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ORIGINAL ARTICLE

Microtensile bond strength to Er:YAG laser pretreated dentin

Avaliação da adesão em dentina irradiada pelo laser de Er:YAG

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ABSTRACT

Objective: Although the effects of Er:YAG (erbium:yttrium aluminium garnet) laser on cavity preparation as well as on dentin bonding to composite have been described in the literature, the longevity of this bond is still unknown. So, this study evaluated the short-term microtensile bond strength to dentin samples after different protocols of surface treatment. **Materials and Methods:** 60 bovine incisors were cleaned, worn to expose a dentin area and subdivided into groups according to treatment conditions: surface treatment (no irradiation – control group; dentin irradiation with Er:YAG laser 250 mJ/4 Hz; 160 mJ/10 Hz), adhesive system (Clearfil SE Bond - Kuraray; Adper Single Bond 2 - 3M/ESPE), and storage time (24 h; 90 days). After adhesive procedures, a block of Z250 composite resin (3M/ESPE) was built-up on each tooth. The teeth were sectioned to obtain samples for the microtensile bond strength test. Half of the samples were tested 24 h after cutting, and the other half were stored in distilled water for 90 days before testing. Intergroup analysis was also performed considering the same variables using ANOVA for multiple comparisons with Tukey test with a significance level of 5%. Data showed weaker bond strength for groups previously treated with laser ($p < 0.05$) compared with control groups, and these were not influenced by adhesive system used, nor by storage period. Stereoscopic microscope observations showed that fractures occurred predominantly at the adhesive interface in the groups irradiated with the Er:YAG laser. **Conclusion:** Within the parameters and variables used in this study, the Er:YAG laser could not provide an additional improvement in dentin-resin bond strength, irrespective of the type of adhesive system used or the storage period evaluated.

KEYWORDS

Adhesive systems; Er:YAG laser; Microtensile bond strength.

RESUMO

Objetivo: Ainda que a ação do laser de Er:YAG no condicionamento e preparo do substrato dentinário, bem como na resistência de união à resina composta já tenha sido descrita na literatura, a longevidade da adesão decorrente deste processo ainda não está bem estabelecida. **Material e Métodos:** Neste estudo, ensaios de microtração foram realizados em palitos obtidos de 60 incisivos bovinos, subdivididos em 12 grupos constituídos pela combinação das variáveis: tratamento dentinário prévio com o laser de Er:YAG (250 mJ / 4 Hz; 160 mJ / 10 Hz) e sem irradiação (grupo controle), sistema adesivo (Clearfil SE Bond / Kuraray; Adper Single Bond / 3M ESPE) e período de armazenagem (24 h; 90 dias). Os resultados mostraram menor resistência à microtração (com diferença estatisticamente significante $p = 0,05$) em relação aos grupos não tratados com o laser, não importando o sistema adesivo empregado, nem o período de armazenagem. A observação ao microscópio estereoscópico mostrou que as fraturas ocorreram predominantemente na interface adesiva para os grupos submetidos ao laser de Er:YAG. **Conclusão:** Portanto, a irradiação com o laser, nos parâmetros e variáveis utilizados e em comparação com os grupos controle, afetou negativamente a adesão à dentina, não havendo alteração relevante na longevidade da adesão para os períodos de armazenagem avaliados.

PALAVRAS-CHAVE

Condicionamento com laser de Er:YAG; Resistência à microtração; Longevidade da interface adesiva..

INTRODUCTION

The modern concepts of adhesion in Dentistry are based on minimally invasive and conservative procedures to restore teeth due to carious lesions or trauma [1]. In view of this, one of the alternatives to the removal of caries is the use of high intensity laser treatment, which interacts with the water and hydroxyapatite present in dental structure [2].

Since the investigation about the effects of high intensity laser on tooth structure by Goldman et al. [3], the Er:YAG laser (which emits light with wavelength of 2.94 μm) has been increasingly studied because of the possibility of providing atraumatic, and in most cases, painless procedures. When used with the correct parameters, this laser can be safely used for conservative restorative dentistry promoting enamel surface etching or even selectively removal of dental tissue by the process called ablation [4-7]. In enamel, this ablation can create a micro retentive pattern, which would favor adhesive procedures. In dentin, scanning electron microscopy analysis of irradiated surface has revealed a rough substrate, without the presence of smear layer, with open dentin tubules and prominent peritubular dentin [8-11]. The effects of Er:YAG laser on cavity preparation have been widely described in the literature, as well as the bond strength of irradiated substrate to composite resin [5,12]; however, its longevity and stability have not yet been well-established.

Due to mechanical and chemical degradation, the hybrid layer created by modern adhesive systems may not be as durable as once believed [13-14]. Despite the constant development of adhesive systems and the achievement of efficient bond strength values [15-16], the bond interface is still the weakest area of restorations [17]. When the interface between dentin and composite resin is exposed to oral environment, marginal discoloration, poor marginal adaptation and consequent loss of restoration retention are still frequently observed.

Factors that may influence degradation of dentin-resin bond include exposure to water, and consequently, monomer degradation [18], incomplete hybridization [19] and presence of solvent or water trapped at the interface [20].

Although these factors may interfere in the conventional bond interface, the influence of Er:YAG prepared dentin on the short-term dentin-resin interface, as regards the longevity and stability of restorations, is still not known. Therefore, the null hypothesis tested was that the short-term bond strength is not affected by dentin preparation method, regardless of the adhesive system.

MATERIAL & METHODS

Experimental Design

The factors under study were the laser parameters at three levels (no laser irradiation, protocol 1: 250 mJ/10 Hz and protocol 2: 160 mJ/4 Hz), the adhesive system at two levels (Adper Single Bond 2 and Clearfil SE Bond), and the storage period at two levels (24 h and 90 days). The experimental units consisted of 60 dentin samples, randomly divided into six groups (n=10). The microtensile bond strength test and fracture pattern analysis were carried out at the interface between dentin prepared with Er:YAG laser at different time intervals, 24 h or 90 days after bonding of composite with different adhesive systems. The distribution of groups is presented in Table 1.

Table 1 - Experimental design and specimen distribution

Groups n = 10	Laser parameters n = 10	Adhesive system n = 10	Storage period n = 5
G1	---	Acid etching + Adper Single Bond 2	24 h / 90 days
G2	---	Clearfil SE Bond	24 h / 90 days
G3	250 mJ / 4 Hz	Acid etching + Adper Single Bond 2	24 h / 90 days
G4	250 mJ / 4 Hz	Clearfil SE Bond	24 h / 90 days
G5	160 mJ / 10 Hz	Acid etching + Adper Single Bond 2	24 h / 90 days
G6	160 mJ / 10 Hz	Clearfil SE Bond	24 h / 90 days

Sample Preparation

The study sample consisted of 60 recently extracted bovine teeth thoroughly cleaned with flour of pumice using a rubber cup in a slow-speed hand piece. Before preparing the specimens, the buccal enamel of each sample was ground flat in a water-cooled polishing machine (Politriz Ecomet 3, Buehler, Lake Bluff, Il, USA) with 600-grit silicon carbide paper (Struers Company, Cleveland, OH, USA) in order to remove the superficial enamel, expose the subjacent shallow dentin and standardize the surface and smear layer formation. All samples were analyzed under an optical microscope at 40x magnification for the verification of enamel absence.

Laser irradiation

The Er:YAG laser used was the Key Laser II (KaVo Dental, Biberach, Germany), with wavelength of 2.94 μm, pulse width of 250–500 microseconds, and handpiece 2051. The samples of G1 and G2 were not irradiated and served as control groups. For dentin preparation, irradiation for groups G3 and G4 was carried out with energy per pulse of 250 mJ, pulse repetition rate of 4 Hz, and fluence of 80.2 J/cm^2. Groups G5 and G6 were irradiated with energy per pulse of 160 mJ, pulse repetition rate of 10 Hz and fluence of 51.3 J/cm^2. The cooling system consisted of a water spray set for 6 mL/min. The spot size diameter was 0.63 mm. The focused laser beam was kept perpendicular to the target area during irradiation, and the handpiece was kept 12 mm from the target area (focused mode). This distance was standardized with a 21 mm file attached to the handpiece. A single operator performed all manual irradiations.

Restoration Procedure

All specimens were randomly assigned to two subgroups, according to adhesive systems used, one water-ethanol based “etch & rinse” adhesive, (Adper Single Bond 2, 3M/ESPE, St. Paul, MN, USA), and one water-based self-etching system (ClearFil SE Bond, Kuraray, Osaka, Japan), which were applied according to the manufacturer’s instructions with microbrush (Table 2). Samples were restored with composite resin Z250 (3M/ESPE, St. Paul, MN, USA) in increments of 2 mm, to form resin blocks measuring 5 x 5 x 5 mm. Each increment was light-activated (Optilux, Demetron, Kerr, Orange, CA, USA), separately for 30 seconds, also in accordance to the respective manufacturer’s instructions and after using its own radiometer. Subsequently, the specimens were stored in distilled water at 37 °C for 24 h or 90 days (n=5). During the 90 days storage, distilled water was renovated every week.

Table 2 - Specifications and procedures for the adhesive systems used

Specifications	ClearFil SE Bond, Kuraray, Japan	Adper Single Bond 2, 3M/ESPE, St. Paul, MN, USA
Composition	Self-etching primer: 10-MDP, HEMA, hydrophilic dimethacrylate, di camphorquinone, N,N-diethanol-p-toluidine, water Adhesive resin: MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, di-camphorquinone, N,N-diethanol-p-toluidine, silanized colloidal silica	Adhesive resin: water, alcohol, HEMA, BisGMA, dimethacrylate, copolymers of polyacrylic acids and acid polyitaconic
Procedures	First step: self-etching primer application on dentin for 20 seconds and gently air-dried Second step: adhesive resin application; air thinned and light polymerized for 10 seconds	First Step: etchant (35% phosphoric acid–3M/ ESPE) for 15 seconds and rinsed with water for 10 seconds Second step: application of two coats of Adper Single Bond 2 adhesive, the surface being gently air-dried for 5 seconds and light polymerized for 10 seconds

Microtensile Bond Strength

After 24 h of the bonding procedure, all samples (G1– G6) were sectioned into beams (1x1 mm) using a slow-speed diamond disc (Buehler Series 15LC Diamond; Buehler Ltd) under constant water coolant, and then subjected to a microtensile test either immediate or after three-months water storage (n = 5) [21-22]. Each beam was attached to a tensile apparatus using a cyanoacrylate adhesive (Super Bonder, Loctite Brasil Ltda, Itapevi, SP, Brazil). At all times, care was taken to ensure that the bonded area remained perpendicular to the long axis of microtensile strength testing apparatus. The beams were then stressed to failure under tension at a cross-head speed of 0.5 mm/min and a load cell of 100 N, with a testing machine (Instron Universal 4442, Canton, MA) adapted for microtensile tests. The exact dimension of each fractured beam was individually measured using a digital caliper. Final bond strength value (MPa) was calculated by dividing the peak force (N) by the cross-sectional area of the failed interface (mm^2).

Fractured surface analysis

The fractured surfaces were visually analyzed using a dental operating microscope (Carl Zeiss 398253 OPMI PICO, Essen, Germany) at 40x magnification to determine the fracture pattern of each specimen. Fracture patterns were classified as Type I: adhesive – failure within the adhesive and dentin or resin interface; Type II: cohesive in dentin - failure within the dentin substrate; Type III: cohesive in resin - failure within the resin; and Type IV: adhesive-cohesive – mixed failure, with both adhesive and cohesive failures within the same fractured surface.

Statistical analysis

The data were evaluated to check the equality of variances and normal distribution of errors (Shapiro-Wilk test). Intragroup analysis regarding the storage period (24 h or 90 days) and the type of adhesive system used was performed using paired-t test with a significance level of 5%. Intergroup analysis was also performed considering the same variables using ANOVA for multiple comparisons (SPSS Statistics, IBM Co., Armonk, NY, USA) with the Tukey test with a significance level of 5%.

RESULTS

The mean values of microtensile bond strength and their respective standard deviations are shown in Figure 1. The storage period did not influence bond strength significantly ($p >$

0.05), independently from adhesive system and the pretreatment protocol of dentin surfaces used. Likewise, adhesive systems did not influence bond strength values, and no statistically significant difference was observed among samples that received same pretreatment protocol and storage time ($p > 0.05$). Non-irradiated samples (G1, G2) presented statistically significant difference ($p < 0.05$) to irradiated ones (G3-G6), but no difference was observed between the irradiation protocols ($p > 0.05$).

As regards the fracture type, the qualitative analysis indicates that the mode of failure did not vary significantly with storage period. Experimental groups (G3 – G6) presented predominantly Type I fracture pattern, with adhesive failure between the dentin and composite resin. Comparatively, G2 (control group/Clearfil SE Bond) presented Type II fractures (cohesive in dentin) more frequently than G1 (control group/Adper Single Bond 2).

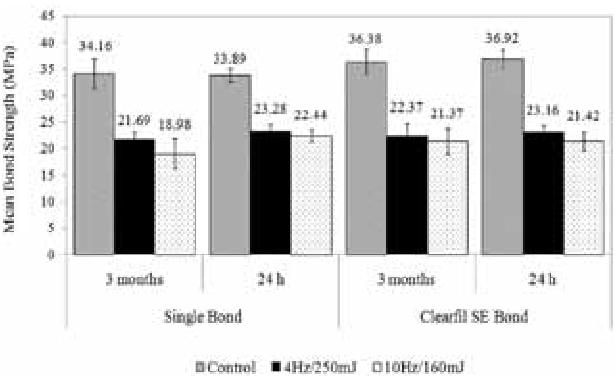

Figure 1 - Microtensile bond strength mean values (± standard deviation) according to dentin pretreatment, adhesive system and storage period

DISCUSSION

When considering ablation of dental substrate with high intensity lasers, important aspects should be taken into account for restorative procedures to be adequately performed, such as bond strength to the composite resin and durability of adhesive restorations.

It is not yet known whether laser irradiation of dentin can guarantee restoration stability over the course of time, retaining the physical and mechanical features of the bond interface. In the present study, lower microtensile bond strength values were found for the irradiated groups; however, these numbers did not decrease with time, and behaved similar to non-irradiated samples. Therefore, under the conditions of this study, the null hypothesis tested has failed to be rejected.

When the dentin/resin interface is exposed to the oral cavity environment, clinical findings such as leakage, marginal discoloration and poor marginal adaptation can be observed [18]. The results of this study have shown that dentin treated with Er:YAG laser presented lower bond strength values compared with the non- irradiated groups ($p < 0.05$); despite these values, the bond strength of irradiated substrates are sufficient to provide adequate bonding procedures (> 20 MPa) [23].

The results of this study are in agreement with those of Brulat et al. [24], although they performed a shear bond strength test using Clearfil SE Bond on dentin previously treated with Er:YAG laser at higher settings (350 mJ/10 Hz). Martinez-Insua et al., [9] could also not notice beneficial effects of Er:YAG laser (160 mJ/4 Hz) on dentin bond strength to brackets. Recent studies on adhesion after dental substrate irradiation with erbium lasers indicate that re-etching irradiated surface with phosphoric acid may remove altered substrate layer and provide higher bond values [25].

It is known that dentinal surface irradiated with the Er:YAG laser shows morphological changes caused by the increase of temperature [2,26]. Despite the beneficial alterations such as smear layer removal and opening of dentinal tubules, adverse modifications resultant from ablation's micro-explosions can also be noticed [2,26,27, 28]. The particles ejected during ablation process can sediment on the irradiated area, forming a debris-rich surface [2]. These factors may contribute to an unfavorable effect on restorative material bonding [26]. Ceballos et al., [27] evaluated irradiated dentin under transmission microscopy and noticed the presence of fused remnants, as well as poorly denatured collagen fibrils attached to the underlying dentin substrate, and lack of interfibrillar spaces. These characteristics are thought to restrict resin diffusion into the subsurface intertubular dentin, thus lowering shear bond strength to resin composite. Moretto et al. [28] showed in an in vitro study that erbium laser interacts with dental hard tissue resulting in a specific morphological pattern of dentin and collagen fibrils that negatively affected the bond strength to composite resin. Some other hypotheses are discussed by De Moor and Delmé [26], who reported that the subsurface alterations produced by Er:YAG ablation were responsible for the decrease in bond strength, as well as cohesive failure in the sub-bonding layer in dentin. Additionally to the denaturation of collagen fibrils, temperature increase may lead to melting and vitrification of dentin substrate. This process should be avoided, due to its negative influence on bond strength to composite. Vitrification results from the recrystallization of dentinal apatite, with possible formation of an additional calcium phosphate phase, caused by the production of heat. This is followed by rapid contraction during the cooling phase, resulting in high internal stress that may contribute to the formation of microfractures in the irradiated dentin [29].

In the current study, one water-ethanol based “etch & rinse” and one water-based self-etching adhesive system were used. No statistically significant difference on bond strength values was observed between them, either immediately or longitudinally, 90 days after adhesive procedures. De Oliveira et al. [30] described that for these same adhesive systems, thick hybrid layers were observed with confocal microscopy for Er:YAG laser-modified dentin surface. However, the authors also suggested that hybrid layers promoted by both adhesive systems were non-uniform and it is well accepted that bond strength and durability rely on the quality of the hybrid layer rather than on the thickness or morphology of hybrid layer/resin tags [31].

The fracture pattern analysis resulting from the microtensile test revealed that the predominant pattern observed was Type I or Adhesive for the Er:YAG laser irradiated groups, while fracture types I, II and III were randomly distributed for the non-irradiated groups. It should be highlighted that very similar findings were found for both evaluated aging periods, showing that the laser treated groups were more susceptible to bond interface fracture. Moreover, Type I fractures occurred predominantly between the adhesive layer and dentin, inferring that the process may have produced some specific difficulty in hybrid layer formation. This could be explained by the laser-induced structural alterations in the collagen fiber network [2,27], which are mainly located in intertubular dentin. This dentin is the most important for hybrid layer formation, and its damage may justify the lower microtensile bond strength values observed for irradiated samples in this study. It could also be hypothesized that a weaker and less stable collagen layer is engineered, leading to a resultant hybrid layer more prone to degradation.

The study of bond longevity after laser irradiation showed that no significant difference could be observed between both storage periods. Further studies should be carried out in order to obtain a microtensile bond curve and its trend towards stability or decreasing of bond strength, even including some biodegradation analysis of the interface, such as nanoleakage under transmission microscopy. This study evaluated bond values after a three-month storage period in water. Studies increasing storage periods and/or adding collagenolytic enzymes have to be conducted in order to provide greater challenges that could better reflect clinical needs for adhesive systems.

The lower bond strength values after laser irradiation found in this study may be attributed to the Er:YAG laser large pulse duration (250-500 μs), which can lead to residual thermal damage of collagen fibers [32]. The increase of temperature results in collagen denaturation, therefore compromising adhesion. Studies on bond strength to dentin after irradiation with ultra-short pulsed lasers are being conducted, and indicate that less increase of temperature and absence of damage to dentin substrate may be expected [28]. Although the effects of laser did not provide an additional improvement in bonding in the present study, other benefits should be considered, such as elimination of the smear layer and microbial reduction [27]. Laser irradiation of dental hard tissues also modifies the calcium phosphate ratio and leads to the formation of more stable and less acid-soluble compounds, thus reducing the susceptibility to acid attack [29].

New adhesive systems are frequently introduced, but they are all designed to be used in conventionally prepared cavities in the presence of a smear layer. The substrate irradiated with the Er:YAG laser is free of smear layer and bacteria, and demands new investigations on different laser irradiation parameters, as well as on new adhesive protocols to be adopted when bonding to the peculiar substrate surfaces, in order to enable proper hybrid layer formation, and therefore, a long lasting restoration.

CONCLUSION

Er:YAG laser irradiation, within the parameters and conditions studied, could not provide an additional improvement in dentin-resin bond strength, irrespective of the type of adhesive system used or the storage period evaluated.

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