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Analysis of adhesive bond strength under simulated pulpal pressure during laser irradiation on different dentin thicknesses

Análise da resistência adesiva sob pressão pulpar simulada durante irradiação laser em diferentes espessuras de dentina

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ABSTRACT

Objective: Evaluate the microtensile bond strength (μTBS) in different dentin thicknesses, under simulated pulpal pressure (SPP), submitted to an adhesive technique using laser irradiation. **Material and methods:** Forty sound human molars were sectioned and randomly divided into two groups (n=20): Group 1 – 1 mm of dentin thickness; Group 2 – 2 mm of dentin thickness. Each group was divided into two subgroups (n=10): Subgroup A – Absence of SPP; Subgroup P – Presence of SPP (15 cm H_2O). The samples were sequentially treated with: 37% phosphoric acid, adhesive system (Adper Single Bond 2), Nd:YAG *laser* irradiation (60 s, 1064 nm, 10 Hz) using 60 and 100 mJ/pulse energy parameters and photopolymerization (10 s). A composite resin block (Filtek Z350) was built up onto the irradiated area. After 30 days stored in water, the samples were sectioned and submitted to microtensile test (10 kgf load cell, 0.5mm/min). Data were analyzed by two-way ANOVA and Tukey tests. **Results:** Two-way ANOVA revealed no significant differences for SPP on bond strength. The laser energy parameters indicated that 100 mJ showed greater μTBS means compared to the group irradiated with 60 mJ. The presence of SPP reduced the mean μTBS values. **Conclusions:** Simulated pulpal pressure did not affect the μTBS using 60 mJ of laser energy parameter. At 100 mJ, the presence of SPP negatively influenced the bond strength, regardless of dentin thickness.

KEYWORDS

Bond strength; Dentin; Intrapulpal pressure; Nd:YAG laser.

RESUMO

Objetivo: O objetivo deste estudo foi avaliar a resistência adesiva (RA) em diferentes espessuras de dentina, associada à pressão pulpar simulada (PPS), quando submetidos à técnica adesiva por irradiação laser. **Material e Métodos:** Quarenta molares humanos hígidos foram seccionados e divididos aleatoriamente em dois grupos (n=20): Grupo 1 – 1 mm de espessura de dentina; Grupo 2 – 2 mm de espessura de dentina. As amostras foram divididas em 2 subgrupos (n=10): Subgrupo A – ausência de PPS; Subgrupo P – presença de PPS (15 cm de H_2O). As amostras foram tratadas seqüencialmente com: ácido fosfórico 37%, sistema adesivo (Adper Single Bond 2), irradiação com Nd:YAG laser (60 s, 1064 nm, 10 Hz) nos parâmetros de energia de 60 e 100 mJ/pulso e fotopolimerização (10 s). Um bloco de resina composta (Filtek Z350, 3M ESPE) foi confeccionado sobre a área irradiada. Após 30 dias armazenados em água, os espécimes foram seccionados e submetidos ao teste de microtração (carga de 10 kgf, 0.5mm/min). Os dados foram analisados pelos testes ANOVA sob 2 fatores e Tukey ($p<0.05$). **Resultados:** ANOVA mostrou que não houve diferenças significativas para PPS na RA. Para os parâmetros de energia do laser, 100 mJ apresentou maiores médias de RA quando comparado ao grupo irradiado à 60 mJ. A presença da PPS reduziu as médias de RA. **Conclusão:** Pressão pulpar simulada não afetou os valores de resistência adesiva para o grupo irradiado com 60 mJ. Para 100 mJ, a presença da pressão pulpar influenciou negativamente na resistência adesiva, independente das espessuras de dentina.

PALAVRAS-CHAVE

Resistência adesiva; Dentina; Pressão hidrostática; Nd:YAG Laser.

INTRODUCTION

The development and regular use of adhesive materials has revolutionized many aspects of restorative and preventive dentistry [1]. The adhesion mechanism to enamel and dentin is essentially an exchange process that involves the replacement of minerals removed from dental tissue, as a result of etching, by resin monomers with low surface tension and high diffusion capacity [2,3]. However, dentin bonding is a complex process and still represents a challenge, when considering morphological and structural differences at different depths [4] and moisture surface characteristics [5]. In an attempt to minimize the negative effects of excess moisture on adhesive procedures and standardize the morphology of hybridization zone, studies had been evaluated the effects of Nd:YAG (neodymium-doped yttrium aluminium garnet) laser irradiation on dental tissues [6–11].

The first applications of Nd:YAG laser on dentin surface resulted in decreased bond strength. The laser was applied to sound dentin prior to the adhesive system, resulting in heat denaturation of the organic components, as well as obliteration of the dentin tubules by melting and recrystallization of the inorganic components and low bond strength values [6,10,12].

In 1999, Gonçalves et al.[6], through an *in vitro* study, developed the technique of Nd:YLF laser irradiation on dentin surface previously etched and impregnated by non-polymerized adhesive system. The authors obtained excellent bond strength results. Further studies had shown that laser irradiation technique significantly increases dentin bond strength, depending on laser energy parameters [2,10,13]. These greater results are due to the fusion and recrystallization of dentin in the presence of resin monomers, providing a more chemically environment for the adhesive process [6]. Laser also seems to optimize adhesion by improving evaporation of residual solvents [14].

Laser may raise the temperature of irradiated substrate, transmitting this heat to the pulp. This increase is directly proportional to the energy emitted by the laser and can result in irreversible damage. The literature believes that the temperature rise above 5,5°C in the pulp chamber represents a risk of pulpal necrosis [15], a fact that has been discussed by some researches due to the extreme conditions which these results were obtained, such as permanence time of the aggressor agent, intermittence or not of the heat application and pulp flow [16–18].

Simulated pulpal pressure effectively reproduces *in vivo* clinical conditions, allowing heat dissipation by renewing fluid within the pulp chamber. Silva et al. [13] observed a decrease in the variation of temperature when laser irradiation was performed in the presence of simulated pulpal pressure. Santis et al. [19] corroborated these results, also verifying the dentin thickness in 1 or 2 mm does not change the temperature differences observed at the pulp level. However, the presence of simulated pulpal pressure in relation to dentin thickness showed great influence in the reduction of the temperature. The question remains whether the same would occur in the bond strength.

Thus, the aim of this study was to evaluate the bond strength (μTBS) in different dentin thicknesses, under simulated pulpal pressure, submitted to an adhesive technique using laser irradiation. The null hypotheses were: (1) dentin thickness did not result in significant influence on μTBS; (2) simulated pulpal pressure, during laser irradiation, did not result in significant influence on μTBS; (3) laser energy parameters did not result in significant influence on μTBS.

MATERIAL AND METHODS

Samples preparation

Forty sound human molars that were extracted for therapeutic reasons were used in this study, under approval of Research Ethical Committee at the São José dos Campos School of Dentistry (protocol number 283.030). The teeth were cleaned and stored in deionized water at 4ºC, for a maximum period of 30 days.

The teeth were fixed into an acrylic holder using dental wax and sectioned using low-speed laboratory cutting machine (Labcut 1010, Extec Technologies Inc., Enfield, CT, EUA) under water cooling. The teeth were sectioned parallel to the occlusal surface to expose dentin and 1 mm below the enamel-cementum junction to separate crown from the roots and exposure the pulp chamber.

Tooth sample was taken to a polishing device (DP-10, Panambra, São Paulo, SP, Brasil), using 600-grit aluminum oxide abrasive disks, under water cooling, to standardize 20 samples with approximately 1 mm of dentin thickness, and 20 samples with approximately 2 mm thickness from the highest pulp horn.

In sequence, the tooth samples were randomly divided into two subgroups (n=10), according to the presence or absence of simulated pulpal pressure (SPP). Each group was divided into other two subgroups (n=5), based on Nd:YAG laser energy parameters (60 mJ and 100 mJ).

Simulated pulpal pressure

For the group submitted to simulated pulpal pressure, the pressure device was adapted from the model proposed by Silva et al. [13]. The device had a reservoir filled with deionized water, placed 15 cm above the level of the pulp chamber.

Restorative procedure

The dentin surface was etched with 37% phosphoric acid A (Magic Acid, Coltene Vigodent SA Industria e Comercio, Rio de Janeiro, RJ, Brazil), for 15 s and rinsed. To remove excess water, the surface was gently dried with jets of air. The adhesive system (Adper Single Bond 2, 3M ESPE Dental Products, Saint Paul, MN, USA) was applied according to the manufacturer's instructions.

Before photopolymerization, the dentin surface impregnated with non-polymerized adhesive system was irradiated with Nd:YAG laser (Neodymium: Yttrium- Aluminium-Garnet) Pulse Master 600 IQ (American Dental Technologies, EUA), at wavelength of 1064 nm and a 320 μm diameter of optical fiber [6]. The frequency of 10 Hz was applied using 60 and 100 mJ pulse of energy parameters [19]. Laser irradiation was applied in noncontact mode and with surface scanning for 60 s vertically and horizontally, similar to a stepping motor. During laser irradiation, the laser fiber tip was positioned at 90º perpendicular to dentin surface and at a distance of 1 mm. The laser irradiation was executed by the same calibrated operator.

In sequence, the adhesive system was photopolymerized (LED Light Curing System, Demi Plus, Kerr Corporation, Middleton, WI, USA), with power density of 1200 mW/cm^2, for 10 s.

Composite resin blocks (4 mm diameter and 2 mm height) were built up on the irradiated dentin surface. Composite resin (Z350 XT, 3M/ESPE, St Paul, MN, USA) was inserted in two increments of about 2 mm each, with each increment photopolymerized for 20 s (LED Light Curing System, Demi Plus, Kerr Corporation, Middleton, WI, USA). All restored samples were stored in deionized water for 30 days, at 37ºC.

Microtensile bond strength (μTBS)

After 30 days, the samples restored were sectioned using a laboratory cutting machine (Labcut 1010, Extec Technologies Inc., Enfield, CT, EUA) to obtain dentin-composite resin sticks (1 mm^2). The sticks were stored in individual and identified vials (Eppendorf, São Paulo, SP, Brasil) at 37ºC, containing deionized water for 24 h, before testing.

The microtensile bond strength test was performed in a universal testing machine (EMIC DL-1000, São José dos Pinhais, PR, Brazil) at a crosshead speed of 0.5 mm/min and using a 10kg load cell. The mean value (in MPa) for the sticks originating from each tooth was calculated and used for the statistical analysis.

Statistical analysis

Data were analyzed by two-way ANOVA (pulpal pressure, laser energy) according to dentin thickness (1 mm and 2 mm), followed by Tukey test (α=5%).

RESULTS

According to the two-way ANOVA (Table 1), laser energy parameters significantly affected ($p < 0.05$) the bond strength in the group of 1 mm of dentin thickness. The interaction ($p = 0.4985$) and the simulated pulpal pressure showed no significant differences ($p = 0.2653$) in the bond strength, under the experimental conditions.

Table 1 - Two-way ANOVA for 1 mm of dentin thickness.

Source	DF	SS	MS	F	*p*
Laser	1	3024	3024	55.97	0.0001*
Pulpal Pressure (SPP)	1	72.33	72.33	1.339	0.2653
Interaction	1	26	26	0.4812	0.4985
Residual	15	810.4	54.03		

* Statistically significant differences (p < 0.05).

When considering the laser energy parameters ($p < 0.0001$), the highest bond mean values was observed in the group irradiated with 100 mJ and restored in the absence of SPP $(49.61 \pm 9.59)^{Ab}$. For the group irradiated with 60 mJ, there were no significant differences between the presence or absence. However, it differed significantly from the group irradiated with 100 mJ (Table 2).

Table 2 - Group: 1 mm of dentin thickness. Means (±SD) of the bond strength (MPa) and the Tukey results (5%).

SPP	*Laser*	
	60 mJ	100 mJ
Absence	21.92 ± 5.20 Aa	49.61 ± 9.59 Ab
Presence	20.35 ± 9.04 Aa	43.34 ± 4,97 Ab

*Different letters show statistically significant differences (p < 0.05); capital letters refer to columns; lowercase letters refer to lines.

In the group of 2 mm of dentin thickness, two-way ANOVA (Table 3) showed laser energy parameters and the interaction between Laser and SPP significantly affected ($p < 0.05$) the bond strength. The simulated pulpal pressure showed no significant differences ($p = 0.2817$) in the bond strength, under the experimental conditions.

Table 3 - Two-way ANOVA for 2 mm of dentin thickness.

Source	DF	SS	MS	F	*p*
Laser	1	3945	3945	153.5	0.0001*
Pulpal Pressure (sPP)	1	31.9	31.9	1.241	0.2817
Interaction	1	425.4	425.4	16.55	0.0009*
Residual	16	411.3	25.71		

* Statistically significant differences (p < 0.05).

When considering the interaction of laser energy parameters and simulated pulpal pressure ($p = 0.0009$), the highest bond mean values was observed in the group irradiated with 100 mJ and restored in the absence of SPP $(55.59 \pm 6.82)^{Ab}$, with significantly statistical difference from the group restored in the presence of SPP (43.84 ± 3.63)Bb . For the group irradiated with 60 mJ, there were no significant differences between the presence or absence. However, it differed significantly from the group irradiated with 100 mJ (Table 4).

Table 4 - Group: 2 mm of dentin thickness. Means (±SD) of the bond strength (MPa) and the Tukey results (5%).

SPP	*Laser*	
	60 mJ	100 mJ
Absence	24.98 ± 5.28 Aa	55.59 ± 6.82 Ab
Presence	18.28 ± 3.89 Aa	43.84 ± 3.63 Bb

*Different letters show statistically significant differences (p < 0.05); capital letters refer to columns; lowercase letters refer to lines.

When considering the interaction of laser energy parameters and simulated pulpal pressure ($p = 0.0088$), the highest bond mean values was observed in the group irradiated with 100 mJ and restored in the absence of SPP $(56.0 \pm 12.4)^{A}$. For the group irradiated with 60 mJ, there were no significant differences between the presence or absence. However, it differed significantly from the group irradiated with 100 mJ (Table 2).

DISCUSSION

Adhesive systems are composed of resinous monomers of different viscosities and organic solvents (ethanol, acetone, water), and are commercially available in different compositions, mechanisms of action and application forms [3,20,21].

The bonding mechanism of adhesive systems to the dentin substrate is still considered complex, when compared to the enamel substrate, because it has a heterogeneous composition, consisting of organic and inorganic components, different proportions of peritubular, intertubular and sclerotic dentin, variations in permeability and communication with the pulpal tissue. These properties may directly or indirectly affect substrate bond strength [4,6,22–28].

To remedy such difficulties, changes in adhesive systems composition and adhesive techniques have been proposed. Regarding composition, new comonomers, hydrophilic and hydrophobic photoinitiators combinations, remineralizing bioactive particles, MMP inhibitors and crosslinkers are observed [27–31]. Among the techniques are ethanol wet-bonding [5], collagen fixatives [32] and the use of various forms of laser energy to treat dental tissues, such as Neodymium [33–38].

The original idea of using the Neodymium laser after applying the adhesive system prior to polymerization, came from the work of Gonçalves et al. [33]. The authors concluded that Nd:YLF laser, in the parameters employed, promoted the fusion and resolidification of dentin hydroxyapatite in the presence of resinous monomers, suggesting the creation of a new hybridization modality, proven by X-ray diffraction and SEM; corroborated by Franke et al. [10] with significant improvement in hybridization quality and, Silva et al. [39] even in the presence of simulated pulpal pressure.

As the objective of this study was to evaluate the bond strength to human dentin submitted to Nd:YAG laser irradiation, the standardization of the remaining dental was fundamental. Dental remnant thickness standardization was performed from the highest pulpal horn. Thus, specimens with 1 mm of dentin thickness were used [25,26,40] and also with 2 mm [41].

Based on the study by Santis et al. [19], this study used simulated pulpal pressure to approach “in vivo” conditions. The results of this study demonstrate that the adhesive Adapter Single Bond 2 (3M ESPE) is sensitive to dentin moisture produced by pulpal pressure simulation, just as other adhesives used in previous studies, as there was a decrease in adhesive strength when compared to restored groups without pulpal pressure [39].

Positive results obtained by laser irradiation of the adhesive system, prior to photopolymerization, may be related to the improvement in the degree of conversion of the systems [11], as well as higher solvent evaporation by increasing surface temperature during irradiation [42], besides the morphological modifications of the adhesive interface as reported by Gonçalves et al. [33].

Laser irradiation at the 100 mJ parameter positively influenced the microtensile bond strength. In this study, a significant increase in adhesive strength was observed with the increase in laser intensity, contrary to the results obtained by Franke et al. [10], and according to what was found by Silva et al.[39], Marimoto et al. [37] e Ribeiro et al. [43].

Although the 60 mJ parameter provides lower adhesive strength values than the 100 mJ parameter, such results approximate those in the control group (without laser) in the presence of simulated pulpal pressure from the study by Silva et al. in 2016 [39]. As laser can improve the degree of conversion of adhesive systems, this result may be reflected in better longitudinal behavior of hydrolytic degradation resistance[11]. Positive results with 100 mJ may also offer better longitudinal perspectives, contrary to the results by Barcellos et al. (2016) [44] that used higher energy, verifying greater longitudinal degradation of collagen fibers with the parameter of 120 mJ.

The non-use of a control group without laser was an option in this study because it has already been analyzed in several studies of this research group, considering that it would constitute another complicating factor of the statistical understanding of the results. Regardless dentin thicknesses, the laser energy parameters and the presence of pulpal pressure influenced the bond strength results, so that the largest parameter positively influenced the means bond values and the presence of pulpal pressure negatively, reducing the values.

The safety in the applicability of the technique in terms of temperature elevation, in the parameter used, was also ratified in the studies by Santis et al. [19] and Silva et al. [39], where, with the parameter 100 mJ and presence of pulpal pressure, no critical pulpal temperature elevations were observed.

Thus, in vivo research can now be made possible to confirm these results, in order to clinically prove the positive effects of the Nd:YAG laser adhesive technique, as laboratory research has confirmed the safety of the technique and its beneficial effects to the adhesive process.

CONCLUSION

Within the limitations of the study, it can be inferred that the dentin bond strength on the adhesive system under laser irradiation and pulpal pressure:

(1) No significant influence of dentin thickness;

(2) Positively interfered with the increase of laser energy parameters;

(3) Negatively influenced by the presence of simulated pulpal pressure.

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