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Adhesion to eroded dentin submitted to different surface treatments

Adesão a dentina erodida submetida a diferentes tratamentos de superfícies

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ABSTRACT

Objective: This in vitro study measured the microshear bond strength (μ SBS) of a composite resin to sound and artificially eroded dentin, submitted to surface treatment with diamond bur (DB) or Er, Cr:YSGG laser (L). Material and Methods: Bovine radicular dentin samples were randomly divided into six groups (n = 11): G1positive control (sound dentin), G2- negative control (eroded dentin), G3-eroded dentin treated with Er, Cr:YSGG laser at 1.5 W, G4-eroded dentin treated with Er, Cr: YSGG laser at 2.0 W, G5-eroded dentin treated with Er, Cr:YSGG laser at 2.5 W and G6-eroded dentin treated with diamond bur. Erosive cycling was performed by immersion in 0.05M citric acid (pH 2.3; 10 min; 6x/day) and in remineralizing solution (pH 7.0, 1 h, between acid attacks), for 5 days. Three composite resin cylinders were bonded to the samples with etchand-rinse adhesive system and after 24 h storage in distilled/deionized water (37 °C), samples were submitted to microshear bond strength test and mean values (MPa) were analyzed by one-way ANOVA and Tukey tests ($\alpha = 0.05$). Results: G1 $(19.9 \pm 7.6^{\text{A}})$ presented the highest μ SBS mean followed by G6 (12.2 \pm 3.8^B), which showed no statistically significant difference compared with the other groups, except from G4. The lowest μ SBS value was found for G4 $(7.1 \pm 1.5^{\circ})$, which did not differ statistically from G2 (7.5 \pm 1.8B,C), G3 $(8.4 \pm 1.8^{B,C})$ and G5 $(8.6 \pm 3.2^{B,C})$. Analysis of the fracture pattern revealed a higher incidence

RESUMO

Objetivo: O presente estudo in vitro visou avaliar a resistência de união (RU) de uma resina composta à dentina hígida e dentina erodida artificialmente, submetidas a diferentes tratamentos de superfície: ponta diamantada (DB) ou Er, Cr: YSGG (L) em diferentes parâmetros. Material e Métodos: Amostras de dentina radicular bovina foram aleatoriamente divididas em seis grupos (n = 11): G1- controle positivo (dentina hígida sem tratamento); G2 - controle negativo (dentina erodida sem tratamento); G3 - dentina erodida condicionada com laser de Er, Cr:YSGG (L) em 1,5W; G4 - dentina erodida condicionada com Er,Cr:YSGG em 2,0 W; G5 - dentina erodida condicionada com Er, Cr: YSGG em 2,5 W e G6 - dentina erodida tratada com ponta diamantada. A formação da lesão de erosão foi realizada através de 5 dias de ciclagem por imersão alternada em solução desmineralizadora (ácido cítrico 0,05 M; pH 2,3; 10 min; 6x/dia) e em solução remineralizadora (pH 7,0; 1 h, entre os ataques de ácido). Três cilindros de resina composta foram confeccionados na superficie plana das amostras com o auxílio de um sistema adesivo tipo "condicione e lave". Após o armazenamento em água destilada/ deionizada por 24 h a 37 °C, os corpos de prova foram submetidos ao ensaio de microcisalhamento e a media dos valores de RU (MPa) obtidos foram analisados pelo teste ANOVA e teste de Tukev (α = 0,05). Resultados: Os resultados mostraram que G1 (19,9 \pm 7,6^A) apresentou os maiores valores de RU seguido do grupo G6 (12,2 \pm 3,8^B), que não apresentou diferença estatisticamente significativa em comparação com os outros grupos, com exceção do G4. O menor valor de RU foi encontrado no grupo G4 (7,1 \pm 1,5^c), que não diferiu estatisticamente do G2 (7,5 \pm 1,8^{B,C}), G3 (8,4 \pm 1,8^{B,C}) e G5 (8,6 \pm 3,2^{B,C}). A análise do padrão de fratura revelou uma maior

of adhesive fractures for all experimental groups. **Conclusion:** The results indicate none of the surface treatments (diamond bur and Er,Cr:YSGG laser irradiation at the parameters used in this *in vitro* study), associated with the etch-and-rinse adhesive system, did not enhance composite resin bonding to eroded dentin.

KEYWORDS

Dentin; Lasers; Tooth erosion.

INTRODUCTION

D ental erosion is a chemical process characterized by the surface dissolution of dental hard tissues, as a result of the exposure to a variety of acids, without the involvement of microorganisms [1]. At earlier stages, the erosive process involves enamel demineralization, which is characterized by initial softening and increased roughness of the surface [2]. As this process continues, there is progressive dissolution of the enamel crystals, leading to a permanent loss of tooth volume with a softened layer persisting at the surface of the remaining tissue [1]. In advanced stages, the dentin substrate becomes exposed1 and restorative procedures may be necessary.

In the most conventional restorative procedures, the method involves the use of a handpiece at low and high speeds, which is fitted with rotary cutting instruments, such as carbide or diamond burs [3]. Nevertheless, in spite of the low cost and shorter time taken to perform this technique, it can cause pain, vibration and discomfort to the patient. This is why new technologies have advanced in dentistry [4].

Laser has been introduced in dental practice for the removal of mineralized dental tissues before the application of restorative materials. Erbium lasers (Er:YAG and Er,Cr:YSGG) are capable of effectively ablating dental hard tissues because of their high coefficient of absorption in both water and incidência de fraturas adesivas para todos os grupos experimentais. **Conclusão:** Os autores concluíram que nenhum dos tratamentos realizados (ponta diamantada e irradiação com laser Er,Cr:YSGG, nos parâmetros utilizados neste estudo *in vitro*), associados ao sistema adesivo tipo *"condicione e lave"* não aumentou a resistência adesiva da resina composta à dentina erodida.

PALAVRAS-CHAVE

Dentina; Lasers; Erosão dentária.

hydroxyapatite. In addition, they only minimally increase the temperature in the surrounding tissues, especially when irradiation is performed under constant cooling with a continuous water spray [5]. As laser irradiation produces surface modifications, it has been suggested that it could be used for dentin pretreatment prior to the bonding procedures [3,6,7].

irradiated The dentin presents microstructural changes that include the formation of a microscopically rough surface without demineralization and irregular and open dentinal tubules without a smear layer, resembling scales [7]. As the peritubular dentin is richer in minerals than in water, it has the appearance of being slightly protruded in relation to surrounding intertubular dentin. This indicates that when used with the correct parameters, these lasers can promote morphological changes in the tooth surface that appear to be advantageous for resin bonding [7-13]. Therefore, the purpose of this in vitro study was to evaluate the microshear bond strength of a etch-and-rinse adhesive system to eroded dentin treated with bur or Er, Cr:YSGG laser prior to restorative procedure.

MATERIALS AND METHODS

Sample Preparation

Sixty-six root dentin slabs (6 x 5 x 2 mm) were obtained from the cervical third of selected bovine incisor teeth, with the use of a

low speed water-cooled diamond saw (Buehler Ltda, Lake Bluff, USA). The slabs were placed in a polyvinylchloride ring and embedded in epoxy resin. The dentin surfaces were flattened and polished with 400- and 600- grit silicon carbide sandpaper discs (Buehler Ltda, Lake Bluff, USA) for 60 s and sonicated for 10 min in deionized water. The specimens were randomly divided into six groups (n = 11): G1 – Positive control (sound dentin); G2 – Negative control (eroded dentin); G3 – Er,Cr:YSGG laser 1.5 W, 20 Hz, 17.1J/cm²; G4 – Er,Cr:YSGG laser 2.0W, 20 Hz, 22.8 J/cm²; G5 – Er,Cr:YSGG laser 2.5 W, 20 Hz, 28.5 J/cm²; G6- Diamond bur.

Erosive Cycling

The erosive pH cycling was performed by immersion in 0.05 M citric acid (C_eH_oO₇. $H_0O; M = 210.14 \text{ g/mol-E.Merck, Darmstadt,}$ Germany) (pH 2.3) for 10 min, 6x/day. Between acid attacks, samples were immersed in a remineralizing solution (1.5 mmol/L CaCl₂, 1.0 mmol/L KH₂PO₄ and 50 mmol/L NaCl) [14], pH 7.0, for 1 h, at room temperature (25 °C), under constant and gentle agitation on a shaker, according to Ganss et al. [15] During the remaining 18 h, samples were stored in remineralizing solution, also under constant and gentle agitation, until the beginning of the next cycle. The cycles were repeated for 5 days [15]. The solutions were renewed everyday, and the pH of the solutions was checked three times daily. Optical Coherent Tomography (OCT), following the method described by Azevedo et al. (2011)

[16], was considered for the evaluation of three dentin samples, which illustrated the erosive patter induced in the present study (Figure 1). The mean surface loss was of approximately 170 μ m. The experimental immersion time of this *in vitro* study (10 min, 6x/daily) represents severe erosive conditions.

Surface treatment

The control groups (G1 and G2) were not submitted to surface treatment, consisting on the positive control group (sound dentin) and negative control group (eroded dentin), respectively. The experimental groups were treated as follows:

• G3, G4 and G5: samples were submitted to laser irradiation using the Er,Cr:YSGG laser (Waterlase Millenium®, Biolase Technologies, San Clemente, CA, USA) at 2.78 μ m, with a pulse duration of 140 – 200 μ s and fixed repetition rate of 20 Hz. Three different power settings no ablative - were used for dentin treatment: 1.5 W (17.1 J/cm²), 2.0 W (22.8 J/cm²) and 2.5 W (28.5 J/cm^2). The irradiation was performed under water/air cooling (65 % water and 55 % air), based on parameters described in previous studies [17,18]. A sapphire tip with 378 μ m in diameter was manually positioned at approximately 1.0 mm (90°) from the dentin surface (focused mode). Before and during the samples irradiation, the output power of the laser beam was measured with a power meter (GSTM FieldMaster Power, Energy Analyzer, Coherent, Inc., Germany) and was found an average power loss of 25 %.

Figure 1 - OCT (Optical Coherent Tomography) image, which represents the erosive pattern induced in the present study.



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• *G6:* samples were treated with a diamond bur (#2135FF, KG Sorensen, Barueri, SP, Brazil), in a water-cooled high-speed turbine (Kavo do Brasil, Joinville, Brazil). The active tip was positioned parallel to the surface and activated for 5 s to remove a thin layer of the surface modified by the erosive cycling. Due to their inherent wear and short durability, new burs were used after every five preparations.

Adhesive and Restorative Procedures

After surface treatments, the dentin samples, previously isolated with an acid/ solvent resistant adhesive tape containing 1 mm diameter aligned perforations, were etched with 37 % phosphoric acid (CondAC 37 %[®], FGM, Joinvile, SC, Brazil) and bonded with an etchand-rinse adhesive system (Adper Single Bond®, 3M ESPE Dental Products, St. Paul, MN, USA), according to manufacturer's instructions. Tygon tubes (R-3603, Norton Performance Plastic Co., Cleveland, Ohio, USA) - 1.0 mm in diameter x 0.5 mm high - were fixed to the dentin surface, with distances of approximately 1.0 mm between them. After this, a flowable composite resin (Filtek Flow[®], 3M ESPE Dental Products, St. Paul, MN, USA) was inserted into the Tygon tubes and light activated for 20s, using a halogen light at approximately 650 mW/cm² (XL3000, 3M ESPE, St. Paul, MN, USA).

Microshear Bond Strength (µSBS) Test

After 24h-storage in distilled/deionized water at 37 °C, the Tygon tubes were removed using a scalpel and steel wire (0.2 mm diameter) was placed around each resin cylinder individually, to fix it to a device. After this, each cylinder was submitted to the microshear bond strength test in a universal testing machine (4411/ Instron Corp., Canton, MA, USA) at a speed of 1.0 mm/min until fracture of the composite resin cylinder. The results were obtained in Kg/cm² and expressed in MPa. Fractured specimens were stained for 5 min with 2% Ponceau S dye [19] and observed at 40x magnification using an optical microscope (Miview Digital Microscope, Cosview Technologies Co., Ltd., Bantian, Longgang

Dist., China) for determination of failure modes (adhesive, cohesive, and mixed failures).

Statistical Analysis

Mean and standard deviation were calculated for each experimental group, considering the mean μ SBS values of each sample. The data were analyzed using the SPSS17.0 (**SPSS** Inc., Chicago, Illinois, USA) software for Windows, by one-way ANOVA with subsequent pairwise comparisons using the Tukey test ($\alpha = 0.05$).

RESULTS

Results are presented in Table 1. G1 (19.9 \pm 7.6) presented the highest μ SBS mean followed by G6 (12.2 \pm 3.8), which showed no statistically significant difference in comparison with the other experimental groups, except from G4. The lowest μ SBS mean value was found for G4 (7.1 \pm 1.5), which did not result in significant differences compared with G2 (7.5 \pm 1.8), G3 (8.4 \pm 1.8) and G5 (8.6 \pm 3.2). In all experimental groups, the failure mode was predominantly adhesive (between adhesive and dentin) but in laser treated groups (2.0 and 2.5 W) cohesive failures in dentin were observed, as shown in Figure 2.

Table 1 - Mean μSBS (MPa) and SD (standard deviation) for the experimental groups

Groups	Surface treatment	µSBS (MPa)
1	Sound dentin	19.89 ± 7.59 ^A
2	Eroded dentin	$7.49 \pm 1.84^{\text{B,C}}$
3	Eroded dentin treated with Er,Cr:YSGG laser 1.5 W	$8.45 \pm 1.83^{\text{B,C}}$
4	Eroded dentin treated with Er,Cr:YSGG laser 2.0 W	7.07 ± 1.55°
5	Eroded dentin treated with Er,Cr:YSGG laser 2.5 W	8.62 ± 3.17 ^{B,C}
6	Eroded dentin treated with diamond bur	12.17 ± 3.85 ^B

Different letters indicate statistical difference between experimental groups (rows).



Figure 2 - Prevalence (in %) of the failure mode in each experimental group.

DISCUSSION

involves Dental erosion complex histological changes in the dentin and the acid causes demineralization of the outer surface [1]. Frequent exposure to acid leads to a completely demineralized organic matrix, followed by a partially demineralized zone until a sound inner layer that has not been affected by the acid, is achieved [1,20]. The present results showed that erosion negatively affected the bond strength of all groups. This can be explained by the presence of denatured collagen that does not favor adhesion [21]. Similar findings were reported by Zimmerli et al. [22].

Restorative treatment may be necessary if the structural integrity of the tooth is threatened, dentin is hypersensitive, the lesion is esthetically unacceptable to the patient and/or there is a likelihood of pulp exposure [23]. In this study, the results showed that the highest bond strength value was obtained by the positive control group (sound dentin), which received no surface treatment associated with the use of the etchand-rinse adhesive system. All other treatments performed on eroded dentin, were unable to effectively remove or treat the demineralized surface and revert the negative effects of erosion on bond strength.

Diamond burs are the rotary instruments most frequently used for cavity preparation, removal of the softened outer surface and for increasing the longevity of the restoration [24]. They are less expensive and can easily be used. However, they have disadvantages, such as producing unpleasant noise and vibration in the dental structure, which can generate pain and tension in patients [25].

In the present study, the treatment that showed a tendency to increase the bond strength of eroded dentin was the diamond bur preparation. However, the diamond bur increased only the numerical bond strength values, which were statistically similar to those of the other irradiated groups (with exception to group G4) and even to those of the negative control group (eroded dentin).

Improvements in laser technology have led the use of erbium lasers for caries removal, cavity preparations, and enamel and dentin surface modifications. Our data suggest that Er,Cr:YSGG laser using the present parameters of irradiation (1.5, 2.0 and 2.5 W), associated

with etch-and-rinse adhesive systems, was not able to change the eroded dentin surface in order to turn it adequate for the adhesive restoration. Considering the parameters selected for dentin irradiation, there are two hypothesis to be raised related to laser effects: the first one is that Er.Cr:YSGG laser was not able to remove the outer surface altered by erosion, and consequently, did not cause positive effects on the bond between the composite and eroded dentin. In agreement with these results, some authors [6,10,11,17,26] have also reported that irradiation with Er, Cr:YSGG laser, for both cavity preparation and surface conditioning, did not improve the procedure of adhesive bonding to the dental substrate. The second hypothesis is that Er,Cr:YSGG removed the demineralized surface but induced surface/subsurface changes that were unfavorable for adhesion [6]. Further investigations on ultrastructural changes on the dentin substrate and on bonding interface are needed to clarify the findings of the present study. Also, the amount of eroded dentin ablated by the Er, Cr: YSGG laser and its comparison with the amount removed by the diamond bur would certainly give important information for the understanding of the interaction of the erbium laser with the eroded dentin surface.

Depending on the energy density used, the thermomechanical effects of the laser can cause microcracks [27], collagen fibril denaturation [6,28], formation of an acid-resistant surface with granular structures, or a carbonized or melted surface, which can hinder infiltration of the adhesive system, hybrid layer formation and reduce the bond strength [6,11,28,29]. Further investigations on the alterations in the dentin structure should be carried out to explain these results.

In contrast to the present study, Ergücü et al. [30] examined the effect of irradiation with Er,Cr:YSGG on the microtensile bond strength of an etch-and-rinse (Scotchbond Multipurpose, 3MESPE) and a self-etching (AdheSE, Ivoclar Vivadent) adhesive system to sound dentin (4 W, 25 Hz, 70 % air and 30 % water) and caries-affected dentin (2 W, 25 Hz, 65 % air and 55 % water). The laser did not influence the clinical performance of adhesive systems and the data obtained showed no statistically significant differences between treatment with conventional rotary instruments and Er, Cr:YSGG laser. Although the parameters used by Ergücü et al. [30] (2 W, 25 Hz, 65 % air and 55 % water) are similar to the ones used in our study, different results could be attributed to different variables, such as the chemical composition of the dentin substrate that was submitted to erosive or cariogenic challenges, exposure time to the laser beam, hydration of the dentin samples, and others. Therefore, studies using different parameters and adhesives systems are necessary, also considering eroded dentin.

Analysis of the fracture pattern revealed a higher incidence of adhesive and mixed fractures for all experimental groups. Interestingly, the groups irradiated with the higher parameters of the erbium laser (2.0 and 2.5 W) leaded to cohesive failures in dentin, supporting the hypothesis that dentin subsurface could have been altered as described by Moretto et al. [6]. This may indicate that the laser treatment at high powers can lead to the fragility of the dentin, which can be unfavorable to adhesion, while 1.5 W was unable to change the dentin surface and did not contribute to the enhancement of the bond strength between composite resin and dentin.

In general, all the treatments for eroded dentin proposed in the present study, showed no differences in microshear bond strength in comparison with the positive control group, which received no surface treatment, suggesting that none of them was able to effectively change the eroded dentin surface. These results are innovative, considering that up to now, there is only one report in the literature as regards the treatment of eroded surfaces, using high power laser - Er,Cr:YSGG [3]. Finally, to identify a protocol that promotes higher bond strength between dentin and restorative material is highly challenging to laser researchers. Given Ramos-Oliveira TM et al.

the results of this study, further studies must be conducted in order to provide valuable clinical guidelines for improving the bond strength between eroded dentin and resin composite.

CONCLUSION

The results of the present *in vitro* study indicate that none of the surface treatments (diamond bur and Er,Cr:YSGG laser irradiation), associated with a etch-and-rinse adhesive system, had a positive effect on the microshear bond strength to eroded dentin.

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