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Adhesion of a self-etching adhesive to caries-affected dentin treated with different methods for caries removal: a preliminary study

Avaliação de um sistema adesivo autocondicionante em dentina afetada por cárie tratada por diferentes mecanismos de remoção: estudo preliminar

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ABSTRACT

Objective: The aim of this study was to evaluate the adhesion of a self-etching system to caries-affected dentin (CAD) and sound dentin (SD) after different caries removal techniques by using microtensile bond strength test (μTBS). **Materials and Methods:** Twenty-four extracted human molars with coronal carious lesions were used. The samples were randomly divided into 5 groups, according to caries removal method: G1 - negative control (no removal); G2 abrasive disc; G3 – slow speed steel bur; G4 - Nd:YAG laser (energy density 21.2 J/cm^2); G5 - Er:YAG laser (energy density 80.24J/cm^2). SD and CAD/ infected dentin substrates were tested. Self-etch system (Clearfil SE Bond) was applied and resin crowns were built up. **Results:** One-way ANOVA showed statistically significant difference between experimental groups ($p < 0.001$), with significantly lower results for infected dentin (G1), when compared to all other experimental groups. A statistically significant higher bond strength value was observed for SD irradiated with Er:YAG laser when compared to CAD. For bur and Nd:YAG laser specimens, SD and CAD showed the same bonding performance. **Conclusion:** infected dentin is not an adequate substrate for bonding; Nd:YAG laser and steel burs showed the same bonding performance for both SD and CAD; while both dentins, when irradiated by Er:YAG laser, did not adequately interact with self-etching system resulting in poor adhesion.

KEYWORDS

Er:YAG laser; Nd:YAG laser; Infected dentin; Dentin.

RESUMO

Objetivo: O objetivo deste estudo foi avaliar a adesão (RU) de sistemas autocondicionantes à dentina afetada por cárie (CAD) e dentina normal (ND), após diferentes técnicas de remoção de tecido cariado. **Materiais e Métodos:** Foram utilizados vinte e quatro molares humanos extraídos, com lesão de cárie oclusal. A amostra foi aleatoriamente dividida em 5 grupos, de acordo com o método de remoção do tecido cariado: G1 - controle negativo (sem remoção); G2 - disco abrasivo; G3 – broca de aço de baixa velocidade; G4 - laser Nd:YAG (21,2 J/cm^2); G5 - laser Er:YAG (80,24 J/cm^2). Dentinas normais e afetados / infectados foram tratadas com o sistema adesivo (Clearfil SE Bond) e coroas de resina foram construídas. **Resultados:** ANOVA um fator mostrou diferença estatisticamente significante entre os grupos experimentais ($p < 0,001$), com um valor menor para a dentina infectada (G1), quando comparado com todos os grupos experimentais. Maior RU foi observada para ND irradiada com laser Er:YAG, quando comparado a CAD. Para os espécimes tratados com broca e laser Nd:YAG, a RU em ND e CAD foi semelhante. **Conclusão:** a dentina infectada não é um substrato adequado para a adesão; laser de Nd:YAG e brocas de aço mostraram-se adequados como ferramenta de tratamento das dentinas normal e afetada prévio à instalação de restaurações adesivas. Contudo, ambas as dentinas tratadas pelos laser de Er:YAG, não interagiram adequadamente com o sistema auto-condicionante resultando em fraca adesão.

PALAVRAS-CHAVE

Laser Er:YAG; Laser Nd:YAG; Dentina infectada; Dentina.

INTRODUCTION

Conservative dentistry claims for the selective removal of carious tissue. Carious dentin lesion is composed by two different layers: the infected dentine and the affected dentin. The infected dentin is the outer layer which is soft, and is composed by a disorganized matrix and non-remineralized necrotic collagen that must be removed. On the other hand, caries-affected dentin (CAD) is the inner layer, less infected and composed of a collagen matrix that keeps its usual cross-banded ultra-structured and, although partially demineralized it is potentially repairable, and it must be preserved on the cavity floor [1,2].

CAD has different structural and compositional features when compared to SD. Higher water content, increased permeability and lower microhardness [2] are features which could interfere on the bonding performance. In fact, authors [3-13] have shown that lower bond strengths, and thicker and porous hybrid layers [14-16] were observed in CAD when compared to SD, which could consider adhesion to CAD challenging and could compromise the long term durability of the formed hybrid layers. Also, structural differences between SD and CAD could influence the pattern of dentin demineralization, adhesive penetration and consequently the bond at the dentin/adhesive interface [17].

Self-etch adhesive systems are widely used in clinical practice due to its simplified technique and less post operative sensitivity [18]. Self-etch systems demineralize smear layer and underlying dentin, and simultaneously infiltrate monomers into the dentin subsurface, which are polymerized in situ, resulting in the creation of a resin–dentin interface [19-21]. However, there is some concern that smear layer could undermine self-etch adhesive penetration into dentin [21, 22].

Dentin smear layer is composed mostly of submicron particles of mineralized collagen debris [23,24]. According to the surface preparation, the smear layer has been shown to vary its thickness, roughness, density and degree of attachment to the underlying tooth structure [25].

It is speculated that there would be differences in the morphological and chemical structures of the smear layer created on SD and CAD [26], because the mineral and organic contents of CAD are different from those of normal dentin as a result of the cyclical process of de- and remineralization [14,26].

A manual cutting instrument associated with a low-speed mechanical rotating device are conventionally used for caries removal, but more comfortable and easy alternatives that cause less pain, discomfort and lesser vibration or oscillation have been proposed [27]. Newly developed techniques such as the use of high-power lasers (Nd:YAG and Er:YAG), chemo-mechanical, and air abrasion can be used in the dental clinic to remove carious dentin [28] specially to prepare ultraconservative cavities, following the principles of minimal intervention.

Nd:YAG laser was claimed to help adhesion process by increasing the surface area when applied on ND over uncured adhesives [29,30]. Er:YAG laser is able to successfully prepare cavities in enamel and dentin, by photo-ablation, producing minimal thermal damage to the dental pulp or surrounding tissues, creating an irregular dentin surface [31] with absence of smear layer and open dentinal tubules. These morphological features suggest that this is a receptive tissue for adhesive procedures [32].

Perdigão et al. [33] stated that the type of dentinal substrate could influence the bonding mechanism. Therefore, the formation of the hybrid layer, as well as, the substrate preparation prior to adhesion are critical, as it could influence the final restorative result [34]. Thus, studies of adhesion to CAD still require further investigation.

As CAD is considered a highly significant relevant substrate [35] studies performed on this tissue can add knowledge to the performance of adhesive systems. The aim of the present research was to evaluate the bonding performance of a self-etch adhesive system in different substrates (SD and CAD) after being treated by distinct methods for caries removal.

MATERIAL AND METHODS

Twenty-four extracted human molars exhibiting carious lesions were used in this study after the consent of the Ethics Committee of the School of Dentistry at the University of Sao Paulo (89/01). Extracted teeth were collected during daily clinical practice, and maintained in distilled water at 4 ºC until use.

Inclusion criteria were teeth with caries lesions limited to the occlusal surface and extending at least half of the distance from enamel-dentin junction to the pulp chamber. The lesions had to be surrounded enough normal dentin to be used as control bonding sites. This characteristics were determined by visual and radiological inspection, as well as by laser fluorescence values of approximately 30 (DIAGNODent, Kavo, Joinville, SC, Brazil) [36].

Experimental Grouping

Teeth were randomly divided into 8 groups (n = 6), according to removal methods of caries infected dentin, as follows:

G1 - No caries removal (negative control). Samples were not treated.

G2 - Abrasive paper

G3 - Burs. Caries was removed by round steel burs (no. 8; Dynadent, Boon, DE, USA) with water-cooled, low speed handpiece (Kavo, Joinville, SC, Brazil).

G4 - Nd:YAG laser (Pulse Master 1000, American Dental Technology, Southfield, MI, USA). Laser was set at power output of 1.5 W, energy level of 60 mJ, frequency of 25 Hz, energy density of 21.2 J/cm2. The non contact and focused mode was used at a constant distance of 1mm, providing a beam diameter of 320 μm.

G5 - Er:YAG laser (Kavo Key 3 Laser GmbH, Jena, Germany) was set at energy level of 250 mJ, frequency of 4 Hz, pulse duration of 250-500 μs and energy density of 80.24 J/cm2. The laser beam (handpiece #2060) was kept perpendicular to the dentin surface during irradiation, in a non-contact and focused mode (irradiation distance range between 12-15 mm) and the cooling system consisted of a water spray set for 24 μl/min.

The infected dentin was completely removed (except for the group 1), leaving caries-affected dentin surrounded by normal dentin. In order to distinguish caries-infected dentin from caries-affected dentin, we removed the dentin by using the combined criteria of visual examination and staining with caries detector solution (Kuraray Co., Ltd., Osaka, Japan) [37,38]. Dark pink dentin was classified as caries-infected dentin, while discolored dentin, which was slightly stained by caries detector solution, was classified as CAD. Surrounding, yellow, normal, dentin was not stained by caries detector solution.

Sample Restoration

A self-etching adhesive system (Clearfil SE Bond Kuraray Co., Ltd., Osaka, Japan) and a Z350 composite resin (3M ESPE Dental Products, St. Paul, MN, USA) were used to build the samples, according to manufacturer's instructions (Table I). A visible light unit (Astralis 3, Ivoclar-Vivadent AG, Schaan, Liechtenstein-light intensity 520 mW/cm^2) was used to cure both adhesive system and composite resin. Resin based composite crowns were constructed with 1.5 mm layers until a height of approximately 4-5 mm. Each layer was cured for 20 s.

Micro-Tensile Bond Strength (μTBS)

After 24 h storage in distilled water (37°C), the specimens were longitudinally sectioned into multiple beams using a low speed diamond saw (Isomet 1000, Buehler Ltd., Lake, Buff, IL, USA), under cooling. Beams with the same staining

Table I - Materials used, manufacturer, composition and manufacturer's instructions

Adhesive system or resin composites	Manufacturer	Composition	Manufacturer's instructions
Clearfil SE Bond	Kuraray, Tokyo, Japan	Primer: 10-MDP, HEMA, hydrophilicdi-methacrylate, camphorquinone, N,N-diethanol-p-toluidine, waterAdhesive: 10-MDP,Bis-GMA, HEMA, hydropho-bicdimethacrylate, CQ, N,N-diethanol-p-toluidine, silanizedcolloidalsilica	Application for 20s (rubbing), 5s air-drying, application of the adhesive, 10s light-curing
Filtek Z350	3M ESPE St. Paul, Minnesota, USA	UDMA, TEGDMA and Bis-EMA, zirco-nium silicate and (0.6 and 1.4 microns) silica 78.5% in weight, 59.5% in volume	1.5-mm Increments Light cure (20 s)

MDP: Methacryloyloxydecyl dihydrogen phosphate; HEMA: 2-hydroxyethyl methacrylate; Bis-GMA: bisphenol A glycidyl methacrylate; Bis-EMA: bisphenol-polyethylene glycol dimethacrylate; TEGDMA: triethylene glycol dimethacrylate.

pattern, as follows: SD, caries infected dentin (only for the negative control group - G1) or CAD (experimental groups). This procedure generated four (±1) beams of SD and four (±1) beams of CAD or caries infected dentin per tooth. Each beam had a cross-sectional area of approximately 1 mm2. The final width and thickness of the bonded area measured to the nearest 0.01 mm by means of a digital micrometer. Specimens were attached to a Geraldelli jig with cyano-acrylate adhesive (Henkel Ltda., Itapevi, SP, Brazil). The device was pulled at tension (Mini Instron 4442 Instron Co., Norwood, MA, USA), at 0.5 mm/min until it failed. Bond strength was calculated by dividing the load at failure by cross-sectional bonding area. The pre-testing failure were considered as 0 MPa.

Analysis of Failure Mode by Light Microscope

All tested samples were examined under an optical microscope at 50X magnification to identify failure mode. The fractures were categorized as follows: adhesive failure between tooth substrate and adhesive system; cohesive failure in composite resin; cohesive failure in dentin substrate; and mixed failure, when in the same specimen part of the failure was adhesive and part was cohesive.

Statistical Analysis

Data from the bond strength test were statistically compared by ANOVA complemented by Tukey's test ($p \leq 0.05$).

Scanning Electron Microscopy

For morphological illustration, samples from distinct dentins were prepared for Scanning Electron Microscopy (SEM). Specimens were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffered solution (SPI – CHEN – Spi supplies, PA, USA) for 24 h, at room temperature. The samples were dehydrated in ascending grades of ethanol and submitted to chemical drying in hexamethyldisilazane (HMDS – Sigma-Aldrich, St. Louis, MO, USA) for 20 min and sputter-coated with gold and examined in a scanning electron microscope (JEOL 6460 – LV, Jeol Ltd. Tokyo, Japan) operating at 20 Kv. Magnifications with 1000X and 2000X were obtained.

RESULTS

Micro-Tensile Test (μTBS)

The results are summarized in Tables II. One-way ANOVA showed statistically significant differences between experimental groups ($p < 0.001$ and $F = 5.43$). The highest bond strengths were observed in SD samples. Caries infected dentin group (G1) showed the worst μTBS result, when compared to all other experimental groups.

Another ANOVA ($p < 0.001$ and $F = 5.34$) was performed comparing the effect of substrate into the same caries removal method. A significant difference was observed just for the groups where Er:YAG laser was used, in which μTBS to CAD is lower than SD. Slow speed bur and Nd:YAG laser bonding performances were not statistically different according to dentin type. Eighty to ninety percent of adhesive failures were observed for all experimental groups.

Figure 1 shows a scanning eletronmicrograph of a direct comparison of the different caries removal methods according to substrate CAD (A, C, E, F) and SD (B, D, F, H). In A, a disorganized amount of debris intermingled with bacteria was observed, while in B standard smear layer was deposited on SD, obliterating dentinal tubules apertures. When affected dentin was ground by steel burs (C), residual bacteria can still be observed (arrows) in some areas of an irregular dentinal surface. On the other hand, smear layer produced by steel burs on SD (D) form a thin smear layer, which permit to identify the exact location of dentinal tubules apertures (arrow head). Both Nd:YAG laser irradiated CAD (E) and SD (F) are fused and re-solidified, with irregular surface pattern, but CAD is much more rougher than SD dentin. Er:YAG irradiated CAD shows an irregular pattern of ablated dentin, with open dentinal tubules into which some deposits are observed (gray arrows) (G). SD ablated by Er:YAG laser was characterized by a more homogeneous surface, smear layer-free, with open dentinal tubules and clear distinction between peri- (black arrow head) and intertubular (asterisk) dentin (H).

Table II - Mean micro-tensile bond strengths of resins to normal vs caries-affected or infected dentin. Values are means ± standard deviation. Different letters indicate significant differences

Removal method of caries infected dentin	Groups	Normal dentin	Caries-affected dentin	Caries infected dentin
No removal	G1	-	-	18.56 ±6.6[c]
Abrasive Disc	G2	37.47 ±10.17[ab]	-	-
Slow speed bur	G3	39.52 ±5.93[ab]	34.71 ±8.43[ab]	-
Nd:YAG laser	G4	45.58 ±7.87[a]	34.98 ±11.03[ab]	-
Er:YAG laser	G5	43.69 ±14.92[a]	21.83 ±12.40[b]	-

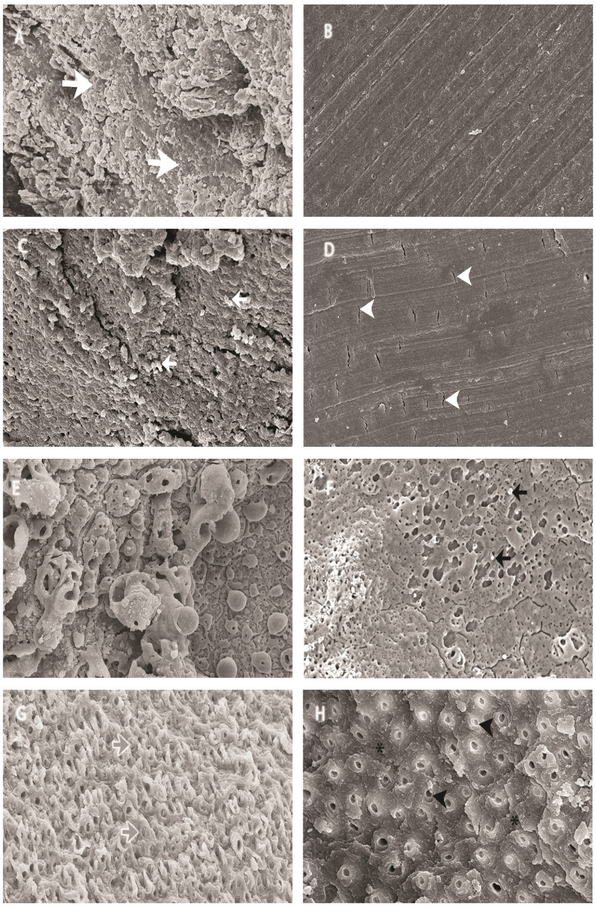

Figure 1 - Electronmicrograph of infected dentin (A), caries-affected dentin (C, E and G), and normal dentin (B, D, F and H) specimens treatment by different caries removal method. A - Surface covered with debris from the carious tissue, with an irregular aspect, without exposure of open dentinal tubules (arrow). (1000X). B – Normal dentin treated with sandpaper abrasive disc under water cooling produced a regular surface covered by standard smear layer that obliterated all dentinal tubules. (1000X). C - Caries-affected dentin obtained treated by low-speed steel bur. Presence of characteristic, heterogeneous, and scaly smear layer where bacteria can still be observed (arrows). (1000X). D - Steel burs on normal dentin form a thin smear layer, which permit to identify the exact location of dentinal tubules apertures (arrows head). (1000X). E – Nd:YAG laser irradiated affected dentin is fused and re-solidified, with most irregular surface pattern observed. (1000X). F - When normal dentin was irradiated by Nd:YAG laser a rough surface can be clearly detected as a sign of fusion and resolidification process (black arrow).(1000X). G – Caries-affected dentin irradiated with Er:YAG laser showing a characteristic image of ablated dentin with open dentinal tubules. Some of them present a crystal deposition into its apertures (gray arrows). (1000X). H - Er:YAG laser irradiated normal dentin was characterized by a more homogeneous surface, smear layer-free, with open dentinal tubules and clear distinction between peri- (black arrow head) and intertubular (black asterisk) dentin. (1000X).

DISCUSSION

Although CAD is a clinically relevant substrate considering conservative approach of dentistry, most of the laboratorial tests are performed using SD [35]. Considering this, adhesion studies using CAD can contribute to determine characteristics of the hybrid layer formed, and the bond strength performance of adhesive systems applied to this substrate [39].

As a slightly infected, able to remineralize substrate, CAD must be left on cavity walls related to the pulp [38]. Smear layers produced in CAD showed acid resistant crystals deposited on dentinal tubules apertures, reducing dentin permeability. This substrate has been considered a protective barrier that reduces ingress of bacteria and its products into the pulp [40]. However, these crystals may also hinder self-etching primer diffusion into underlying sound dentin, impairing hybrid layer formation. Nevertheless, adhesive systems with additional chemical bonding and antimicrobial properties, such as Clearfil SE used in this study, are the best choices for CAD hybridization [36].

This study evaluated different caries removal methods – low-speed steel bur, Nd:YAG and Er:YAG lasers – which changed the morphological aspect of dentin. While spherical steel bur preparations result in a regular surface, with dentinal tubules obliterated by a thin smear layer [41], Nd:YAG laser irradiation generates an irregular melted and re-solidified dentin surface with obliterated dentinal tubules [30]. On the other hand, Er:YAG laser irradiation produces an irregular surface, but with open dentinal tubules and no smear layer [32,42]. The qualitative results obtained in this study clearly illustrate the above-mentioned features. Therefore, one should be aware of all these distinct features when choosing the caries removal method, bearing in mind the mechanism of action of the chosen adhesive system. Self-etching systems show a slight penetration into dentin, while etch-and-rinse systems, due to a separate acid conditioning step, completely remove smear layer and expose dentinal tubules apertures [22, 23].

Regarding to bond strength performance of the self-etching system tested, low-speed steel burs did not influence bond strength with bonding values statistically similar for both CAD and SD. Maybe this result is due to the composition of Clearfil SE Bond system that has an acidic monomer (10-methacryloyloxydecyl dihydrogen phosphate - MDP) in its composition. This monomer chemically bonds to calcium present in hydroxyapatite [43] which is embedded in the hybrid layer and may explain its performance. While some authors [40] observed lower bond strength values in CAD in comparison with SD, it is fundamental to point out that they used different adhesive systems in their studies. Thus, the chemical composition of the adhesive was decisive for the results obtained here [39]. It is noteworthy that the use of a low-speed round bur is still the most widely technique used by dentists [39].

When both CAD and SD were irradiated by Nd:YAG laser, bond strengths were similar. The beneficial action of this laser on adhesion of restorative materials to dentin has not been fully understood [44]. It is known that Nd:YAG laser causes surface melting and recrystalization of dentin. This photo thermal effect promotes a rough surface of amorphous craters, melted margins and obliterated dentinal tubules [44]. This rough surface with increased contact area available for bonding could be responsible for the similar bonding performance. In the present study, even though dentinal tubules were obliterated by melted and re-solidified dentin, self-etching system used was able to similarly interact with both CAD and SD. The additional advantage of bacterial count reduction of Nd:YAG laser [45] could add value to its indication as a caries removal method. The results of this study suggest that the small thickness of the debris left by the Nd:YAG irradiation allowed the correct impregnation of the underlying dentin by resin monomers, resulting in an effective hybrid layer formation, which can be confirmed by the similar results obtained in CAD and SD when using self-etching system. It is important to highlight that the thickness of the hybrid layer is no longer related to high bond strength values, but rather to its correct interlocking with dentin structure [46]. Thus, it can be considered that the Nd:YAG is a valid and feasible tool that, when used by a trained operator, with correct parameters, can stimulate adhesion properties [47]. As we did not perform a transversal evaluation of the formed hybrid layer in Nd:YAG irradiated samples, we would like to point out that this is a limitation of our research, which can be further explored in future studies.

Er:YAG laser irradiated SD shows exposed dentinal tubules with total absence of smear layer, characteristics that suggest an adequate substrate for bonding. On CAD electronmicrographs similar features were observed, but the surface was more irregular. In this study, the irradiation of CAD by Er:YAG laser resulted in lower bond strength when compared to SD samples. Structural changes in the organic matrix of dentin [32] and a possible production of subsurface microcracks, caused by the laser ablation process can explain this result [48,49].

Since CAD has a higher amount of water than SD, the Er:YAG acted more intensely on CAD causing more damage and fusion of the collagen network [42], which directly affected bonding [50]. This result is a surprise because as dentinal tubules apertures were all open, one could think that the surface was more adhesion-friendly, but the organic matrix alteration counteracted the morphological convenience.

Lower bond strengths to infected dentin (G1) indicated that it should be removed so that bonding can be performed on the underlying dentin, such as CAD or SD, according to the clinical situation. Based on the obtained results of this study, we could indicate that removing infected dentin with both burs or Nd:YAG laser are valid, because it is extremely desirable to obtain similar bonding results when working in such different substrates. In the daily clinic it is impossible to separate this tissues and also highly desirable to achieve similar bonding to all tissues that are present in the cavity walls. Together with a perfect margin seal this step plays an important role in the success of the restorative treatment.

Our study limitations concern to the fact as an in vitro study that used natural carious teeth, inherent differences of substrate could occur. In this sense, a new effort on the development of an in vitro standardized CAD [51,52] to produce a pattern substrate to perform future adhesion studies.

CONCLUSIONS

Infected dentin is not an adequate substrate for bonding and must be removed from cavity walls. The selective removal of carious tissue should be performed using round steel burs or Nd:YAG laser when in both SD and CAD. On the other hand, the use of Er:YAG laser on CAD is not indicated, because it creates a substrate that cannot adequately interact with adhesive system, significantly decreasing bonding values.

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