Original Article

Efficacy of Using Erbium, Chromium-Doped:Yttrium Scandium Gallium Garnet Laser-Treated Dentine in a Dentine Barrier Test Device

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ABSTRACT

Objectives: The aim of this study was to evaluate the efficacy of using erbium, chromium-doped:yttrium scandium gallium garnet (Er,Cr:YSGG) laser-treated dentine in a dentine barrier test device. **Materials and Methods:** The test materials (G-Bond™ and Vitrebond™) were applied onto laser-treated or laser-untreated dentine discs. After 24 h of exposure with perfusion of the test chamber, cell survival was evaluated based on enzyme activity and compared to a nontoxic control material. The mean of the control was set to 100% viability. Data were analyzed using the one-way analysis of variance and the Tukey's honest significant difference tests. **Results:** The responses of bovine pulp-derived cells after exposure to G-Bond and Vitrebond on Er,Cr:YSGG laser-treated and laser-untreated dentin were statistically different from negative control group ($P < 0.05$). **Conclusion:** Er,Cr:YSGG laser treatment was not successful enough in decreasing the cytotoxic effects of the dental materials. Different parameters of Er,Cr:YSGG laser or different laser types could be investigated as an alternative to minimizing the cytotoxic effects of dental materials.

KEYWORDS: *Cytotoxicity, dentine barrier, erbium, chromium-doped:yttrium scandium gallium garnet laser*

INTRODUCTION

The physical and biological properties of dental filling materials in contact with dentine may be modified by the nature of dentine. Dentine permeability is of particular interest due to the toxicity of some dental materials.[1,2] Permeability measurements have shown dentine to be a diffusion and adsorption barrier, thereby reducing the concentration of eluted substances that reach the pulp and the possibility of reacting with tissue.[3,4] Sclerotic dentine containing a hypermineralized surface is even less permeable due to partial or total occlusion of tubules.[5] Conversely, the protective effect of dentine seems to be limited, as small hydrophilic molecules have been shown to diffuse through sclerotic dentine.[6]

In most experiments, lasers, including helium–neon, neodymium-doped:yttrium aluminum garnet (Nd:yAG), erbium-doped:yAG (Er:YAG), and carbon dioxide (CO_2), have shown desensitizing effects.[7-9] An erbium, chromium-doped:yttrium scandium gallium garnet (Er,Cr:YSGG) laser is expected to be effective for dental applications.[10-12] Laser desensitization has been introduced as an effective tool to rapidly eliminate or reduce dentine hypersensitivity. Numerous studies have reported relative success, despite variations in the methods and type of laser.[13,14] The mechanism of laser irradiation in dentine is the occlusion, by partial melting, of exposed dentine tubules after low-intensity irradiation.[15]

Based on these findings, Er,Cr:YSGG laser treatment of dentine may decrease the diffusion of toxic substances released from dental materials through dentine to the pulp by partially or even totally occluding the tubules. The objectives of this study were to assess the efficacy of an Er,Cr:YSGG laser to seal dentinal tubule orifices

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in vitro and to determine the cytotoxicity of dental materials evaluated in a dentine barrier test device using Er,Cr:YSGG laser-treated dentine. The null hypothesis of this study was that Er,Cr:YSGG laser treatment of dentine would not affect the toxicity of dental materials in a dentine barrier test device.

MATERIALS AND METHODS

Preparation of dentine discs

Dentine discs of 500 ± 20 µm thick thickness were cut from the first incisors of freshly slaughtered bovines (2–4 years of age) with a low-speed wheel saw (Isomet, Buehler, Lake Bluff, IL, USA) under constant water flow. The smear layer on the pulpal side of the dentine discs was removed by applying 50% citric acid for 30 s. The dentine slices were rinsed with physiological saline and sterilized by autoclaving (121°C for 25 min).

Laser apparatus

A commercially available pulsed Er,Cr:YSGG laser (Waterlase MD, Biolase Technology, San Clemente, CA, USA) was used in this study. Dentine discs were irradiated at 2780 nm with Er,Cr:YSGG laser in the hard tissue mode with the MZ6 sapphire tip (600 mm diameter, 6 mm length) using noncontact mode at an energy level of 0.25 W, repetition rate of 20 pulses/s and pulse duration of 140 ms, 0% water, and 10% air. To simulate the clinical conditions, the dentine specimens were manually irradiated in scanning movement perpendicular to the surface approximately 4 mm away from the surface (30 s/cm²).

Dentine barrier test

The dentine contacting materials, their composition, their batch numbers, and their manufacturers are described in Table 1. The cytotoxicity of the one dentine bonding agent and one resin-modified glass ionomer cement was evaluated in a dentine barrier test device using the three-dimensional cell culture of bovine dental pulp-derived cells. Clonal SV40 large T-antigen-transfected cells,[16] derived from bovine dental papilla, were maintained in growth medium (MEMα, Gibco Invitrogen Paisley, UK), supplemented with 20% fetal bovine serum (Biological Industries, Beit Haemek, Israel), 150 IU/ml penicillin, 150 mg/ml streptomycin (Biological Industries, Beit Haemek, Israel), and 0.1 mg/ml geneticin (Gibco Invitrogen Paisley, UK), in a humidified atmosphere at 37°C in 5% CO_2. Three-dimensional cultures of bovine dental pulp-derived cells were prepared on meshes. Polyamide meshes (0.5 cm²; Reichelt Chemietechnik, Heidelberg, Germany) were immersed in 0.1 M acetic acid for 30 min, washed three times with phosphate-buffered saline, and air-dried. Next, meshes were coated with fibronectin (0.03 mg/ml; Sigma, Deisenhofen, Germany) and air-dried. Cell culture inserts (Millipore, Eschborn, Germany) were placed into six-well plates with 1.25 ml of growth medium per well. The meshes were placed on the inserts and 20 µl of cell suspension (8×10^4 cells/ml) were seeded on them. After 48-h incubation (37°C, 5% CO_2, 100% humidity), meshes were transferred to 24 well plates and incubated until they were used for cytotoxicity experiments (14 ± 2 days). The culture medium (growth medium supplemented with 0.05 mg/ml ascorbic acid) was changed three times a week.

A commercially available cell culture perfusion chamber (Minucells and Minutissue GmbH, Bad Abbach, Germany) made of polycarbonate with a base of 40 mm × 40 mm and a height of 36 mm was modified. The three-dimensional cultures were placed on Er,Cr:YSGG laser treated discs and normal dentine discs were held in place by a special biocompatible stainless-steel holder, resulting in a dentine barrier test situation. Thus, the cell culture chamber was separated into two compartments by the dentine disc. The cell culture tissues were placed in direct contact with the etched side of the dentine disc and held in place by the stainless-steel holder. All chambers were perfused with 0.3 ml assay medium (growth medium with 5.96 g/L HEPES buffer, Merck, Germany) per hour for 24 h at 37°C.

Perfusion was switched off; test materials were introduced into the upper compartment in direct contact with the "cavity" side of the dentine disc. G-Bond was applied to the prepared dentine surfaces using the disposable applicator and was left undisturbed for 5–10 s after the end of the application. Then, it was dried thoroughly for 5 s with oil-free air under maximum air pressure, and finally, light cured for 10 s using a visible light curing unit (Monitex, BlueLex GT1200). Vitrebond dispensed 1 level powder scoop and dispensed 1 drop liquid, then mixed within 10 s. Mixed Vitrebond was applied to the prepared dentine surfaces using the disposable injectors. A nontoxic polyvinyl siloxane impression material (President, Coltene AG, Alstatten, Switzerland) was used as a negative control (100% cell viability). Cytotoxicity of test materials was recorded after the pulpal part of the *in vitro* pulp chamber was perfused with cell culture medium (2 ml/h) for 24 h of incubation at 37°C. Each material was tested six times. Cell viability of three-dimensional cultures was determined by enzyme activity. The tissues were removed from the pulp chambers, placed into 24-well plates containing 1 ml of prewarmed 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution (0.5 mg/ml in growth medium) and

incubated for 2 h at 37°C. Then, the cells were washed twice with phosphate-buffered saline. The blue formazan precipitate was extracted from the mitochondria using 0.5 ml of dimethyl sulfoxide on a shaker at room temperature for 30 min. Of this solution, 200 µl was transferred to a 96-well plate and the absorption at 540 nm (µQuant, Bio-Tek Instruments, Inc., Winooski, VT, USA) was determined spectrophotometrically. The mean values of control tissues (cell cultures exposed to the polyvinyl siloxane impression material) were set to represent 100% viability.

Statistical analysis

Results of cytotoxicity experiments were expressed as a percentage of matching control tissue. Statistical analyses were performed with SPSS for Windows software (ver. 21.0; SPSS Inc., Chicago, IL, USA). The Shapiro–Wilk test was used to evaluate the homogeneity of variables. One-way analysis of variance and *post hoc* Tukey's tests were used to compare cell survival data. The significance level was set to $P < 0.05$.

Scanning electron microscopy analysis

For scanning electron microscopy (SEM) analysis, 500 ± 20 µm dentine discs were cut from first incisors of freshly slaughtered bovines. To remove the smear layer, each dentine discs was submerged into a 17% ethylenediaminetetraacetic acid solution (pH = 7.8) for 5 min, rinsed with distilled water, immersed into a 5.25% NaOCl solution for 5 min, and then stored in distilled water until use. Dentine discs were irradiated by Er,Cr:YSGG laser, as mentioned above. Untreated samples were used as control. Samples were gold sputtered and evaluated by SEM (Zeiss EVO Ls10).

RESULTS

Cytotoxicity testing

The cytotoxicity of dentine-contacting materials in a dentine barrier test device using Er,Cr:YSGG laser-treated dentine is summarized in Figure 1. A polyvinyl siloxane material (President) was used as the negative control. Vitrebond reduced cell survival by 24.04% in untreated dentine and 26.13% in Er,Cr:YSGG laser-treated dentine. Vitrebond was the most toxic material for both laser-treated and laser-untreated dentine. In addition, G-Bond reduced cell survival by 44.83% in untreated dentine and 51.63% in Er,Cr:YSGG laser-treated dentine which was considered to be toxic compared to the negative control ($P < 0.05$). The responses of bovine pulp-derived cells after exposure to G-Bond on Er,Cr:YSGG laser-treated dentine were statistically different from Vitrebond groups on untreated dentine and Er,Cr:YSGG laser-treated dentine ($P < 0.05$).
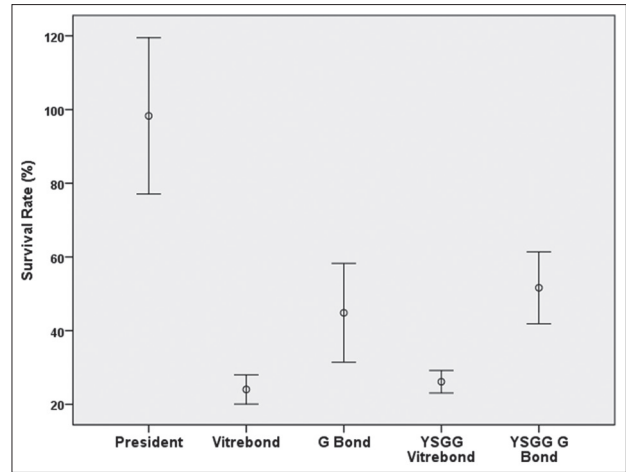

Figure 1: Cytotoxicity of dentin-contacting materials on three-dimensional cultures of SV40 large T-antigen-transfected bovine pulp-derived cells using Er,Cr:YSGG laser-treated dentin. Data are expressed as percentage of the negative-control cultures ($n = 6$). Er,Cr:YSGG: Erbium, chromium-doped: yttrium scandium gallium garnet
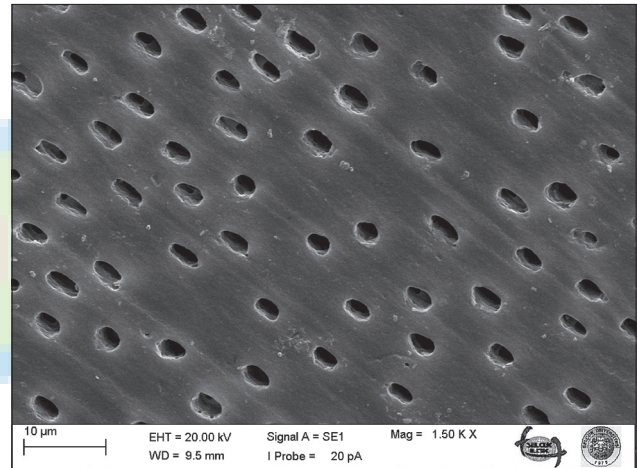

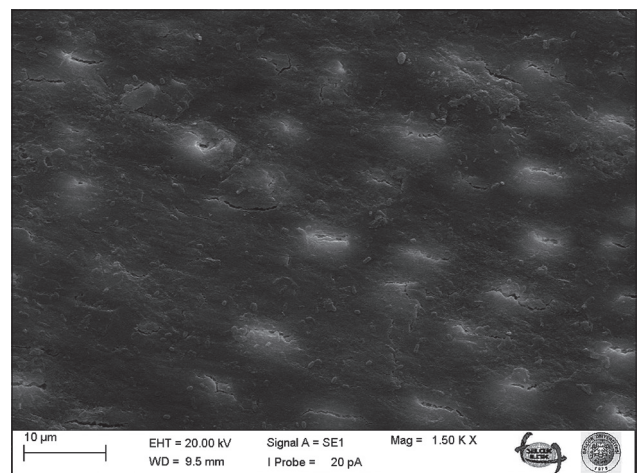**Figure 2:** Control group showing open dentinal tubules before irradiation of the laser therapy (×1500)

Figure 3: Er,Cr:YSGG laser-treated dentin, energy level of 0.25 W, repetition rate of 20 pulses/s, and pulse duration of 140 ms (×1500). Er,Cr:YSGG: Erbium, chromium-doped: yttrium scandium gallium garnet

Table 1: Composition and manufacturers of the tested materials

Material	Composition
Vitrebond	
3M ESPE Dental Products, St. Paul, MN, USA N246883	Powder: Glass powder, Diphenyliodonium Chloride (DPICI) Liquid: Copolymer of Acrylic and Itaconic Acids, HEMA (2-hydroxyethylmetacrylate), water
G Bond	
GC Corp., Tokyo, Japan 1011131	Acetone, Diurethane dimethacrylate, Triethylene glycol dimethacrylate (TEG-DMA), methacryloyloxydecyl dihydrogen phosphate (MDP), Diphenyl (2,4,6-trimethylbenzoyl) phosphine oxide

Scanning electron microscopy surface analysis

The most distinct images of the Er,Cr:YSGG laser-treated dentine and control group are shown in Figures 2 and 3. The control group presented a smooth appearance and open tubule orifices. Moreover, no smear layer covering the dentine or smear plugs blocking the tubules were observed [Figure 2]. SEM observations of the laser group showed a homogeneous area with less-exposed dentinal tubules, the diameter of which was significantly lower compared with the control group [Figure 3].

DISCUSSION

Our results indicate that Er;Cr:YSGG laser treatment of dentine discs occludes the open dentinal tubules partially that are thought to conduct cytotoxic extracts of dental materials. However, the laser treatment of dentine does not seem to protect pulp cells from toxic substances of dental materials in three-dimensional bovine pulp-derived cells; therefore, our hypothesis was accepted. From SEM analysis, some areas could be described as having undergone melting. Gholami *et al*. showed that the Er,Cr:YSGG laser was able to melt peritubular dentine and occlude dentinal tubules partially or completely.[17]

It has been consistently demonstrated that dentine is an effective barrier, preventing cell damage from a variety of materials and chemicals. Studies have shown that dentine can reduce the toxicity of resins and bonding adhesives by limiting their diffusion from the cavity preparation to the pulp.[18-20] Dentine most likely adsorbs substances in the tubules and further limits the passage of substances.[3] Some adhesive components diffuse rapidly through dentine,[21] and there is compelling evidence to question whether these adhesives can cause cytotoxicity through diffusion. Bouillaguet *et al*. showed that the high permeability of dentine generally allowed greater diffusion of dental adhesives.[22] Galler *et al*. suggested

that the protective properties of dentine are selective and depend on the chemical nature of the dentine-contacting material. Therefore, cytotoxic materials intended for use on dentine should be tested in simulated dentine barriers.[23]

Bovine dentin was used as the substrate because of the appropriate size of the teeth. Moreover, the use of the bovine teeth makes it easy to obtain uniform surfaces for bonding. It has been confirmed that bovine teeth are a suitable substitute for human teeth and provide similar bond strength of human teeth.[24,25]

In this study, we used a resin-modified glass ionomer liner/base material and one-component self-etching light-cured adhesive, as these materials normally come into close contact with dentine. In this study, Vitrebond showed a substantial reduction in cells, similar to previous studies, in which it has been consistently found to be cytotoxic to different cell lines and based on different evaluation methods.[26,27] In addition, Vitrebond was cytotoxic in other dentine barrier tests[20,28] and toxicity increased with decreasing dentine thickness between the test material and target culture.[23] It has been suggested that its pronounced cytotoxicity is due to the catalyst diphenyliodonium chloride and 2-hydroxyethyl methacrylate (HEMA).[29,30] Morisbak *et al*. found that HEMA-induced cell proliferation disturbances and toxicity through glutathione depletion and subsequent reactive oxidative species formation.[31] Among methacrylate monomers, HEMA has been documented to be a potent contact allergen and is known to penetrate conventional dental gloves in a relatively short period.[32,33]

G-Bond was developed as a one-step, one-component self-etch adhesive for fast and easy bonding procedures and to provide a bonding agent without HEMA. HEMA is often present in relatively substantial amounts in numerous commercial adhesives as it promotes tooth surface wetting and can infiltrate etched dentine surfaces up to several micrometers deep, which is required with relatively deeply etching etch-and-rinse adhesives. Nevertheless, G-Bond was formulated without HEMA to avoid allergic reactions in practitioners and patients, which is a steadily increasing biocompatibility problem associated with the increased use of adhesives, as well as to improve the hydrolytic resistance and long-term stability of the resulting bond.[34,35]

Several *in vitro* studies have evaluated the cytotoxicity of G bond. Sun *et al*.[36] tested four one-step self-etching dental adhesives (Adper Easy One, iBond, Clearfil S³ Bond, and G-Bond) on cultured human periodontal ligament fibroblast cell culture and reported that they

caused a reduction in cell vitality and morphological changes. Şengun *et al*. evaluated cytotoxicity of four dentin-bonding agents (G-Bond, Adper Prompt Self-Etch, Clearfil DC Bond System, and Quadrant University-1-Bond) on bovine dental papilla-derived cells by dentin barrier test device. G-Bond and Adper Prompt Self-Etch were cytotoxic for the pulp-derived cell cultures in spite of a dentin barrier. Dentin-bonding agents include biologically active ingredients and may modify pulp cell metabolism when the materials are used in deep cavities. If these adhesive agents are used in deep cavities, a biocompatible cavity liner should be used.[20] In this study, we tried to modify the nature of dentin using Er,Cr:YSGG laser. The Er,Cr:YSGG laser irradiation of dentine can obliterate exposed dentin tubules [Figure 3]. Hence, the permeability of dentin can be reduced.

The literature shows that dentine surfaces prepared by Er:YAG and Er,Cr:YSGG lasers are similar in micromorphology,[37] speculation on the different forms of action of the two lasers are still discussed. The greater absorption of the OH− ions from hydroxyapatite by Er,Cr:YSGG laser could promote a different interaction between the laser and the dentine surface. Moreover, the higher and fixed repetition rate of 20 Hz could involve more leakage into the irradiated windows. In our previous study the use of Er:YAG laser treatment of dentine reduced the cytotoxic effects of dentine-contacting materials in three-dimensional bovine pulp-derived cells, G Bond and I Bond were not cytotoxic when they were applied to Er:YAG laser treated dentine;[26] however in this study Er,Cr:YSGG laser treatment of dentine was not successful enough in decreasing the cytotoxic effects of G Bond and Vitrebond.

Aranha and Eduardo analyzed dentine permeability and the morphology of exposed dentine surfaces irradiated with Er:YAG and Er,Cr:YSGG lasers using different parameters. The Er,Cr:YSGG laser used has a fixed repetition rate of 20 Hz. Similar to the Er:YAG laser, the power settings used were lower, however, the Er:YAG laser promoted less permeability and in some cases, the values were negative, meaning that the dye solution penetrated more into the irradiated window than into the control window.[38]

The Er,Cr:YSGG laser promotes chemical surface alteration and can change the mineral content of enamel and dentine.[37] A previous study reported that the high absorption of the Er,Cr:YSGG laser emission wavelength (2.78 μm) by water may result in the evaporation of tubule fluid and the smear layer.[39] Therefore, studies on this laser have focused on finding a treatment mechanism or compared it with other

types of lasers in terms of its efficacy as an *in vitro* desensitization treatment.[40,41]

CONCLUSION

Many studies have examined the cytotoxicity of dentine-contacting materials; however, all of these materials have been used for various indications, raising the question of how to minimize their toxic effects. To help address this concern, in this study, the Er,Cr:YSGG laser treatment of dentine evaluated in a dentine barrier test device; however, it seems that Er,Cr:YSGG laser treatment was not successful enough in decreasing the cytotoxic effects of the dental materials. Different parameters of Er,Cr:YSGG laser or different laser types could be investigated as an alternative to minimizing the cytotoxic effects of dental materials.

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Conflicts of interest

There are no conflicts of interest.

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