

Effect of duration of Er,Cr:YSGG laser etching on dentine morphology

An *in vitro* study

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The conventional method of cavity preparation by rotary instruments is not favoured by many patients. On the other hand, dentine prepared as such is covered with smear layer, which is composed of dental hard tissue, carious debris, and residual bacteria. This decreases the dentine surface energy and prevents adequate adhesion to dentine.^{1,2} Dentine is a major component of teeth. It is a complex substrate for bonding due to its heterogeneous composition, mainly organic structure, hydrophilic nature, and morphological variations. Introduction of adhesive primers with enhanced hydrophilicity for dentine surface conditioning and providing a stronger bond to more hydrophobic adhesive resins largely resolved this issue.^{3–6} The conventional method of forming a strong bond to dentine is via phosphoric acid etching and removal of the mineral content to create microporosities within the collagen network. Upon removal of the hydroxyapatite crystals of the outer layer of dentine, about 50 % unfilled space and about 20 % of water remain in the dentine surface. In order to obtain a strong bond, resin should infiltrate into the collagen scaffold and form a hybrid layer. The primer also penetrates into the dentinal tubules concurrent with the formation of the hybrid layer. This results in formation of quite large resin tags. After etching, the tooth should be rinsed with air and water spray to thoroughly re-

move the acid and stop the etching process.^{7–9} Otherwise, cysteine cathepsins, which can be activated in mildly acidic environments, may also activate matrix-bound matrix metalloproteinases and destabilise the hybrid layer in long term.^{10,11} If the etching time is too long and the etched zone is too deep, decalcified dentine may not be fully impregnated. The etched but not impregnated space may serve as a mechanically weak zone.

After rinsing, drying of dentine must be performed cautiously. Even a short air blast from an air–water spray can inadvertently dehydrate the outer surface and cause the remaining collagen scaffold to collapse. Once it happens, the collagen mesh prevents the penetration of primer and bonding will fail. On the other hand, excess moisture tends to dilute the primer and interfere with resin penetration.^{7–9} Excessive acid conditioning causes incomplete infiltration of resin monomers and creates a gap between resin tags and dental structure that decreases the bond strength by creating a weak zone.¹² In conventional surface treatment, the primer penetrates into the fluid-filled dentinal tubules. It is generally under-cured and forms soft flexible tags.^{7,8} Today, laser system, as a novel modality, has been suggested for use as an alternative to dentine surface etching. Among laser systems, the erbium family of lasers is believed to be the most successful. There are several studies that have explored various parameters such as laser power and frequency for dentine etching and surface conditioning for proper bonding.^{3–5} But no study has investigated the effects of duration of Er,Cr:YSGG laser etching on dentine surface morphology. The aim of this study was to evaluate ultrastructural morphological changes in dentine following different durations of Er,Cr:YSGG laser irradiation using scanning electron microscopy (SEM).

Materials and methods

Sample preparation

Twenty-five extracted human-impacted permanent third molars were used in this study. Soft tissue residues were completely removed from the tooth surfaces with a dental scaler. All teeth were then stored in distilled water

Duration of irradiation	T1	T2	T3	T4	T0 = control
Exposure 1	1	1	1	0	1
Exposure 2	1	1	1	0	1
Exposure 3	1	1	1	0	1
Exposure 4	1	1	1	0	1
Exposure 5	1	1	1	0	1

0 = smear layer was not observed
1 = smear layer was observed
T1–T4 = different durations of irradiation: T1, 5 s; T2, 10 s; T3, 20 s; T4, 40 s;
T0, no irradiation
Exposure 1–5 = number of irradiated areas by Er,Cr:YSGG laser

Table 1: Effect of duration of irradiation on the smear layer.

containing 0.4% thymol for one week for disinfection. Then, samples were stored in distilled water at room temperature until the experiment. Each tooth was cut below the occlusal pit and fissure level, perpendicular to the longitudinal axis of the tooth by means of a high-speed handpiece and silicon carbide disc to remove the occlusal enamel and expose the superficial dentine surface. Next, an area measuring 5 mm in length and 5 mm in width was prepared on the occlusal surface of each tooth for laser irradiation.

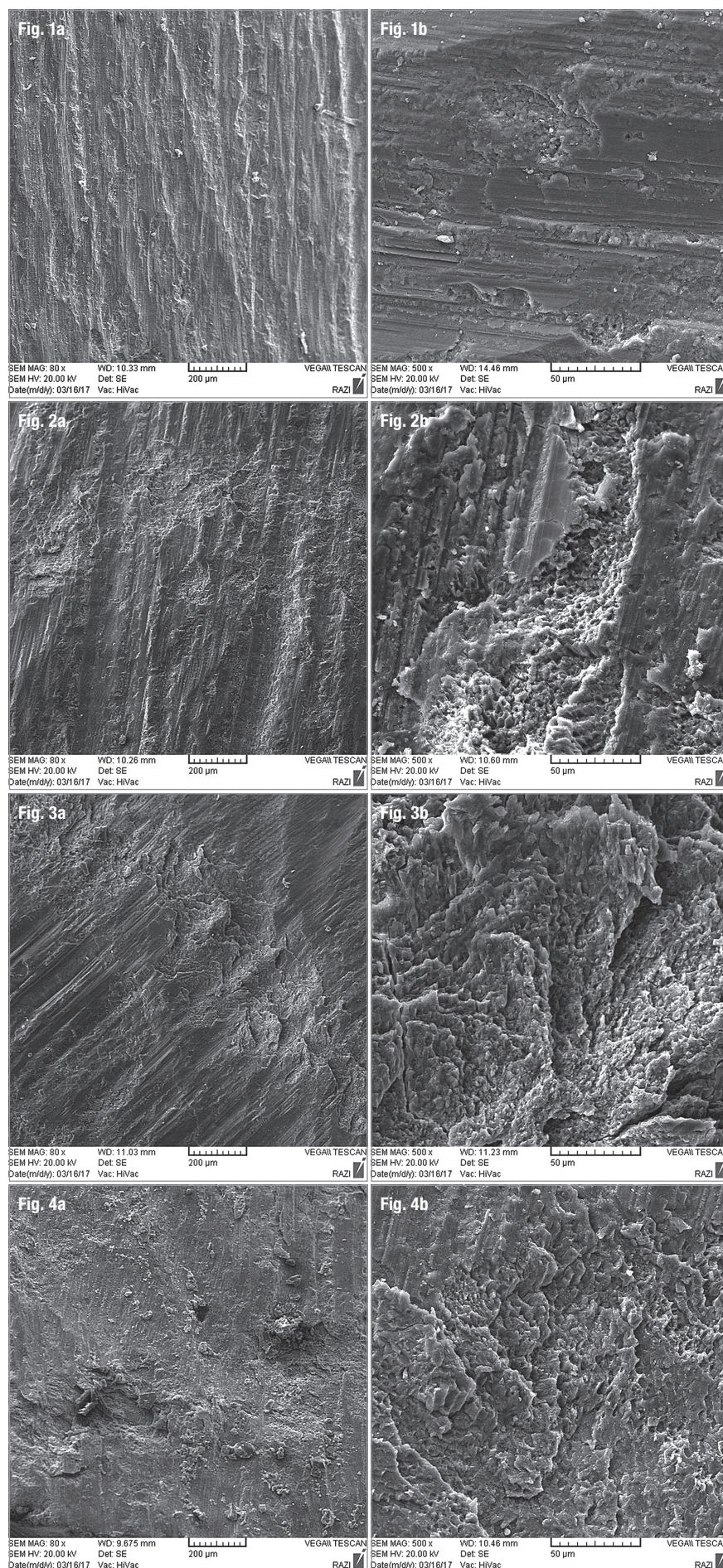
Laser application

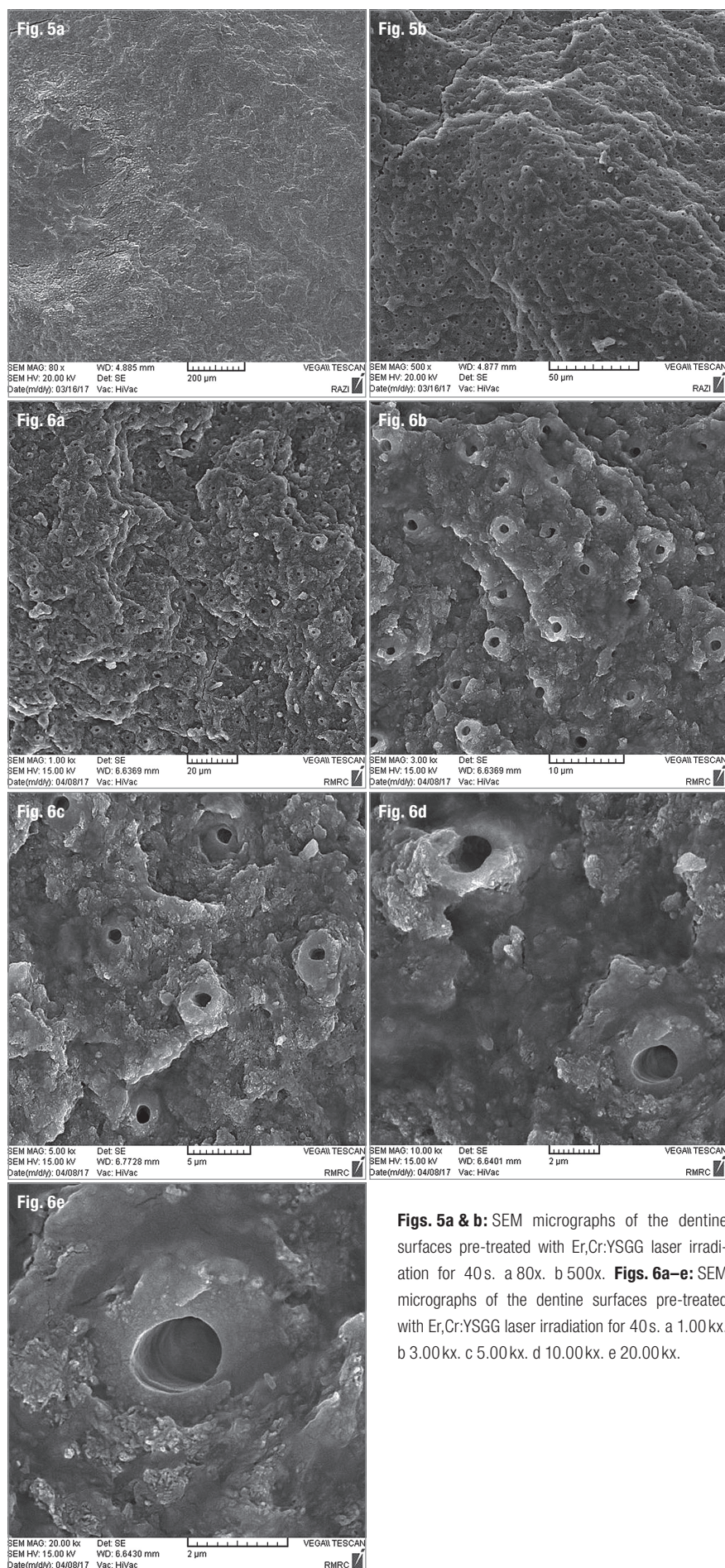
The marked occlusal area was irradiated with Er,Cr:YSGG laser (BIOLASE) at a wavelength of 2,780nm. The laser parameters were as follows: Output power 4.5W, peak power 1,500W, energy density per pulse 8.57 J/cm², energy per pulse 0.09 J, frequency 50Hz, water 80, air 60, pulse duration 60µs, tip diameter 600µm, cross section of tip 0.028cm², angle of radiation 8, irradiation surface 1.16mm, distance 2mm. The teeth were randomly divided into five groups according to the duration of laser irradiation: T1, 5s; T2, 10s; T3, 20s; T4, 40s; T0, no laser irradiation. After laser irradiation, the samples were stored in distilled water.

SEM analysis

The effects of laser irradiation on dentine surfaces were evaluated using SEM at 80x and 500x magnifications. Prior to SEM analysis, the samples were vacuum-dried and sputter-coated with gold for 180s. SEM observations were carried out at an accelerated voltage of 20kV with 25mm working distance. SEM findings were scored to evaluate the effect of duration of laser irradiation on the smear layer as follows: Score 0 = absence of smear layer; Score 1 = presence of smear layer. More SEM images were obtained from sample number 4 at 1.00kx, 3.00kx, 5.00kx, 10.00kx, and 20.00kx magnifications.

Figs. 1a & b: SEM micrographs of the dentine surfaces pre-treated only with silicon disc (control group). a 80x. b 500x. **Figs. 2a & b:** SEM micrographs of the dentine surfaces pre-treated with Er,Cr:YSGG laser irradiation for 5s. a 80x. b 500x. **Figs. 3a & b:** SEM micrographs of the dentine surfaces pre-treated with Er,Cr:YSGG laser irradiation for 10s. a 80x. b 500x. **Figs. 4a & b:** SEM micrographs of the dentine surfaces pre-treated with Er,Cr:YSGG laser irradiation for 20s. a 80x. b 500x.





Figs. 5a & b: SEM micrographs of the dentine surfaces pre-treated with Er,Cr:YSGG laser irradiation for 40s. a 80x. b 500x. **Figs. 6a–e:** SEM micrographs of the dentine surfaces pre-treated with Er,Cr:YSGG laser irradiation for 40s. a 1.00kx. b 3.00kx. c 5.00kx. d 10.00kx. e 20.00kx.

Results

Analysis of the results with the Mann–Whitney U test showed that 40s of irradiation in T4 group caused significant removal of the smear layer compared to T0 group ($P = 0.008$). Other durations of radiation did not completely remove the smear layer ($P = 1$, Table 1). SEM morphological analysis of the specimens showed different characteristics according to the surface pretreatment, as described below: Control group (T0): The surface was covered with smear layer (Fig. 1). Er,Cr:YSGG laser irradiation for 5, 10, and 20s: The dentine surface in these groups revealed different amounts of the smear layer (Figs. 2–4). Er,Cr:YSGG laser irradiation for 40s: Dentine surface in this group showed an irregular pattern without the smear layer, with open dentinal tubules and no enlargement. A prominent peritubular dentine appearance suggested greater removal of intertubular dentine due to its higher water sorption. There were no evident signs of melting or microcracks (Fig. 5). Among the different time durations of Er,Cr:YSGG laser irradiation, only 40s of laser irradiation caused smear layer removal from the dentinal tubules. According to the results in group 4, further SEM analyses at 80kx, 500kx, 1.00kx, 3.00kx, 5.00kx, 10.00kx, and 20.00kx magnifications were performed in this group (Fig. 6).

Discussion

The quality of the dentine-resin interface plays an important role in achieving a high quality and durable composite restoration.¹³ Dentine preparation by rotary instruments creates smear layer on dentine surface that causes problems in obtaining suitable bond between the adhesives and dentine. On the other hand, the conventional method of smear layer removal includes the use of phosphoric acid on dentine for 15s. This method has limitations such as (1) demineralisation that occurs with the removal of dentine mineral content, (2) over-etching since by increasing the duration of etching, greater depth of dentine is demineralised, (3) inadequate washing of the etchant results in unwanted continuation of the etching process, and (4) over-drying causes the collagen network to collapse and under-drying dilutes the primer. After the application of bonding agent, resin tags form by penetration of

primer into the fluid-filled dentinal tubules. These resin tags are generally under-cured, soft, and flexible. In addition, the interface is prone to nano-leakage because of gap formation between tags and dentine due to incomplete penetration of adhesive.^{3,7}

In the 1990s, erbium lasers were introduced for preparation of hard tissue as an alternative to rotary instruments. Er,Cr:YSGG laser (emitting at a wavelength of 2.79 μm) is an effective tool for removal of dental hard tissues.^{14,15} This wavelength is absorbed by the hydroxyapatite and water. The hydroxyl radicals and water in hydroxyapatite crystals receive most of the laser energy. By water evaporation in the tooth mineral components, a large volumetric expansion occurs.^{1,2} Next, micro-explosions occur that remove the hard tissue from the irradiated regions.¹⁶ It has minimal side effects on the sound tooth structure.¹ Dentine conditioning with laser has advantages. As reported in some studies, the laser settings can be adjusted to physically etch the dentine surface. Power, frequency, and other parameters can be adjusted to prevent smear layer formation on the dentine surface. Laser does not cause dentine demineralisation. It does not have the risk of over-etching or over-/under-drying. The erbium laser-treated dentine is dehydrated prior to priming and bonding; thus, the resin tags are more likely to be long and strong.^{1,17,18} Of studies on the effect of different laser parameters on dentine morphology, no study investigated the effect of various durations of Er,Cr:YSGG laser irradiation on dentine surface morphology. Dentine irradiated with Er,Cr:YSGG laser shows a microscopically rough surface without demineralisation,^{19,20} open dentinal tubules,^{21–23} no smear layer, and satisfactory sterilisation of the cavity.²⁴ These characteristics are considered as an advantage of laser preparation if composite resins are to be applied as the filling materials.²⁵

The Er,Cr:YSGG laser setting used in this study included 4.5W average power, 1,500W peak power, 0.09J energy per pulse, 50Hz frequency, 8.57 J/cm² energy density, 80% water and 60% air, pulse duration of 60 μs , and distance of 2 mm above the surface. The energy density used in our study was not within the ablation range. Only dentine surface was etched and conditioned for the bonding process. Five, 10, and 20 s of laser irradiation caused different amounts of smear layer. The applied Er,Cr:YSGG laser setting with 40 s of duration caused a scaly-like appearance on the surface with less homogenous and less regular surface creating a micro-retentive pattern on dentine without heat injury or melting, which is favourable for bonding process. The dentine surface showed no smear layer; dentinal tubules were open; and the sub-surface was not demineralised. Open tubules and absence of smear layer are additional factors that enhance bonding to laser-treated dentine.¹⁴ This can be explained by micro-explosions at the tissue surface, resulting from the sudden boiling of water within the tissue (thermo-

mechanical ablation).²⁶ The results obtained from this study can be used in further studies to evaluate the composite bond strength with different bonding systems.

Conclusion

Forty seconds of laser irradiation with the aforementioned parameters eliminated the smear layer from the dentine surface, and the obtained surface had micro-retentive pattern on dentine and open tubules without heat injury or melting and demineralisation which was suitable morphology for bond to composite resin. Laser irradiation for less than 40 s could not completely remove the smear layer from the surface. Each one of these surfaces could have optimum bonding with composite by applying different adhesives systems which should be investigated in further studies.

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