# $CO_2$ Laser Improves 45S5 Bioglass Interaction with Dentin

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## ABSTRACT

Bioglass 45S5 is a bioactive glass that can create a layer of calcium-phosphate crystals on mineralized hard tissues. In this study, 45S5 bioglass was mixed with phosphoric acid and irradiated with $CO_2$ laser and examined as a possible aid in the treatment of dentin hypersensitivity. The dentinal surface modified by the aforementioned technique was chemically and micro-morphologically examined with a field emission scanning electron microscope (FE-SEM) equipped with an energy-dispersive x-ray spectroscope (EDS), and the crystalline structures of the examined dentinal surfaces were examined by x-ray diffraction (XRD). Moreover, the mechanical properties of the newly formed layer were examined by nanoindentation. The results showed that 45S5 bioglass could occlude the dentinal tubule orifices with calcium-phosphate crystals. The application of $CO_2$ laser potentially improved the mechanical organization of these crystals.

**KEY WORDS:** 45S5 bioglass, $CO_2$ laser, bioactive glass.

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## INTRODUCTION

Dentin hypersensitivity is one of the major challenges in dental practice (Orchardson and Gillam, 2006) that affects from 4% (Rees and Addy, 2002) to 57% of the world population (Chabanski *et al*., 1996; Irwin and McCusker, 1997). The hydrodynamic theory (Brännström *et al*., 1968; Brännström, 1986) explains the phenomenon of dentin hypersensitivity as an increase in the flow of the fluids present in dentinal tubules that have patent orifices, thereby activating nerves situated in the outer layers of the pulp. Exposure of dentinal tubule orifices may be caused by many factors, such as acid erosion (Brunton *et al*., 2000; Prati *et al*., 2003), attrition, abrasion (West *et al*., 1998), parafunctional habits, or gingival recession. These dentinal tubule orifices require permanent occlusion as a treatment approach (Orchardson and Gillam, 2006; Markowitz and Pashley, 2008; Pashley *et al*., 2008).

Agents used for the treatment of dentin hypersensitivity show only temporary success clinically, because they are gradually removed by daily brushing, food friction, and changes in the pH in the oral cavity (Brunton *et al*., 2000).

Bioactive glasses such as 45S5 bioglass can interact with hard tissues by forming a calcium-phosphate-rich layer that can bond chemically to these hard tissues (Hulbert *et al*., 1987; Yamamuro *et al*., 1990; Hench, 1991). Moreover, the energy available in a $CO_2$ laser is absorbed by the hydrated calcium-phosphate crystals (Sasaki *et al*., 1998). The thermal effects of the $CO_2$ laser remove the water of crystallization and subsequently improve the physical properties of the calcium-phosphate crystals. The aim of this study was to examine the micro-morphological features and chemical changes that take place on the dentinal surface after 45S5 bioglass application before and after $CO_2$ laser treatment, by field emission scanning electron microscopy (FE-SEM) coupled with energy-dispersive x-ray spectroscopy (EDS). The crystalline structures of the tested dentinal surfaces were examined by x-ray diffraction (XRD). Moreover, the hardness and the modulus of elasticity of the newly formed interaction layer were evaluated by nanoindentation.

The hypothesis in this study was that $CO_2$ laser irradiation will modify the calcium-phosphate "interaction layer" formed on the dentin surface.

## MATERIALS & METHODS

### Dentin Specimens' Preparation

Seventy-six freshly extracted non-carious third molars were used following guidelines approved by the Tokyo Medical and Dental University Ethical

**Table 1.** Summary for the Experimental Groups

| | Group I | Group II | Group III | Group IV |
|---|---|---|---|---|
| 45S5 bioglass application | – | – | + | + |
| $CO_2$ laser application | – | + | – | + |

(–) Not applied, (+) Applied

Committee. We sectioned the teeth to obtain their buccal surfaces, which were ground flat to expose dentin at the cervical region (Paes Leme *et al*., 2004). All the dentin surfaces were utlrasonicated in de-ionized water for 30 sec, then etched in 0.5 M EDTA (pH 7.4) for 2 min and rinsed with water spray for 30 sec (Paes Leme *et al*., 2004).

The teeth were divided into 4 groups (Table 1): Group I, no bioglass was applied and no laser irradiation was done; Group II, no bioglass was applied, but the dentin surface was irradiated with a $CO_2$ laser; Group III, bioglass was applied, but there was no $CO_2$ laser irradiation; and Group IV, bioglass was applied followed by $CO_2$ laser irradiation. All specimens were stored in de-ionized water for 24 hrs after the experimental procedures.

## 45S5 Bioglass Application

One-tenth of a gram of 45S5 bioglass powder (NovaMin®, 5 μm average particle, NovaMin Technology, Alachua, FL, USA), composed of 24.5 wt% $Na_2O$, 24.4 wt% CaO, 6 wt% $P_2O_5$, and 45 wt% $SiO_2$, was mixed on a glass slab for 1 min by spatula with 0.2 mL of 50 wt% phosphoric acid that was prepared by the dilution of 85 wt% phosphoric acid (Wako Chemicals, Osaka, Japan) in distilled water to form a gel (pH 2). The acidic gel was immediately applied to the dentin surfaces of group III and IV specimens by microbrush (Microbrush International, Grafton, WI, USA). A layer of bonding agent (Clearfil SE Bond, Kuraray-Medical, Tokyo, Japan) was immediately applied over the 45S5 bioglass-phosphoric-acid gel and then light-cured (Table 2).

After storage in de-ionized water for 24 hrs, the thin layer of the bonding agent was gently removed by means of an excavator, then rinsed with water spray for 30 sec.

## $CO_2$ Laser Application

We used a pulsed $CO_2$ laser system (Lasery 15 Z-Niic, Tokyo, Japan), with a wavelength of 10.6 μm, and having a non-contact handpiece (F50, Niic), to irradiate the dentin surfaces of group II specimens and the newly formed layer of group IV specimens. The output energy used was 0.5 W, with pulse width of 0.12 msec and pulse rate of 100 Hz. The distance between the target area and the laser beam source was 50 mm, and the diameter of the $CO_2$ laser beam at this distance was 5.3 mm. The time for laser irradiation/specimen was 1 min, and a sweeping motion was used to ensure irradiation of all treated surfaces; thus, the calculated energy density according to the aforementioned parameters was 136 J/cm$^2$.

## SEM Examination for Tubule Orifice Closure

Five specimens from each group were dehydrated in ascending concentrations of ethanol, then gold-coated. The specimens' surfaces were evaluated by SEM (SEM JSM-5310LV, JEOL, Tokyo, Japan).

## XRD Examination of the Dentin Surfaces

Five specimens from each group were examined by x-ray diffractometry (RAD IIA, Rigaku Denki, Tokyo, Japan) with CuKα radiation of 40 KV and Ni filter. The diffraction intensities were measured 4 times for each specimen by a scanning technique in the range of $2\theta = 10°$- $60°$ by a 0.1° step for 2 sec/point.

## FE-SEM/EDS Interface Examination

Five specimens from each group were conditioned for 20 sec by the self-etching primer of Clearfil SE-Bond, bonded according to manufacturer's instructions, and then light-cured. Building up of light-curing resin composite (Clearfil AP-X, Kuraray-Medical) was carried out. The specimens were stored in de-ionized water for 24 hrs, then sectioned perpendicular to the interface to give 1.5-mm-thick slabs. The cut surfaces were polished, etched (Bakry *et al*., 2007), gold-coated, and then examined by FE-SEM/EDS (S-4500, Hitachi, Hitachinaka, Japan). Line scans were done across the treated dentinal surfaces with an EDS attachment for

**Table 2.** Materials Used in This Study

| Materials | | Composition | Procedures |
|---|---|---|---|
| 45S5 Bioglass (NovaMin® Technology, USA) | | $SiO_2$ (45 wt%), $Na_2O$ (24.5 wt%), CaO (24.4 wt%), $P_2O_5$ (6 wt%) | Mix 0.1 g of 45S5 bioglass with 0.2 mL of phosphoric acid |
| Clearfil SE Bond (Kuraray, Osaka, Japan) | Primer: | MDP,HEMA, Water, PI, accelerators, CA. | Apply self-etching primer (20 sec) |
| | Bond: | MDP, HEMA, MFM, PI, accelerators, CA. microfiller | Apply adhesive, gently air dry, light cure (10 sec) |
| Clearfil AP-X (Kuraray, Osaka, Japan) | | Bis-GMA, TEG-DMA barium glass filler (85 wt%), PI, accelerators | Apply and light cure for (40 sec) |

Abbreviations: HEMA = 2-Hydroxyethyl methacrylate; bis-GMA = bisphenyl glycidyl methacrylate; MDP = 10-methacryloxydecyl dihydrogen phosphate; TEG-DMA = Triethylene glycol dimethacrylate; MFM = Multifunctional methacrylate; PI = Photoinitiator; CA = Catalyst.

the following elements: phosphate, calcium, and silicon.

### Nanoindentation Test

Four specimens were selected from each group. The specimens were treated with bonding system (Clearfil SE-Bond, Kuraray-Medical), and then built up with light-curing resin composite (Clearfil AP-X, Kuraray-Medical). The hardness and the modulus of elasticity for the "interaction layer" in addition to dentin with/without laser irradiation were measured with a nanoindentation device (ENT-1100a, Elionix, Tokyo, Japan). A minimum of 10 indentations was performed across the polished cross-section (Paital *et al*., 2009) of the interaction layer in groups III and IV, and on the superficial 10 μm of the cross-section of dentin with/without $CO_2$ irradiation in groups I and II. The maximum load used was 5 mN, with a loading rate of 0.5 mN/sec. The maximum load was held constant for 1 sec for each indentation.

### Statistical Analysis

Student's *t* test was used to compare hardness and modulus of elasticity for the dentin with/without laser irradiation, and the interaction layer with/without laser irradiation ($p < 0.05$).

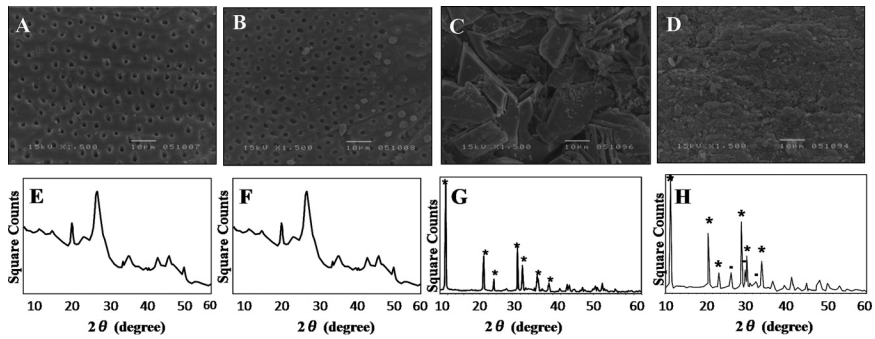


**Figure 1.** FE-SEM top surface examination and XRD analysis. **(A)** Top surface view for group I (no bioglass, no laser treatment) specimens, showing patent dentinal tubules with complete absence of any smear layer. **(B)** Top surface view of dentin of group II (no bioglass, but laser treatment), showing obliteration of some dentinal tubule orifices; however, no cracking was observed. **(C)** Top surface view of dentin of group III (bioglass application, but no laser irradiation), with complete coverage of the whole dentin surface with large crystalline structures. **(D)** Top surface view of dentin of group IV (bioglass and laser application), with complete coverage of the whole dentin surface with a compact layer of smaller crystalline structures. **(E,F)** XRD diffraction of the top dentin surfaces for groups I and II, showing normal hydroxyapatite crystal diffraction patterns. **(G)** XRD diffraction of the top dentin surface for group III, showing brushite crystal diffraction patterns; peaks are demarcated with (*). **(H)** XRD diffraction of the top dentin surface for group IV, showing brushite crystal diffraction patterns demarcated with (*) and monetite crystals diffraction patterns demarcated with (■).

## RESULTS

### SEM Examination for Dentinal Orifice Closure

Group I specimens (control) that did not receive bioglass or laser treatment (Fig. 1A) showed patent dentinal tubules with the absence of a smear layer due to the etching effect of EDTA. Specimens of group II (Fig. 1B) that had received laser treatment but no bioglass showed occlusion of a few dentinal tubules; however, most of the dentinal surfaces showed micro- morphological features similar to those of the control group.

The interaction layer covered 100% of the dentin surfaces of all of the bioglass-treated dentinal surfaces in groups III and IV. The interaction layer formed on dentinal surfaces of group III specimens showed that this layer is composed of flat crystal plates as large as 10 x 50 μm. However, the layer formed on top of the dentinal surfaces of laser-treated group IV seemed to be more compact, in contrast to the layer formed on specimens of group III, which did not receive laser treatment (Figs. 1C, 1D) and exhibited smaller crystals.

### XRD Examination of Dentinal Surfaces

The crystalline structure of dentin surfaces in groups I and II showed the x-ray diffraction pattern typical of hydroxyapatite crystals, confirming the minimal effect of the $CO_2$ laser on the dentin when operated with the parameters used in this study (Figs. 1E, 1F).

The crystalline structure of the interaction layer formed in group III specimens (bioglass only) showed the formation of ($CaHPO_4$. $2H_2O$) brushite crystals (Fig. 1G).

The crystalline structure of the interaction layer formed in group IV (bioglass and $CO_2$ laser) was assigned as brushite ($CaHPO_4$ .$2H_2O$) and monetite ($CaHPO_4$) crystals (Fig. 1H).

### FE-SEM/EDS Interface Examination

Specimens in groups I and II (Figs. 2A, 2B) showed good adaptation between dentin and the bonding agent. Specimens of group II (laser treatment but no bioglass) showed no cracks in dentin. EDS line-scan analysis in groups I and II showed a drop in the calcium and phosphate contents as line scans crossed from the dentin to the bonding agent. Specimens of group III (bioglass but no laser) showed a precipitated layer extending over the whole surface of the dentin which penetrated about 4 μm into the dentinal tubules (Fig. 2C). Specimens of group IV (bioglass with laser treatment) showed a compact layer (Fig. 2D) formed on the dentinal surface, having a thickness of approximately 5 μm, covering the dentin and penetrating about 3 μm into the dentinal tubules. EDS line-scan analysis for groups III and IV showed that the interaction layer covering the dentinal surfaces was a calcium-phosphate-rich layer with trace amounts of silica.

### Nanoindentation Examination

Student's *t* test showed that treatment of the interaction layer with the $CO_2$ laser significantly increased the hardness and the modulus of elasticity for that layer ($p < 0.05$), while it did not significantly affect the dentin hardness or its modulus of elasticity ($p > 0.05$). The means and standard deviations of hardness and modulus of elasticity for all groups are presented in Fig. 2E.

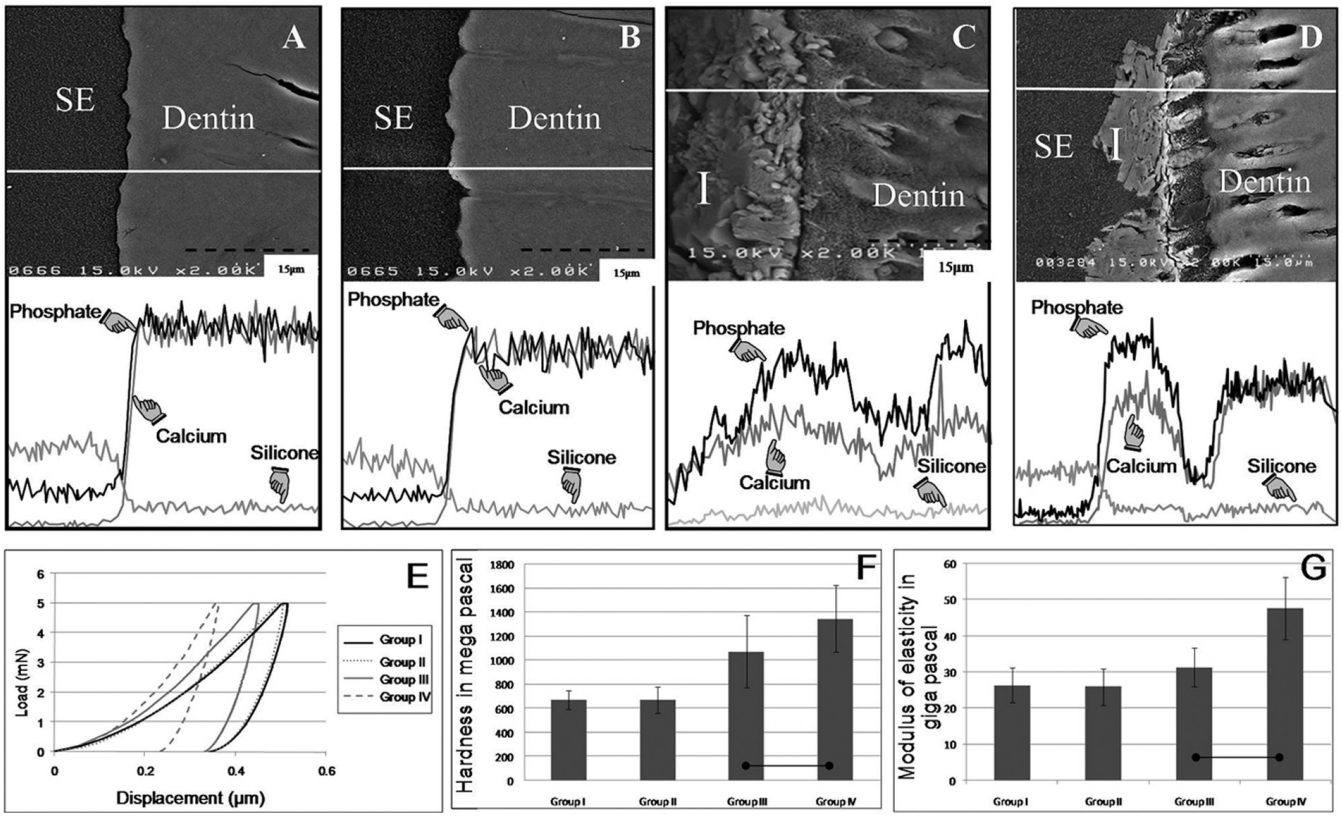


**Figure 2.** FE-SEM/EDS interface examination and nanoindentation results. **(A,B)** Interface between dentin (Dentin) and bonding agent (SE). EDS analysis for A and B shows the increase in calcium and phosphate peaks and the decrease in silicon peak as the line scans cross the interface. **(C,D)** Complete coverage of the whole dentin surface (Dentin) by a calcium-phosphate-rich "interaction layer" (I) that penetrated into the dentinal tubules. **(E)** Characteristic loading and unloading curves for the nanohardness of all groups. **(F)** The hardness results for all groups. Connected bars are statistically significant ($p < 0.05$). **(G)** Results of the modulus of elasticity for all groups. Connected bars are statistically significant ($p < 0.05$).

## DISCUSSION

The results showed that the application of 45S5 bioglass can create particles that occlude and penetrate the dentinal tubule orifices, suggesting the use of this technique to diminish the dentinal tubule fluid movement responsible for pulpal nerve irritation. These calcified plugs are not remnants of the smear layer, because, prior to 45S5 bioglass application, all of the specimens' dentinal surfaces were ultrasonicated and acid-conditioned to simulate the micro-morphological features of hypersensitive dentin clinical cases (Paes Leme *et al*., 2004).

The current technique provided a way for applying 45S5 to be in contact with hypersensitive dentin surfaces for 24 hrs to complete bioactive reactions that have been recorded as taking at least 2 hrs (Hench, 1991).

It is suggested that the mechanism of calcium-phosphate crystal formation on dentinal surfaces by the current technique is as follows: 45S5 bioglass powder, upon being mixed with the aqueous solution of 50% phosphoric acid, will leach out calcium, phosphate, and sodium crystals into the aqueous acidic media (Bunker *et al*., 1988). Simultaneously, the acidic gel may mobilize calcium and phosphate ions from the underlying dentin. The phosphate ions released from 45S5 bioglass and those abundant in the phosphoric acid solution will react with the calcium ions from bioglass and dentin to form acidic calcium-phosphate salts (*i.e*., brushite). These inorganic salts will precipitate on top of the dentinal surface, with smaller crystals penetrating the dentinal tubules. The silica network of the 45S5 bioglass is thought to react with hydroxyl ions released from aqueous storage media to form silanol compounds (Beletskii and Sventskaya, 2009), which are soluble in water. It is speculated that the silica network formed silanol compounds upon being exposed to water, which was easily washed out upon application of an air-water jet.

This explains why only trace amounts of silica were detected upon elemental analysis of the interaction layer by FE-SEM/EDS.

XRD analysis of the calcium-phosphate salts in group III showed the formation of brushite crystals ($CaHPO_4.2H_2O$), due to the low pH of 45S5 bioglass-phosphoric-acid gel. The pH of the gel directly after being mixed was measured in a pilot study to be 2.0; this low pH is suitable for the rapid formation of brushite crystals (Kani *et al*., 1983; Abraham *et al*., 2005).

Water-free monetite crystals ($CaHPO_4$) appeared with brushite crystals in group IV specimens, which may be attributed to irradiation of group IV samples by the $CO_2$ laser. Brushite crystals lose water abundant between its crystals at temperatures > 50°C, to form anhydride monetite ($CaHPO_4$) (Klammert *et al*., 2009). Apparently, $CO_2$ laser irradiation may have raised the temperature

of the interaction layer of group IV specimens above 50°C, allowing for partial conversion of brushite crystals into monetite crystals. The $CO_2$ laser parameters used in this study were reported to be safely used on dentin *in vivo* (Moritz *et al*., 1998), and thus the hypothesis in this study was accepted.

Moreover, this temperature increase might have caused some water to evaporate from the interaction layer, causing some improvement in its hardness and modulus of elasticity. This was evident in the results of a nanoindentation experiment which showed that group IV specimens recorded higher mechanical properties when compared with group III. Moreover, it was recently reported that the interaction layer was able to resist abrasion challenge (Bakry *et al*., 2009).

The observation of less stable forms of the calcium-phosphate crystals, *i.e*., brushite and monetite, and the absence of hydroxyapatite crystals, which are considered a more stable form of calcium-phosphate crystal, may be attributed to absence of any storage period for the specimens after removal of the residues of 45S5 bioglass. We speculate that a storage medium rich in calcium and fluoride, instead of the water storage used in this study, would have helped in the transformation of brushite crystals into hydroxyapatite crystals (Kani *et al*., 1983; Monma and Kamiya, 1987; Kumar *et al*., 1999; Abraham *et al*., 2005; Štulajterová and Medvecký, 2008).

The application of white 45S5 bioglass gel by the current technique simulates the application of temporary filling materials in conservative dentistry procedures. However, further studies are needed to determine the acceptance of this technique by patients. Moreover, studies are needed to examine the hydraulic conductance of dentin after bioglass application.

Application of 45S5 bioglass to dentin was able to occlude patent dentinal tubule orifices with a layer of calcium phosphate crystals to create what we call an 'interaction layer'. The application of a $CO_2$ laser to the interaction layer modified the crystals by removing the hydration water and improved its quality.

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