Effect of Calcium Phosphate Glass on Dentinal Tubule Sealing after Irradiation with the Carbon Dioxide Laser

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Abstract: The aim of this study was to evaluate the dentinal tubule sealing and acid resistance of dentin specimens following the application of calcium phosphate glass powder prior to irradiation with a CO2 (carbon dioxide) laser. Dentin models simulating open dentinal tubules were divided into two groups: experimental (calcium phosphate glass slurry applied to the dentin surface) and control (no slurry applied to the surface). All specimens in the experimental group and five specimens in the control group were irradiated with a CO2 laser. The defocused laser beams (0.5 and 1 W) were applied (spot size, 5 mm in diameter) from a distance of 20 mm for 10 s. The surfaces and cross-sectional areas of the specimens were examined using an SEM (scanning electron microscope). In addition, the resistance to acid was evaluated in these specimens. The open dentinal tubules in the control groups were sealed following irradiation with the CO2 laser at 0.5 W and 1.0 W. Likewise, sealing of open dentinal tubules was observed in the experimental group after CO2 laser irradiation. The acid resistance of the dentin surface was improved after CO2 laser irradiation; specimens in the experimental group presented with significantly lower amounts of Ca ion release compared to those in the control group. These findings indicate that CO2 laser irradiation alone or after the application of calcium phosphate glass powder can effectively seal the dentinal tubules and alleviate dentin hypersensitivity.

Key words: CO2 laser, dentinal tubules, calcium phosphate glass.

1. Introduction

The application of lasers in various fields has gained popularity following the successful oscillation of a ruby laser by Theodore Maiman in 1960 [1]. Goldman et al. [2] were the first to apply this technology in the dental field and report that laser irradiation can remove carious lesions from the teeth. Since then, the effects of different lasers on hard tissues have been reported [3-5]. Recently, the preventive effects of laser treatment on dental caries have gained attention in the field of clinical dentistry. After Sognnaes and Stern [6] demonstrated an increase in the acid resistance of the enamel following ruby laser irradiation, Stern et al. [7] and Yamamoto and Ohyla [8] reported similar effects using CO2 (carbon dioxide) and YAG lasers, respectively. Subsequently, Winkler [9] reported that the laser can be focused on the margin between the restoration and the enamel to improve the marginal sealing and prevent secondary caries. Furthermore, Stewart et al. [10] reported the fusion of the powdered hydroxyapatite to the enamel when a CO2 laser beam was applied to the pits and fissures on the occlusal surface of an extracted tooth. It was suggested that the improvement in acid resistance after laser irradiation was due to the surface smoothening effect, which resulted in a reduction in the tooth surface area and slowing down of the
dissolution rate. Moreover, substantial improvement in the acid resistance of the tooth structures could not be achieved by the fusion of the apatite powder only.

Thus, considering the possibility of enhancing the acid resistance of the tooth structure by laser irradiation, we demonstrated the fusion of calcium phosphate glass to the enamel surface using a CO$_2$ laser in our previous studies [11-14]. This method enables the use of an active approach for the treatment of incipient carious lesions, such as pit and fissure sealants, in accordance with the recent minimal intervention concept in restorative dentistry. Furthermore, since bonding agents are not used in this method, it is possible to prevent microleakage and the subsequent development of secondary caries. In addition, it could be a viable option for treating dentin hypersensitivity, which is common in the current super-aging society.

A recent study reported the alleviation of hypersensitivity via laser irradiation in clinical dentistry [15]; however, evidence in support of this technique has not been adequately presented. In the case of the CO$_2$ laser, a char layer is formed after irradiation, which causes a dark discoloration and compromises the esthetics. Therefore, it is mainly used for the irradiation of gingival tissues or the mucosa. Following the elimination of this issue using the high-density energy of the CO$_2$ laser, it was possible to fuse the exposed dentin surfaces and seal the opened dentinal tubules.

Improvements in the acid resistance of both the dentin and cementum leading to the prevention and treatment of root surface caries are of importance, particularly, due to the super-aging society in Japan. Additional studies evaluating the potential for enhancement of the acid resistance of the tooth structures using laser irradiation are merited.

In the present study, dentin models simulating open dentinal tubules were fabricated to investigate the morphological changes in the tubules after CO$_2$ laser irradiation; additionally, the sealing of the tubules and the acid resistance of the dentin surface were examined after the application of calcium phosphate glass powder prior to the laser irradiation.

2. Materials and Methods

2.1 Materials

Dentin was obtained from the roots of cryopreserved bovine mandibular anterior teeth following approval from the animal experimentation committee of the Kanagawa Dental College (19-0002). An ingot of calcium phosphate glass (Asahi Glass Co., Tokyo, Japan) was ground to 400-mesh pass with an automatic mortar (Type 03501; Ito Products, Tokyo, Japan). Subsequently, 10 mg of the powder was mixed with 200 µL distilled water for the experiments.

2.2 Methods

2.2.1 Specimen Fabrication

The pulp and soft tissues attached to the root surface and cementum were removed using a periodontal curette to expose the dentin. Donut-shaped specimens were fabricated by sectioning the root immediately below the cervical line until 5 mm apical to the first section. The specimens were sagitally sectioned using a wire saw and polished using a 2,000 grit waterproof sandpaper to obtain a flat outer surface. Following the standard method for sample fabrication based on previous dentin hypersensitivity experiments, the specimens were placed in 10% phosphoric acid for 10 s and rinsed with water. Thereafter, they were mechanically polished for 1 min at a speed of 1,000 rpm using a gel containing 30% crushed apatite (average size, 100-200 µm in diameter), 49% propylene glycol, and 21% polyvinyl pyrrolidone. Immediately after polishing, the specimens were cleaned with deionized water in an ultrasonic bath for 30 min and washed with deionized water in a stirrer for another 24 h to remove the smear layer and smear plugs for the fabrication of specimens containing open dentinal tubules.
2.2.2 Treatment with Calcium Phosphate Glass

Crushed calcium phosphate glass powder (1 mg) was added to a slurry of distilled water (50 mL) and applied to half of the specimens (experimental group) with open dentinal tubules using a small brush. The remaining half of the specimens without calcium phosphate glass powder formed the control group.

2.2.3 Laser Irradiation

After drying the specimens, the head of a CO₂ laser (Opelaser Pro, Yoshida Dental Mfg Co., Tokyo, Japan) was placed perpendicular to the surfaces. The oscillation mode was set as continuous irradiation and the output power was changed to 0.5 W and 1.0 W. The probe was defocused and the laser beam was applied (irradiation distance, 20 mm; spot size, 5 mm in diameter); the mounted specimens were moved randomly. The group without the laser irradiation was considered as the control and each group comprised five samples.

2.2.4 Observation

After the laser-irradiated specimens were coated with a gold film (100 Å) using the Quick Auto Coater (SC-701 AT, Sanyu Electron, Tokyo, Japan), the surfaces were observed using an SEM (scanning electron microscope, Superscan SS-550, Shimadzu, Kyoto, Japan) at a power of 5 kV. Additionally, the surfaces of the sectioned specimens were observed in the same manner.

2.2.5 Acid Resistance Testing

Nail varnish was applied to the irradiated surface of each specimen in the experimental and control groups, excluding the inside of a circle 3 mm in diameter. A fish line was attached with varnish to the other side of the specimen within a defined circle. After drying, each specimen was hung in a Styrofoam sample bottle containing 0.1 mol/L acetic acid-sodium acetate buffer solution (10 mL; pH 4.0) with the aid of the fish line. The specimens were immersed in the solution for 30 min using a rotating magnetic stirrer (200 rpm). After 30 min, 1 mL of 1% KNO₃ solution was added to the buffer solution; a flame analysis was conducted using the Atomic Absorption Flame Photometer (AA-610, Shimadzu Co., Kyoto, Japan) at an analytical wavelength of 422.6 nm to measure the amount of Ca released from the dentin surface into the solution. The acid resistance of a calcium phosphate glass ingot was tested in the same manner. The sample size in each group was 5.

2.2.6 Statistical Analysis

After the mean and standard deviations were obtained, the values were statistically analyzed using one-way analysis of variance and Fisher’s least significant difference test. A p-value of < 0.05 was considered significant.

3. Results

3.1 Surface Observation

Fig. 1 shows the SEM images of the specimens in the control group (without glass powder). Open dentinal tubules were observed in specimens that were not irradiated with the CO₂ laser, whereas the irradiated specimens presented with sealed dentin tubules. The surface and intertubular dentin were fused in the specimens that were irradiated with an output power of 0.5 W; in addition, smooth surfaces and sealed dentinal tubules were observed in these specimens. On the other hand, the location of each dentin tubule was clear in the 1.0 W group, and each tubule was sealed.

SEM images of the specimens irradiated by the CO₂ laser after the application of the glass powder slurry (experimental group) are presented in Fig. 2. Sealed dentinal tubules were observed in the specimens that were irradiated at a power of 0.5 W. Likewise, the dentinal tubules were sealed in the specimens irradiated at 1.0 W; however, they demonstrated a thicker sealing layer compared to those in the 0.5 W group.

3.2 Acid Resistance

The concentrations of Ca released after acid resistance testing in each specimen in the experimental and
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Fig. 1  SEM images of the dentin surface in the control group before and after CO₂ laser irradiation. Open dentinal tubes were observed in the non-irradiated control specimens (surface and cross-sectional view). The dentin surfaces of the specimens irradiated at 0.5 W or 1.0 W presented with closed dentinal tubes (arrow). Scale bar: 5 μm.

Fig. 2  SEM images of the dentin surface irradiated with a CO₂ laser after the application of the ceramic powder (experimental group). A coating on the surfaces along with the presence of sealed dentinal tubes in the specimens irradiated with 0.5 W and 1.0 W after the application of glass powder (arrows). Scale bar: 10 μm.
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4. Discussion

According to previous studies, including the one on hydrodynamic theory reported by Pashley et al. [16], the main cause of dentinal hypersensitivity is the opening and exposure of the dentinal tubules. Therefore, a desensitizing agent or a restorative material is commonly used to seal the tubules [17-19].

Among the various dental devices developed in recent years, the laser is most widely used in clinical dentistry. The opened dentinal tubules are sealed when the surface of the dentin is irradiated with the unique high energy density of the laser, resulting in the alleviation of the symptoms of dentin hypersensitivity without the use of any medicaments.

Based on this phenomenon, we fabricated dentin models simulating open dentinal tubules and causing dentin hypersensitivity, and investigated the morphological changes in the tubules after CO₂ laser irradiation. Additionally, the sealing of the tubules, as well as the alterations in the acid resistance of the dentin surface after the application of calcium phosphate glass powder prior to laser irradiation, was evaluated.

The dentinal tubules remained open in the non-irradiated specimens in the control group indicating the adequacy of our dentin hypersensitivity model. Furthermore, the tubules were sealed after continuous laser irradiation at 0.5 W. Likewise, the open dentinal tubules were sealed in the group that received continuous laser irradiation at 1.0 W; however, the surface of the dentin in this group was...
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less smooth compared to that in the 0.5 W irradiation group suggesting that excess irradiation can destroy the normal structure of the dentin surface.

Studies using a CO₂ laser to remove carious dentin reported that the dentin immediately below the laser-treated area was visibly hardened compared to other areas, signifying an improvement in the acid resistance of the surface [20-22]. Gomi et al. [23] reported that the surface dentin had evaporated after CO₂ laser irradiation, and the dentin immediately below the irradiated area had melted and exposed its carbonized organic contents. Additionally, they found inorganic substances that bound and recrystallized after deeply penetrating the dentinal tubules resulting in the closure of the tubules.

In accordance with these previous findings, the results of the present study showed improvements in the acid resistance of the dentin after laser irradiation when compared to the non-irradiated specimens. Several studies have reported similar findings using the CO₂ laser [24-27] and other lasers [28-31]; nevertheless, the application of these lasers in a clinical setting is a matter of concern due to the high irradiation power. In this study, the sealing of the open dentinal tubules was achieved by the application of a defocused laser beam at 0.5 W and an irradiation distance of 20 mm for 10 s. Thus, this method may aid in reducing pulpal irritation when applied to the tooth.

Adequate sealing of the dentinal tubules was observed in specimens that were irradiated with the CO₂ laser after the application of calcium phosphate glass powder. The composition of calcium phosphate glass is similar to that of natural enamel (32% CaO, 11% SrO, 4% Al₂O₃, 52% P₂O₅, and 1% CeO₃). Previous studies have reported the fusion of the enamel with the calcium phosphate glass creating an indistinct margin in a gradient manner when the enamel was used as an adherend [11-14]. Therefore, calcium phosphate glass is considered to possess the ability to merge with the highly inorganic contents of the enamel.

In the present study, the decrease in Ca ion release in the CO₂ irradiated specimens belonging to the experimental group may be attributed to the fact that a part of the dentin was enameled. Although the thickness of the sealed layer in the dentinal tubules varied depending upon the output power used, no significant difference in acid resistance was observed indicating the clinical effectiveness of the irradiation at 0.5 W. The actual amount of Ca ions released from the sealed dentinal tubules would be lower if the amount released from the calcium phosphate glass ingot was deducted from the total value obtained in the acid resistance test, indicating an improvement in the acid resistance of the surface.

The findings of this study indicate that CO₂ laser irradiation on the dentin surface is effective for the sealing of open dentinal tubules. Moreover, pretreatment with calcium phosphate glass powder prior to laser irradiation improves the sealing of the tubules thereby alleviating dentin hypersensitivity. In the clinical setting, the laser irradiation area (0.5 mm in diameter) should be widened to some extent to achieve uniform sealing of the open dentinal tubules across the extensive surface of the dentin.

5. Conclusion

In the present study, dentin models simulating open dentinal tubules were fabricated to investigate the morphological changes in the tubules after CO₂ laser irradiation. The sealing of the tubules, as well as changes in the acid resistance of the dentin surface, was evaluated after the application of calcium phosphate glass powder to the surface before laser irradiation. The following conclusions were reached:

(1) Open dentinal tubules in the dentin models were sealed following the application of a CO₂ laser at 0.5 W and 1.0 W.

(2) Similarly, sealing of open dentinal tubules was observed in specimens treated with calcium phosphate glass powder prior to CO₂ laser irradiation.

(3) The acid resistance of the dentin surface was
improved after CO₂ laser irradiation. The specimens in the group treated with calcium phosphate glass presented with considerably lower amounts of Ca ion release compared to those in the control group.

These findings indicate that CO₂ laser irradiation alone or after the application of calcium phosphate glass powder can effectively seal the dentinal tubules and alleviate dentin hypersensitivity.

**Conflicts of Interest**

The authors declare that there is no conflict of interest in the present study.

**Acknowledgment**

This study was supported by the Japan Society for the Promotion of Science, Grants-in-Aid for Scientific Research Fundamental Research (C)19K10161.

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