Effect of Er:YAG Laser Pulse Repetition Rate Variation on Bond Strength to Re-wet Dentin

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Purpose: To evaluate composite/dentin tensile bond strength on pretreated dentin surfaces using 2940-nm Er:YAG laser operating under a microsecond pulsed regime with different laser pulse repetition rates, followed by a re-wetting dentin solution.

Materials and Methods: Forty extracted human third molars were randomly distributed into four groups, according to dentin pretreatment: G1: (control) 37% phosphoric acid + adhesive; G2: Er:YAG laser irradiation with 80 mJ energy for 20 s at a repetition rate of 5 Hz + acid + re-wetting agent + adhesive; G3: irradiation at 10 Hz + acid + re-wetting agent + adhesive; G4: irradiation at 15 Hz + acid + re-wetting agent + adhesive. The adhesive system was Adper Single Bond (3M ESPE) and the hybrid composite resin was Z100 (3M ESPE). The obtained values from tensile bond strength test in MPa were submitted to statistical analysis with ANOVA followed by the Tukey test.

Results: G2 showed the best results of the lasered groups, demonstrating significant bond strength resistance similar to that of the control group.

Conclusion: Varying the laser pulse repetition rate (pps) can lead to different composite/dentin tensile bond strengths, even followed by re-wetting HEMA solution. The lowest pulse repetition rate (5 Hz) promoted the highest tensile bond strength between the adhesive restoration and irradiated dentin.

Keywords: Er:YAG laser, re-wetting agent, dentin, repetition rate, tensile bond strength.

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Materials and adhesive procedures were originally developed to be used on enamel and dentin substrates prepared by conventional techniques (hand cutting and rotating instruments). However, research today is focussing on alternative and selective techniques with a higher conservation of the dental tissues, which could also produce more effective adhesion to dentin. Er:YAG laser is one of these innovations.[1-9]

An Er:YAG (erbium:yttrium-aluminum-garnet) laser system emitting at a wavelength of 2940 nm coincides with the water absorption peak.[10] Therefore, the stored energy is well absorbed by hydroxyapatite, and it has proven to be effective in removing dental tissues. The great advantage of using this kind of laser in dentistry is its safety and efficiency. It does not cause thermal damage to the pulp,[11] produces no vibration (thus increasing patient comfort),[12] and in some clinical situations, it can improve enamel's caries resistance.[13]

Regarding Er:YAG laser efficacy for conditioning dentin, the literature has shown that the effect of this laser depends on many factors, such as parameters of absorbed energy, pulse repetition rate (pulses per second), pulse mode, pulse width, focal distance, irradiation time and water cooling.[14-16] It is known that Er:YAG laser does not demineralize dentin; it opens dentin tubules, creating a microretentive pattern with superficial irregularities. Nevertheless, it does not constitute an alternative treatment to the use of 37% phosphoric acid.[17] As opposed to acid conditioning, Er:YAG laser irradiation acts on the surface through a photomechanical effect, removing superficial dentin and producing a poor smear layer.[18-20] However, a thermal effect at the molecular level has been noted.[10] Additionally, the use of 2940-nm Er:YAG laser irradiation on the dentin surface just before restorative procedures can reduce the number of bacteria.[21,22]

Furthermore, when dental tissue is ablated, Er:YAG laser energy is absorbed by the water contained in the target tissue, which dehydrates the irradiated surface and thus works against adhesion. The current adhesive systems use a hydrophilic adhesive agent which must contact a moist surface to be able to micromechanically penetrate into it. In this way, an incompatible relationship between substrate and adhesive agent would probably arise. Some studies[2,23,24] suggest re-wetting the irradiated surface immediately before adhesive application, using a HEMA (hydroxyethyl methacrylate) solution, in order to enhance the adhesion and reduce marginal microleakage when Er:YAG laser is used.

Because of the concern about achieving Er:YAG laser conditioning under biologically tolerable parameters, the purpose of the present study was to evaluate the resin/dentin tensile bond strength, examining the effect of varied laser pulse repetition rates or pulses per second (pps) associated with re-wetting dentin with HEMA solution just before the restorative procedure.

MATERIALS AND METHODS

Forty healthy human third molars were used, which had been extracted for orthodontic reasons. These teeth were stored in 0.5% thymol solution and kept at a temperature of ca 5°C until used in this study.

The teeth were selected based on the quality and size of the crown, discarding unhealthy ones and those with anatomic crowns that were not at least 10.0 mm mesiodistally and 8.0 mm buccolingually. Each tooth was fixed with a low fusion compound on a wooden base for mounting in an Isomet saw (Isomet 1000, Buehler; Lake Bulff, IL, USA), bearing a diamond disk 0.3 mm thick. A complete transversal section was made at the medium third of the crown, using a speed of 300 rotations per minute.

Following this, all teeth were imbedded in acrylic resin (cylinders 20.0 mm high and 25.0 mm in diameter). Each prepared tooth section was positioned at the center of the cylinder, according to the size of the crown.

After complete acrylic polymerization, the cylinder was polished (LaboSystem; Struers, Denmark), in order to create uniform dentin surfaces, with a sequence of 320-, 500-, and 600-grit sandpapers (Acqua-Flex, Norton; USA), under water cooling.

After this procedure, all the samples were stored in distilled water and kept cold until their distribution into groups, which was random but for the final distribution of all samples into the 4 groups by different dentin surface treatments (Table 1).

The Er:YAG laser system (Twin Light, Fotona Medical Lasers; Ljubljana, Slovenia) operating in the microsecond regimen with pulse duration between 200 and 450×10^{-6} s emitting at a 2940×10^{-9} m wavelength, maximum energy per pulse of 500 mJ, repetition rate or pulse frequency varying from 2 to 15 Hz, was used. Its delivery system is composed of an articulated arm, with a sapphire window handpiece operating through a noncontact beam with a focused transversal section area of 0.0038×10^{-4} m^2 with an air/water spray cooling system.

As there are so many comparative morphological and bond-strength studies about conventional burs vs Er:YAG laser on dentin tissue before adhesive procedures, the current study evaluated only the different repetition rates before the adhesive procedure using a re-wetting agent. This methodology is supported by another study;[18] therefore, there is no "control group" just with Er:YAG laser treatment or just with re-wetting agent application.

In this study, the following irradiation parameters were used: focused mode, 80 mJ energy for 20 s, pulse repetition rates of 5, 10, and 15 Hz. The experimental setup was such that the laser beam was applied perpendicularly to the sample surface and positioned at a focal distance between 12.0 and 15.0 mm, under water flux of 24 ml/min, irradiating in both directions, under powerful suction.

Energy per pulse was determined, as FT-Raman spectroscopy showed that the mineral and organic content were less affected by the acid etching and by the

Table 1 Groups according to superficial dentin treatment

Groups	Superficial dentin treatment
G1 (control)	37% phosphoric acid + adhesive
G2	Er:YAG laser (80 mJ, 5 Hz); 37% phosphoric acid + re-wetting agent + adhesive
G3	Er:YAG laser (80 mJ, 10 Hz); 37% phosphoric acid + re-wetting agent + adhesive
G4	Er:YAG laser (80 mJ, 15 Hz); 37% phosphoric acid + re-wetting agent + adhesive

Table 2 ANOVA of average bond strengths

Variation sources	Degrees of Freedom	Sum of degrees	Medium squares	F squares	P
Interaction	3	538,1611	179,3870	13.78	0.003
Residue	36	468,6230	13,0173		
Total		39	1006,7842		

Significance level at 0.01 %.

Er:YAG laser irradiation with 80 mJ.[25,26] Irradiation time was determined because the irradiated area was pre-determined in 25.0 mm^2 (5.0 x 5.0 mm).

After irradiating all samples with the different parameters, all adhesive procedures were done: the application of the re-wetting agent (Primer from Scotchbond Multi-Purpose Plus, 3M-ESPE; St Paul, MN, USA) and adhesive system (Adper Single Bond, 3M- ESPE).

When the dentin surface treatments were finished, the composite resin restorations were done. For this purpose, a metal mold was used, 25.0 mm diameter and 5.0 mm deep, bi-split, and supported by a 1.0-mm-long threaded ring, which created inverse trunk-conical restorations, this being the shape necessary for the posterior tensile bond strength test. A hybrid composite resin was (Z100, 3M-ESPE) inserted into the mold by increments; each increment was photopolymerized for 20 s with a halogen curing light (Ultralux, Dabi Atlante, Brazil) of > 400 mW/cm^2 intensity, as previously checked with a radiometer.

After all restorations were complete, the samples were stored in distilled water at room temperature (25°C) up to the moment of the test.

The tensile bond strength test was performed by a universal testing machine (MTS-810, Material Test System; Minneapolis, MN, USA) at a crosshead speed of 2 mm/min and 1 KN load cell, using an appropriate metal device to fix the sample into the machine.

The values obtained from the tensile test were the quotient of the applied power divided by the area of the tooth/composite resin unit. The calculations were performed by Test Works 4 (MTS) software, using the data previously set for the test and the data obtained from the test machine. In addition, scanning electron microscopy (model JSM – T 330 A, JEOL; Tokyo, Japan) was used to analyze the samples prepared from the sectioning of the middle third of the dental crown, with the same dentin pretreatments, yielding three samples for each group.

RESULTS

The values obtained from the tensile test were submitted to ANOVA at 0.01% significance level in order to verify whether differences existed among the mean bond strengths. Because of the differences found, a

Table 3 Tukey Test of average bond strengths in MPa

Groups	Standard deviation	Tukey (1%)
G1	20.04 (± 4.20)	
G2	16.82 (± 4.03)	2.8
G3	11.49 (± 2.96)	
G4	11.42 (± 3.07)	

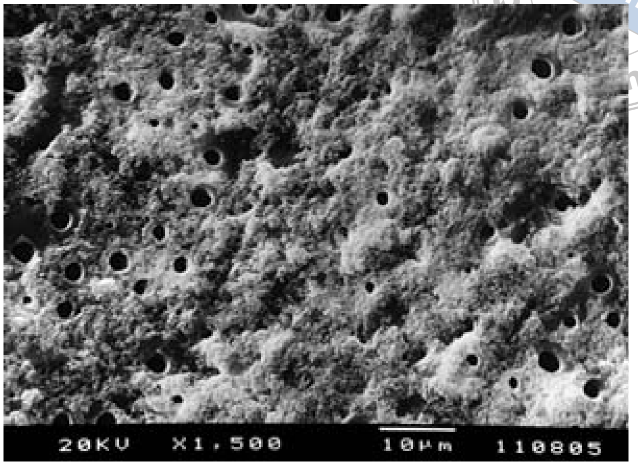

Fig 1 Irradiated dentin under a repetition rate of 5 Hz (magnification 1500X).

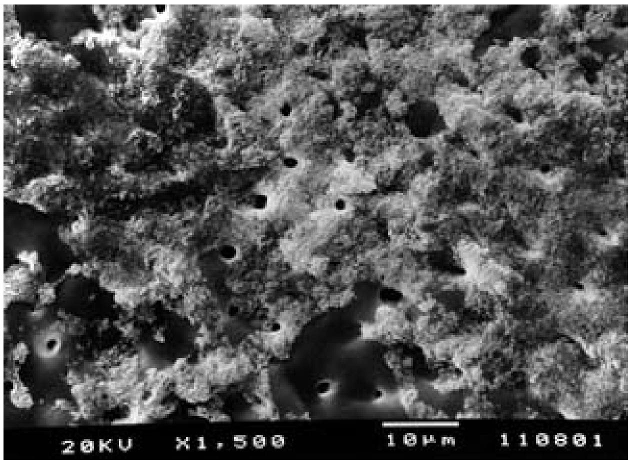

Fig 2 Irradiated dentin under a repetition rate of 10 Hz (magnification 1500X).

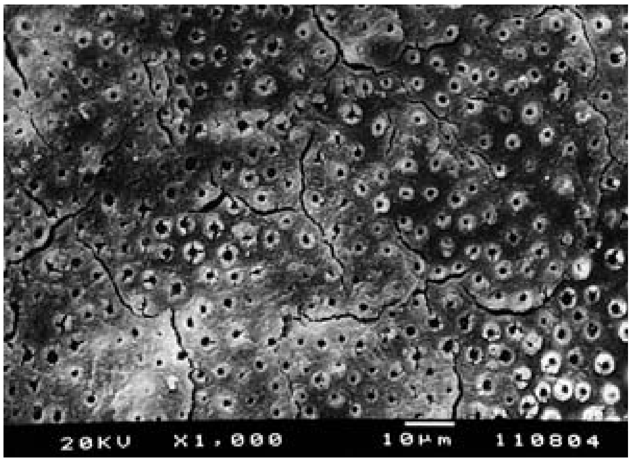

Fig 3 Irradiated dentin under a repetition rate of 15 Hz (magnification 1500X).

Tukey test was also performed. The results are shown in Tables 2 and 3. The bond strength values showed that there is a statistically significantly difference among the groups (Table 3).

Table 3 shows that the highest mean value was presented by G1, while G2 showed an intermediate mean value, and groups G3 and G4 were statistically similar, with the lowest values of tensile bond strength.

Superficial morphological dentin patterns after pretreatment were examined by scanning electron microscopy (SEM) under 1500X magnification. It is interesting to compare results between the three different pulse repetition rates used. In Figs 1 and 2, with 5 and 10 Hz, respectively, it is possible to see partially opened dentin tubules, some "islands" of smear layer, and intertubular dentin with irregular surfaces. Increasing the repetition rate to 15 Hz (Fig 3), it was possible to observe a higher density (number/area) of opened dentin tubules; peritubular dentin is very pronounced and several cracks are present. Most of these cracks originate at the center of dentin tubules.

DISCUSSION

The use of alternative options, such as Er:YAG laser, for dentin surface pretreatment before adhesive restorations have yielded different results, which should prompt further studies on parameters appropriate for optimizing bond strength.

Any Er:YAG laser damage to the collagen may not impair the bond strength of resin-modified glass-ionomer cement restorations to dentin.[27,28] However, when the restorative material is a composite resin, the obtained results are very different. The collapse of the collagen fibers, even when it is caused by laser irradiation or excessive air drying during cavity preparation, limits the possibility of micromechanical retention of the adhesive system on the conditioned dentin.[29] This happens because the most recent adhesive systems need a moist surface, maintaining the mesh of collagen fibers, in order to permit an efficient diffusion of the adhesive. Collapsed collagen is related to dentin dehydration; it can compromise the success of adhesive techniques currently used.[30]

However, if it is possible to re-expand the collagen network, enabling better micromechanical retention, adequate bond strength still can be obtained.[31] Thus, this study re-wet the irradiated dentin with the primer from Scotchbond Multi-purpose Plus immediately after irradiation and before the application of the adhesive system. Scotchbond Multi-purpose Plus is chemically composed of HEMA (hydroxyethyl methacrylate), polyalkenoate acid copolymer and water.

In our investigation, the rewetting of the dentin with HEMA solution was used in all laser groups, except for the control group, because the literature shows HEMA can enhance the adhesion of dentin with collapsed collagen fibers.

The energy per pulse of 80 mJ used here to precondition dentin is considered biologically acceptable, because it does excessively heat the dental tissues. It causes a superficial ablation with no signs of carbonization, and sustains superficial grinding in the micrometer range.[32] Moreover, dentin treated by Er:YAG laser at 80 mJ per pulse plus 37% phosphoric acid etching for 15 s is the most conservative procedure in terms of changing the proportion of phosphate and organic components.[25,26,33]

In clinical practice, the movement and focussing of the laser on such small areas make evident the difficulty of setting the pulse rate high enough to ablate effectively but low enough to avoid unwanted tissue damage. Because of the doubts still present related to the influence of the laser pulse repetition rate on dentin adhesion, we decided to verify its effects on the adhesive procedure. In the conventional protocol (acid conditioning + adhesive system) or control group, we found mean tensile bond strength values statistically higher and similar to group 2 (pulse repition rate = 5 Hz). In the groups where 10 and 15 Hz were used (groups 3 and 4), lower values of tensile bond strength were found. The reason for the most effective adhesion being found in the 5 Hz group may be as follows: 80 mJ energy and a low repetition rate are able to control the pulse distribution on the conditioned area, avoiding an irreversible hydrolysis of the collagen fibers, which could inhibit the adhesive penetration and yield weak adhesion. Gonçalves et al[34] and Aizawa et al[35] also verified that an increase in Er:YAG laser pulse repetition rate can significantly decrease the bond strength.

The SEM images (1500X) obtained in this study showed that laser ablation opened the dentinal tubules. For instance, the dentin treated with 5-Hz Er:YAG laser was more gently and superficially ablated. This group, when associated with re-wetting agent and acid conditioning, had better adhesion. However, increasing the pulse repetition rate to 10 and 15Hz produced a higher number of opened dentin tubules and a total absence of smear layer, besides the presence of microcracks. Using 15 pulses per second to deliver laser energy, the bond strength was significantly lower. We believe that collagen could possibly have been denatured because of the energy density used and, consequently, even with the use of the re-wetting agent; it was not possible to promote the reticular re-expansion of the collagen fibers, which compromised the adhesion.

In fact, as the repetition rate increases, the possibility of temperature increase also does. High repetition rates resulted in heat accumulation, as mentioned by Brygo et al.[36]

Cracks originating from tubule centers under 15 Hz could be explained by rapid, intense dentin tubule fluid ejection, promoting greater dehydration of dentin tissue surface.

In order to increase the use of this kind of procedure in restorative and esthetic dentistry, more studies are needed on a molecular level to understand the collagen alteration related to this type of irradiation and photoactivation. Such results may influence the development of new restorative materials that physically and chemically interact with substrates modified by Er:YAG laser.

CONCLUSION

Based on tensile bond strength test and the scanning electron microscopy results, we conclude that variations in Er:YAG laser repetition rate is a very important factor to be considered when performing adhesive procedures. Our results suggest that Er:YAG laser can be employed as an anti-bacterial dentin pretreatment, if it is followed by chemical treatment with phosphoric acid and re -wetting with HEMA solution.

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