post-treatment solution, approximately $15\mu\text{m}$ of sealing depth into dentinal tubules could be produced. In this study, application of DP-bioglass paste that was mixed with 30% phosphoric acid could produce a sealing depth of $60\mu\text{m}$ (Fig. 2(a)) and could evenly cover the orifices of dentinal tubules (Fig. 1(b)). DP-bioglass that was mixed with 40%, 50%, and 60% phosphoric acid resulted in similar occlusive effect, indicating the 30% phosphoric acid was the lowest effective concentration. With 20% phosphoric acid, such a homogeneous sealing effect was not obtained (Figs. 1(c), 2(b)). As 37% phosphoric acid is generally employed to etch the dental hard tissues, DP-bioglass with 30% phosphoric acid should be tolerable and safe for pulp tissue.

DP-bioglass paste with CO_2 laser irradiation has been proposed to treat tooth fractures^{22,23}. By means of transient high energy induced by CO_2 laser, DP-bioglass paste was anticipated to melt and flow into

dentinal tubules. The results revealed that the dentinal surfaces were homogeneously covered by melted DP-bioglass paste and about $10\mu\text{m}$ of sealing depth could be achieved (Figs. 3(a), (b)). In addition, no recrystallization or grain growth was found after CO_2 laser irradiation. This implied that CO_2 laser energy could sufficiently melt the DP-bioglass paste and possibly the underlying dentin structure, hence making the two materials fuse together. The rapid cooling rate after CO_2 laser irradiation could not allow any well-crystallized product to be formed²⁴, and thus the XRD diffraction patterns of DP-bioglass paste lased by CO_2 laser exhibited no sharp characteristic peaks (Figs. 5(a), (b)).

Using CO_2 laser may raise another concern if the temperature rise proved harmful to the pulp tissue. To evaluate the thermal effect, we simulated the clinical condition by selecting premolars as the specimens since they are the teeth most likely to be afflicted. The cervical area was irradiated, and temperature rise in the pulp cavity was measured. The results demonstrated that temperature rise was $17.75 \pm 1.12^\circ\text{C}$ when DP-bioglass paste was not applied. Nevertheless, the temperature rise decreased to $4.86 \pm 0.47^\circ\text{C}$ when DP-bioglass paste was used. Zach and Cohen²⁵ have reported on pulp response to externally applied heat. Their results showed that 15% of teeth failed to recover from an intrapulpal temperature increase of 5.5°C and 60% of teeth could not recover if the temperature increase was 11°C . Therefore, temperature increase below 5.5°C produced only minimal intrapulpal changes and has been considered a safe threshold. Based on this report, temperature rise of $4.86 \pm 0.47^\circ\text{C}$ would therefore cause no injury to pulp tissue when DP-bioglass paste was irradiated by CO_2 laser. Furthermore, *in vivo* temperature rise due to laser treatment should be much lower because blood flow, blood perfusion, and heat conduction through gingival and bone would help dissipate the heat to the surrounding tissues²⁶.

When the concentration of phosphoric acid was 40% and above, the XRD diffraction patterns of DP-bioglass paste showed that the major crystalline phase was calcium phosphate monohydrate ($\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$) (Figs. 4(b), (c), (d)). As the concentration of phosphoric acid was decreased to 30% and 20%, the major crystalline compound of DP-bioglass paste was dicalcium phosphate dihydrate ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) (Figs. 4(e), (f)). The reactions were proposed as follows²⁷:

SEM-EDX examination revealed that the Ca/P ratio of DP-bioglass precipitation inside dentinal

tubules ranged from 1.10 to 1.42 (Table 1). As the depth of the examined site increased, the Ca/P ratio also became greater. This implied that the deeper precipitate was more apatite-like and more resistant to hydrolysis.

The mechanism of precipitate formation inside the dentinal tubules was assumed to proceed as follows. First, DP-bioglass was melted by phosphoric acid, and then calcium as well as phosphate ions were released. Second, the ions and remnant phosphoric acid diffused into the dentinal tubules, and peritubular dentin was dissolved by phosphoric acid. Third, the dissolved ions from peritubular dentin, calcium ions, and phosphate ions altogether formed the precipitate. If the precipitate could receive Ca^{2+} from the pulpal fluid *in vivo*, it is possible for dicalcium phosphate dihydrate ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) to transform to calcium-deficient hydroxyapatite²⁸⁾, a more thermodynamically stable, hydrolysis-resistant compound. The reaction was proposed as follows²⁸⁾:

Since the composition of the precipitate compound was similar to that of human teeth, and if a continuous mineralization process through the above reaction formula (3) could take place, the longevity of the therapeutic effect could be substantially prolonged. Nevertheless, further *in vivo* study needs to be conducted to evaluate the influences of dentinal fluids and pulpal pressure on the occlusive effect of DP-bioglass paste.

CONCLUSION

DP-bioglass paste was fabricated by mixing 30% phosphoric acid and DP-bioglass to treat dentin hypersensitivity. Consequently, homogeneous covering on exposed dentinal tubules and 60 μm of sealing depth into the dentinal tubules could be observed. CO_2 laser irradiation was employed to melt the DP-bioglass paste and create about 10 μm of sealing depth. Moreover, temperature rise during CO_2 laser irradiation was below 5.5°C.

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