Evaluation of the Morphological Characteristics of Laser-Irradiated Dentin

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Abstract

Objective: The aim of this study was to investigate the effect of different energy settings of Er:YAG laser irradiation on dentin surface morphology with respect to the number of opened dentinal tubules. **Background data:** An ideally prepared dentin surface with opened dentinal tubules is a prerequisite for adhesive fixation. No study, however, has yet compared the numbers of opened dentinal tubules with regard to statistical differences. **Methods:** Conventional preparations using a bur with or without additional acid etching acted as control groups. Dentin specimens were prepared from human third molars and randomly divided into eight groups according to the energy settings of the laser (1, 1.5, 4, 6, 7.5, and 8 W) and two controls (bur and bur plus acid etching). After surface preparation, dentin surfaces were analyzed with a scanning electron microscope, and the number of opened dentinal tubules in a defined area was counted. **Results:** The control groups showed smooth surfaces with (bur plus acid etching) and without opened dentinal tubules (bur), whereas all laser-irradiated surfaces showed rough surfaces. Using the energy setting of 4 W resulted in significantly more opened dentinal tubules than the conventional preparation technique using the bur with additional acid etching. In contrast, the energy setting of 8 W showed significantly fewer opened dentinal tubules, and also exhibited signs of thermal damage. **Conclusions:** The Er:YAG laser with an energy setting of 4 W generates a dentin surface with opened dentinal tubules, a prerequisite for adhesive fixation.

Introduction

ESTHETICS HAVE BEEN A SUBSTANTIAL part of the daily life of every dental practitioner since modern dentistry is no longer only responsible for the treatment of dental diseases. Adhesive fixation of tooth-colored restorations is, therefore, an essential part of restorative dentistry. One of the most important factors of adhesive fixation is bond strength between tooth structure and restoration, traditionally obtained by an appropriate adhesive system.¹ Stability and durability of the adhesive fixation between dentin and the adhesive systems is mainly gained through a micromechanical retention between monomers of the adhesive systems and areas within the dentin, resulting in a hybrid layer and resin tags.^{2,3} The only possible areas in mineralized dentin facilitating infiltration of the monomers are the dentinal tubules.

Conventional cavity preparations with rotating instruments, however, produce smear layers that seal the dentinal tubules.⁴ Removing the smear layer (e.g., by acid etching) is therefore a prerequisite, which dissolves the smear layer, demineralizes the peritubular and intertubular dentin, and exposes the collagen matrix.³ Acid etching also causes some negative side

effects, depending upon the concentration of the acid. Strong acids such as phosphoric acid can demineralize the dentin surface deeper than monomers would be able to infiltrate, which results in unprotected tissue layers underlying the restoration.⁵ A further critical point associated with the use of phosphoric acid is that water rinsing after application of the acid is followed by air drying, which is a very sensitive step, because excessive drying results in collapsed collagen fibers, with subsequently inhibited monomer infiltration.⁶

To overcome the issues with the use of high-concentration acids, self-etch adhesives were invented. These self-etch adhesives, also known as all-in-one adhesives, combine the conditioner, the primer, and the bonding agent in the same liquid.^{7,8} The benefit is that the application is, therefore, less technique sensitive; the depth of the demineralization and infiltration is mutually adapted, and patients report less postoperative sensitivity.⁹ Self-etch adhesives, however, cannot completely dissolve thick smear layers and, therefore, partially fail in obtaining the ideal surface for adhesive fixation.^{10,11} Another option for removing the smear layer or preventing the formation of a smear layer during cavity preparation is the use of an erbium laser.

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The radiation of erbium lasers is absorbed to a significant extent by water molecules in the dental hard tissue, which leads to sudden boiling and water evaporation resulting in microexplosions. These microexplosions are the reason for the very specific morphology of laser-treated dentin.¹² Erbium lasers create a microretentive pattern with opened dentinal tubules and no smear layer, apparently providing ideal conditions for adhesive fixation.^{13,14} In the literature, however, the results on shear bond strength, which is one possible parameter for assessing adhesive fixation, are inconclusive. Some investigators report equal or higher shear bond values after laser treatment, compared with the combination of rotary instruments and acid etching,^{15,16} whereas others report that laser treatment adversely affects adhesion to dentin.^{17,18}

The lack of clarity regarding laser treatment and adhesive fixation is attributed to the highly differing results obtained with different study designs, methods, varying laser parameters, and the different adhesive systems used.¹ It is assumed that bond strength data substantially vary among different investigations as a result of several factors influencing adhesive fixation.^{19,20} One possible reason for these discrepancies might be the different laser settings being used, which results in the production of different dentin surfaces with respect to the number of opened dentinal tubules. To our knowledge, no study has yet compared the numbers of opened dentinal tubules with regard to statistical differences. Therefore, the information about the elementary requirements for adhesive fixation, which are, *inter alia*, an ideally prepared dentin surface with opened dentinal tubules, is crucial. Consequently, the aim of this study was to evaluate, using scanning electron microscopy (SEM), the influence of erbium laser irradiation on the morphology of the dentin surface compared with conventionally rotating instruments with or without acid etching.

Materials and Methods

Specimen preparation

Sixty-five sound and restoration-free extracted human third molars were selected, cleaned and stored in distilled water at 4°C. The patients (between 18 and 40 years of age) were informed about the possibility of using their teeth for research purposes, and consent was obtained. An ethical committee approved the use of teeth for research purposes (Ethical Committee, Medical University of Vienna). Prior to preparation of dentin specimens, the teeth were rinsed thoroughly under running tap water and the enamel cusps were removed using a precision diamond band cutting device (EXAKT Advanced Technologies GmbH, Norderstedt, Germany). Afterwards, two dentinal discs of each tooth were cut off under constant irrigation with distilled water. Finally, enamel was removed with a handpiece and a diamond bur (Komet Dental, Lemgo, Germany) to assure pure dentinal specimens with a thickness of 1 mm and a diameter of 3–4 mm. A total of 120 dentin specimens were randomly divided into eight groups according to the surface treatments to be used.

Laser irradiation and control group preparation

Laser irradiation was performed with an Er:YAG laser (LiteTouchTM, Syneron Medical Ltd., Irvine, CA) using a

600 µm tip. To guarantee standardized irradiation of the dentinal specimens, a special XYZ table was invented and manufactured in collaboration with the Vienna University of Technology (Vienna, Austria). The position of the table was computer controlled and enabled a precise height and angle adjustment for the laser handpiece. The software controlling the handpiece (Measurement & Automation Inc.) allowed a defined movement of the laser along the x- and y-axis and was preprogrammed with the start and end-point. Working with this device, a standardized continuous irradiation of the dentinal specimens in noncontact mode could be assured. Laser treatment was realized in strict accordance with safety precautions. Dentin specimens treated with a diamond bur for simulating conventional preparation with resulting smear layer served as positive control group. Dentin specimens treated with a diamond bur and subsequently phosphoric acid etching served as negative control group, which simulates the golden standard procedure for smear layer removal.

The following treatment parameters were used for 15 dentin specimens in each group:

Positive control group: Dentin specimens were treated with a conventional handpiece (W&H Dentalwerk, Bürmoos, Salzburg, Austria) and a 45 µm grit diamond bur (Komet Dental).

Negative control group: Dentin specimens were treated with a conventional handpiece (W&H) and a 45 µm grit diamond bur (Komet Dental), and etched with 35% phosphoric acid (Scotchbond, 3M ESPE, St. Paul, MN).

Laser group 1: Dentin specimens were irradiated with a pulsed Er:YAG laser (1 W, 100 mJ, 10 Hz, water and air cooled) at an irradiation angle of 60 degrees.

Laser group 2: Dentin specimens were irradiated with a pulsed Er:YAG laser (1.5 W, 100 mJ, 15 Hz, water and air cooled) at an irradiation angle of 60 degrees.

Laser group 3: Dentin specimens were irradiated with a pulsed Er:YAG laser (4 W, 200 mJ, 20 Hz, water and air cooled) at an irradiation angle of 60 degrees.

Laser group 4: Dentin specimens were irradiated with a pulsed Er:YAG laser (6 W, 300 mJ, 20 Hz, water and air cooled) at an irradiation angle of 60 degrees.

Laser group 5: Dentin specimens were irradiated with a pulsed Er:YAG laser (7.5 W, 250 mJ, 30 Hz, water and air cooled) at an irradiation angle of 60 degrees.

Laser group 6: Dentin specimens were irradiated with a pulsed Er:YAG laser (8 W, 400 mJ, 20 Hz, water and air cooled) at an irradiation angle of 60 degrees.

Afterwards, all dentinal discs were stored in distilled water at 4°C until examination using SEM.

SEM analysis

The surface of each dentinal specimen was examined under a scanning electron microscope (Hitachi Tabletop Microscope TM-1000, Chiyoda, Tokyo, Japan). Images were always taken of a defined area of each dentinal specimen to assure similar conditions. Three independent observers analyzed the images using the software ImageJ (imagej.nih.gov/ij). Dentinal surfaces were evaluated regarding the number of opened dentinal tubules. In addition, the surface condition of the dentinal specimens was inspected for signs of thermal damage.

Statistical analysis

Statistical analysis was performed using SPSS version 21.0 (SPSS Inc., Chicago, IL). The nonparametric Mann–Whitney *U* test for paired samples was used to test for differences in the number of opened dentinal tubules between either of both control groups using the bur with or without additional acid etching, and each of the laser-treated groups (with Bonferroni correction for multiple comparisons).

Results

The median, 25th, and 75th percentiles, and minimum and maximum of the number of opened dentinal tubules of 15 specimens in each group are presented in Fig. 1.

The mean number of opened dentinal tubules of 0.7 ± 1 in the bur group (positive control) and 62.7 ± 9 in the bur plus acid etching group (negative control) were significantly different ($p < 0.001$). The bur group (positive control) also showed significantly fewer opened dentinal tubules than all laser groups, with p values < 0.001 using laser settings between 1 and 7.5 W and a p value of 0.006 using a laser setting of 8 W. The mean number of opened dentinal tubules in the laser-treated groups was similar in the groups of 1 W (85.2 ± 2), 1.5 W (81.7 ± 3), 4 W (99.9 ± 2), 6 W (71.2 ± 3), and 7.5 W (67.3 ± 3). The laser setting of 4 W achieved the highest number of opened dentinal tubules, showing significant differences compared with the conventionally prepared dentin surface with acid etching ($p = 0.003$). The laser setting of 8 W (31.1 ± 2) achieved the fewest number of opened dentinal tubules in the laser groups, showing significant differences compared with the conventionally prepared dentin surface with acid etching ($p = 0.003$).

Scanning electron micrographs of representative samples of the investigated dentin surfaces are shown in Fig. 2a–h. Both bur-treated control groups showed a smooth surface. However, the bur treatment alone (Fig. 2a) presented almost no opened dentinal tubules, whereas the bur treatment plus acid etching (Fig. 2b) presented a surface with opened dentinal tubules without any signs of smear layer. All of the laser-irradiated surfaces showed a rough surface with protruded dentinal tubules (Fig. 2c–h). Increasing signs of thermal damage such as cracks and melting could be identified in groups 6 W (Fig. 2f), 7.5 W (Fig. 2g), and 8 W (Fig. 2h).

Discussion

The present study compared the morphology of dentin surfaces after eight different surface treatment procedures, seeking the ideal dentin surface for adhesive fixation. The criterion for this ideal surface was opened dentinal tubules, because adhesive fixation between dentin and adhesive systems is primarily achieved by micromechanical retention.^{2,3} The dentin surfaces treated with rotary instruments alone (positive control) did not show opened dentinal tubules, whereas the samples of the group with subsequent acid etching (negative control) showed a smooth surface with significantly more opened dentinal tubules. All laser-treated surfaces (experimental groups) resulted in a typically irregular and microretentive surface without smear layer, which is in accordance with the literature.¹⁴ The number of opened dentinal tubules in the laser groups using up to 7.5 W was basically similar to the acid-etched dentin surfaces treated with rotary instruments. Only the laser-treated group using 4 W showed significantly more opened dentinal tubules than the acid-etched dentin surfaces after treatment with rotary instruments, whereas the laser-treated group using 8 W showed significantly fewer opened dentinal tubules than the acid-etched dentin surfaces treated with rotary instruments.

The question of whether a greater number of opened dentinal tubules will result in better adhesive fixation still remains open. It is known, however, that opened dentinal tubules are one prerequisite for successful adhesive fixation. The fact that the highest number of opened tubules was found in the laser group using 4 W, which might result in good adhesive fixation, supports the findings of other studies on shear bond testing after laser irradiation. A similar setting, using an erbium laser with 4 and 5 W, resulted in high shear bond strength in human primary teeth.²¹ Furthermore, it was shown that laser irradiation at 5 W produced significantly increased surface roughness compared with acid-etched dentin with similar shear bond values in both groups.²² Our results when using 8 W presented significantly fewer opened dentinal tubules than in the other laser groups and the conventionally prepared dentin surface with acid etching. Further, melting and cracks were discovered in the SEM analysis of this group. It would be interesting to examine the precise causes and impact of these damages in a further study using confocal laser microscopy.

FIG. 1. Number of opened dentinal tubules after treatment. Dentin specimens were analyzed with respect to the number of opened dentinal tubules after laser irradiation using different laser settings from 1 W up to 8 W compared with preparation with a bur with or without additional acid etching. Dentin treated with a bur alone showed almost no opened dentinal tubules, whereas all laser groups up to 7.5 W showed similar numbers of opened dentinal tubules like the bur and acid etch group. Laser irradiation with 4 W showed a significantly higher number of opened dentinal tubules, whereas laser irradiation with 8 W showed significantly fewer opened dentinal tubules.

FIG. 2. Representative scanning electron micrographs (SEM) of dentin surfaces. SEM of representative samples of dentin surfaces. Bur-treated control groups showed a smooth surface in which the surfaces without acid etching presented almost no opened dentinal tubules (a), whereas the bur plus acid etching group presented surfaces with opened dentinal tubules without any signs of smear layer (b). All laser-irradiated surfaces showed a rough surface with protruding dentinal tubules (c–h). Increasing signs of thermal damage such as cracks and melting could be identified corresponding to the increasing energy used in groups 6 W (f), 7.5 W (g), and 8 W (h).

These findings are in accordance with studies investigating dentin surfaces after laser irradiation. It has been reported that the degree of vitrification, diagnosed as melted tooth substance, is associated with the use of increasing laser energy.^{23,24} The first signs of vitrification appeared at 300 mJ for dentin samples, which corroborates our observations of melting and cracks in the 8 W group using 400 mJ. Further, a study on the morphological characteristics of lased dentinal surfaces revealed microfractures when using similar energy outputs.²⁵ The same study, however, also showed completely closed dentinal tubules when using lower energy levels such as 200 mJ. These findings are in

distinct contrast with the results of our study, in which the 4 W group using 200 mJ showed the highest number of opened dentinal tubules; significantly more than all other groups. One explanation for these discrepancies might be the different laser frequency settings. The setting was 1 Hz in the previous study, whereas 20 Hz was used in the present study.

The results of the present study are of clinical relevance, because the group with the highest number of opened dentinal tubules corresponded with laser settings that were clinically appropriate and approved for dental treatment. Moreover, the laser setting with the most opened tubules was also recommended by the manufacturer for cavity preparation of dentin. Consequently, the advantage is that a laser setting can be used that is neither too low nor too high for clinical use in order to achieve an ideal surface for adhesive fixation. Lower laser settings would lengthen the treatment time unnecessarily,²⁶ whereas higher laser settings would be harmful to the dental hard tissue and the dental pulp.²⁷ The present results, therefore, do not indicate that changes in the recommended protocol are required to enhance surface properties.

In the present study, dentin specimens from different teeth obtained from patients of different ages (between 18 and 40 years) were used for the various experimental groups. This is a limitation of the study, because the number and diameter of dentin tubules partially vary in different teeth, and there was no possibility of directly comparing the treatment groups on the same dentin surface. It is not possible, however, to obtain all dentin specimens for eight treatment groups from the same tooth. To minimize the effect of these limitations, the sample size was increased accordingly; only third molars without caries were used and a randomization of the specimens to the different groups was performed. It should also be remembered that for caries removal, the use of carbide burs is also an option, which might result in a different surface morphology. Nevertheless, the final dentin surface morphology will be a result of the etching technique used. Further, there is no evidence of the direct correlation between the number of opened dentinal tubules after laser irradiation and the success of the adhesive fixation. For example, it is reported that bond strength decreased after laser irradiation when no additional conditioning of the dentin surface was performed.²⁸ Higher acid resistance of the lased dentin and no formation of a hybrid layer were discussed to be the reasons for these results. The main problem is that a comparison of the outcomes of different investigations is not possible, because of the multiple factors influencing the results. The conclusion of the present study, that 4 W is a favorable energy level for dentin treatment, might, therefore, act as a primer for future studies. Using this basic laser setting, other parameters of adhesive fixation, such as different adhesive materials, could be tested with shear-bond testing methods.

Conclusions

This morphological *in vitro* study showed that the use of a Er:YAG laser at an energy setting of 4 W generates a dentin surface with a higher number of opened dentin tubules than other laser settings and conventional preparation with acid etching.

Author Disclosure Statement

No competing financial interests exist.

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