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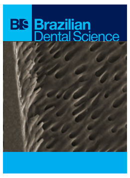

ORIGINAL ARTICLE

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Evaluation of bond strength of composite resin to enamel demineralized, exposed to remineralization and subjected to caries infiltration

Avaliação da resistência de união da resina composta ao esmalte desmineralizado submetido à remineralização e infiltração de cárie

Bruna Chrispim dos REIS[1], Ana Júlia Faria de LACERDA[1], Taciana Marco Ferraz CANEPELE[1], Alessandra Buhler BORGES[1], Karen Cristina Kazue YUI[1], Carlos Rocha Gomes TORRES[1], César Rogério PUCCI[1]

1 Department of Restorative Dentistry – School of Dentistry – Institute of Science and Technology – UNESP – UnivEstadualPaulista— São José dos Campos – SP – Brazil.

ABSTRACT

Objective: To evaluate the bond strength between resin composite and differentenamel substrates: sound enamel; demineralized enamel submitted or not to remineralization; and demineralized enamel infiltrated with an infiltrating resin. **Materials and Methods:** 120 bovine teeth were selected, the root portion was removed and the enamel finished. Specimens were divided into the following groups: (A) Control (n=24): adhesively treated and restored; (B) (n=96): the samples were immersed in a demineralization solution to create white spot lesions and divided into four subgroups: (B1) demineralized enamel; (B2) samples were stored in artificial saliva (8 weeks); (B3) samples were stored in a 0.05% sodium fluoride solution (1 min day/8 weeks); (B4) samples were treated with an infiltrantresin (Icon, DMG).The groups were treated with one of the following adhesives: Clearfil S3 Bond Plus (Kuraray)or Single Bond Universal (3M ESPE), followed by the resin composite application (Filtek Z 350 XT, 3M ESPE).The specimens were submitted to thermalcycling aging(10,000 ×; 2±5ºC, 50±55ºC and 37°±2°C). The specimens were sectioned into prism shapes with ~1mm^2 of base and submitted to microtensile test.The collected data were submitted to ANOVA and Tukey´s test (α= 5%). **Results:** The Means (±SD) in MPawere: Clearfill S3 Bond Plus: Control (17.17±3.52); B1 (11.60±0.74); B2 (6.83±1.87); B3 (8.38±1.59) and B4 (27.00±1.76); Single Bond Universal: Control (26.26±3.19); B1 (10.94±2.00); B2 (11.05±1.74); B3 (15.63±1.25) and B4 (22.60±2.29). **Conclusion:** The surface infiltrated withaninfiltrating resin(Icon) did not

RESUMO

Objetivo: Avaliar a resistência de união entre a resina composta e diferentes substratos de esmalte: hígido, esmalte desmineralizado submetido ou não a remineralização, e desmineralizado infiltrado com Icon (DMG). **Material e Métodos:** 120 dentes bovinos, cujas raízes foram removidas e o esmalte vestibular foi lixado. Os espécimes foram divididos em: Grupo A, controle, (n=24) que recebeu os procedimentos adesivos e restauradores para posterior teste de microtração; Grupo B (n=96), onde os corpos de prova (cdps) permaneceram imersos em solução desmineralizadora para produzir lesões subsuperficiais de cárie artificial, sendo subdividido em 4: B1, cdps somente desmineralizados; B2, cdps imersos em saliva artificial (8 semanas); B3, cdps imersos em solução de fluoreto 0,05% (1 minuto diariamente por 8 semanas); B4, cdps infiltrados com material resinoso (Icon, DMG). Todos os grupos receberam aplicação do sistema adesivo Clearfil S3 Bond Plus (Kuraray) ou Single Bond Universal (3M ESPE), seguida da resina composta Filtek Z 350 XT (3M ESPE). Os espécimes foram submetidos a ciclagem térmica e seccionados em prismas com dimensões aproximadas de 1mm^2 de base e submetidos ao teste de microtração. **Resultados:** os dados foram submetidos à ANOVA e ao Teste de Tukey (α= 5%). As médias em Mpa (±desvio padrão) foram: Clearfill S3 Bond Plus: Grupo Controle (17,17±3,52); B1 (11,60±0,74); B2 (6,83±1,87); B3 (8,38±1,59) e B4 (27,00±1,76); Single Bond Universal: Grupo Controle (26,26±3,19); B1 (10,94±2,00); B2 (11,05±1,74); B3 (15,63±1,25) e B4 (22,60±2,29). **Conclusão:** a superfície infiltrada com Icon não negatively affect the bond strength between resin composite and enamel. The demineralized and remineralized groups with sodium fluoride and artificial saliva presented statistically lower results when compared to the other groups.

KEYWORDS

Enamel; Caries; Bond strenght.

interferiu negativamente na resistência de união da resina composta ao esmalte e os grupos que foram desmineralizados e remineralizados com flúor e saliva artificial apresentaram valores estatisticamente inferiores aos demais.

PALAVRAS-CHAVE

Esmalte; Carie; Resistencia de união.

INTRODUCTION

Tooth enamel is an acellular mineralized tissue, with crystal structures with composition similar to the mineral hydroxyapatite. $Ca_{10}(PO_4)6(OH)_2$ and inclusions of carbonate, sodium, fluoride and other ions that make it an impure form of mineral. On the enamel crystals, some phosphate ions are replaced by carbonate ions, frequently with the simultaneous replacement of calcium by sodium and, furthermore, some hydroxyl ions are replaced by fluoride ions. The enamel apatite and the majority of other biological apatite is, consequently, a carbonated fluor-hydroxyapatite [1].

By the dietary physical-chemical metabolic action and physiological conditions from the oral environment such as saliva, temperature, pH and the presence of other fluids, the enamel constantly suffers a process of demineralization and remineralization[2].

Enamel hydroxyapatite becomes susceptible to dissolution when exposed to a significant amount of acids in a critical pH (pH=5.5). As a result, the concentration of calcium and phosphate ions (Ca^{2+} and HPO_4^{2-},respectively)decrease when compared to the hydroxyapatite solubility product, stimulating a physico-chemical tendency of the enamel to loseCa^{2+} and$HPO_4^{2-}$to the oral environment, trying to recover the balance, a phenomenon known as demineralization, improving the solubility and starting an caries lesion, with submicroscopic changes[3].

The caries process formation, beginning with the subclinical mineral loss (demineralization),progresses to an active white spot lesion and the lesion formation has been studied [3-6]. There is a concordance between the authors that under ideal conditions caries lesions on the enamel surface can stop[3-6].

A new philosophy for the management of the dental caries disease emerged with the aim of creating a more favorable environment for the caries control with minimal intervention[7] and comfort for the patient, anoutcome that has become a concern for the modern dentist [8-9].Icon (DMG) was introduced as a novel treatment to the established ones, indicated by white spot lesionsurfaces limited to the enamel and non-cavitated caries, for both smooth surfaces and proximal areas. The material is indicated for lesions that exceed treatment with preventive therapies, however, this treatment works when there is not a need for a conventional restorative intervention using carbide or other burs. This less invasive treatment avoids the loss of dental structure and prohibits the transition from the initial demineralization to the cavitation. The material acts through a microinfiltration technology, filling the demineralized structure, avoiding the caries progression and reinforcing the mineral structure, giving back the characteristics similar to the adjacentsound enamel.

When the enamel areahas been infiltrated by the infiltratingresin (Icon, DMG) and there is an need for an adhesive procedure such as: bonding orthodontic brackets; the need for a conservative restorative procedure; restorations due to dental fractures, once the infiltrant material will not be removed, there is a question about the bonding between the treated surface and the compatibility of this material to current bond systems [10-11].

Faced with these challenges, the aim of the present study was to investigate the bond strength between resin-composite, two types of adhesives systems and different enamel substrates: sound and demineralized enamel, submitted or not to remineralization, or enamel infiltrated with Icon®.

MATERIALS E METHODS

Sample preparation

One hundred and twenty freshly extracted bovine incisor teeth were cleaned, stored in physiologic solution with 0.1% thymol at -18°C [12], no longer than 28 days [13].The roots were removed with a perpendicular section.

The roots were removed with a cross section perpendicular to the long-axis, 2.0 mm from the cement enamel junction with a diamond disk (Dremel, Breda, Holanda) using a mandrel mounted high speed (Nevoni, São Paulo, SP, Brazil). The enamel surface was flattened with 400 and 600 grit sandpaper, in a polishing machine (DP-10, Panambra, São Paulo-SP, Brazil), under water cooling and constant pressure to obtain a smooth flat surface with 5 × 5 mm.

The tooth were positioned in a silicon mold (Industrial silicon, Rodhorsil, Clássico, São Paulo, SP, Brazil) with a diameter of 2 cm and a height of 1.5 cm and with their buccal surface facing the mold. Self-polymerizable acrylic resin (Jet-ArtigosOdontológicos, Clássico, São Paulo, SP, Brazil) was inserted into the mold. The tooth embedded in acrylic resin was removed from the mold and polished under water-cooling to standardize the smear layer.

Experimental groups

Specimens (N=120) were randomly allocated in two groups according to the adhesive system applied: (SAC) - Adper Single Bond Universal; and (SAA) -Self-etching adhesive system: EasyClearfil S3.

These groups were subdivided into five groups according to the enamel substrate, Group A (N=24): Soundenamel (control group) without treatment.All the other groups were submitted to demineralization treatment in order to form a superficial caries lesion, being immersed in demineralization solution[14], with 3mM $CaCl_2 2H_2O$, 3mM KH_2PO_4 and 50mM CH_3COOH (pH 5.0). The samples were individually immersed in the solution at 37 °C during 7 days, changing the solution daily to keep the pH constant. The total volume of the solution was calculated by using 2 $ml/mm^2$of the enamel area immersed into the acid solution, using a multifunctional shaker (Kline)at ~120 rpm, avoiding the saturation of the calcium acid in contact with the sample in order to reduce the activity.

Group B1 (n=24):demineralized enamel without remineralization.

Group B2 (n=24):demineralized enamel remineralized with saliva. The samples were stored in artificial saliva for 8 weeks [15]. The saliva solution was replaced daily.

Group B3 (n=24):enamel was demineralized and remineralized with fluoride. The specimens were immersed in 0.05% sodium fluoride solution for 1 min, washed with deionized water and once again immersed in artificial saliva. The protocol was repeated during 8 weeks.

Group B4 (n=24):The demineralized enamel was treated with aninfiltrating resin

(Icon, DMG). Phosphoric acid at 35% (Magic Acid, Vigodent/Rio de Janeiro-RJ, Brazil) was applied for 30 s[16], the specimens were washed with water and dried during 30s, followed by the resin-based infiltrant application for 3 min, cured for 40 s[17,18].

Phosphoric acid was used to verify if the combination with the infiltrant resin (Icon, DMG) would provide a satisfactory performance.

The 24 specimens of each group were divided into two subgroups (n=12) according to the adhesive system (Table 1).

Table 1 - Group experimental designs

120 teeth	Group A (control) (24 teeth)	AE: Easy Clearfil SE (ECSE) + Filtek (12 teeth)	
		AA: Adper Single Bond U (ASBU) + Filtek (12 teeth)	
	Group B (demineralized) (96 teeth)	B1: demineralized (24 teeth)	B1E: ECSE + Filtek (12 teeth)
			B1A: ASBU + Filtek (12 teeth)
		B2: remineralized with artificial saliva (24 teeth)	B2E: ECSE + Filtek (12 teeth)
			B2A: ASBU + Filtek (12 teeth)
		B3: remineralized with fluoride (24 teeth)	B3E: ECSE + Filtek (12 teeth)
			B3A: ASBU + Filtek (12 teeth)
		B4: infiltrated with Icon (24 teeth)	B4E: ECSE + Filtek (12 teeth)
			B4A: ASBU + Filtek (12 teeth)

Dentin-adhesive application

The dentin-adhesive system was applied on the treated enamel surface:Clearfil S3 Bond Plus(Kuraray Medical Inc. Okayama,Japan) and Single Bond Universal (3M ESPE, St. Paul, MN, EUA) were selected and appliedaccording to the manufacturer´s instructions.

Composite resin insertion and polymerization

The resin composite (Filtek Z350 XT, 3M ESPE, St. Paul, MN, EUA, A3) was inserted on a silicon mold(4 mm x 4 mm x 4 mm) in 2mm increments, light cured with a LED curing unit with light intensity of 500 mW/cm^2 (Emitter A, Schuster) during 20s/increment. The silicon mold was removed and each face of the resin blocks was cured for an extra 20s. The restored teeth were stored in distilled water at 37ºC for 24h.

Thermal-cycling

The specimens were submitted to thermal-cycling(Thermal-cycling Machine ER 26000, Erios, Brazil) in three water baths with different temperatures: the specimens were immersed in water at 2±5ºC, followed by 50±55ºCand an intermediary bath at 37°±2°C.The number of cycles were 10,000 and each cycle consisted of a 15 s interval[19].

Specimen preparation for microtensile bond strength test

Each specimen was sectioned along with the tooth´s long axis on the mesiodistal and vestibulo-buccal with approximately one millimiter thick, using a diamond disk at low speed mounted in a cutting saw machine (Labcut 1010, Extec Technologies Inc., Enfield, CT, EUA) under water cooling. Sticks containing resin composite and dental structure were obtaining.

Each tooth originated around 4 to 9 sticks. The selected stickschosen for the microtensile test were the ones that the bondedarea between the substrateswas intact and presents adjacent area without any superficial defect. The stick cross-sectinal area was measured before the mechanical test using an electronic digital caliper(StarrettIndústria e ComércioLtda, São Paulo, SP, Brazil).

The stick specimens were attached to the microtensile test device (Odeme, Joaçaba, SC, Brazil) using cyanoacrylate gel glue (Loctite 454, Henkel Loctite AdesivosLtda, Itapevi, SP, Brazil) and the test was conducted in a universal testing machine (DL-200, EMIC, São José dos Pinhais, PR, Brazil) at 0.5 mm/min according to the ISO/TR 11405.

The bond area and the force applied to break the bond were used to calculate the bond tensile strength in Mega Pascal (MPa) applying the following formula: Rm=F/A, where: "Rm" is the microtensile bond strength; "F" is the applied force in Kgf; and "A" is the bond area in mm.

The tested specimens were stained with hematoxylin at 7%for 3 min and analyzed with a steromicroscope (Discovery V20, Karl Zeiss, Jena, Alemanha) with 40× magnification to determine the failure mode:

- **Adhesive:** Failure on the adhesive/dentin structure or on the adhesive/resin composite interface in more than 75% of the analyzed area.
- **Mixed:** Failure without a predominance higher than 75% of any type of failure.
- **Cohesive on the dentin substrate:** Failure predominant on the dentin substrate (~75%)
- **Cohesive on the resin composite:** Failure predominant on the resin composite (~75%).

During the statistical analysis, the data from adhesive and mixed failures were considered and the data resulting from the cohesive failures on the resin composite or in the dentin were discarded.

The results of the cohesive(dentin/resin composite) pre-test failure was not included in the statistical analysis. For the pre-test failures, it was considered the lower value obtained in each group, in order to obtain normality between the results[19].

The results of bond strength (MPa) were analyzed using the two-way parametrical ANOVA and Tukey´s test ($p<0.05$).

RESULTS

Table 2 shows the values of mean and standard deviation of microtensile bond strength test (MPa) of the groups submitted to thermal cycling. The highest mean was presented by the group Icon/Clearfil S3 (27±1.76MPa) followed by the Control/Single Bond Universal (26.26±3.19). While the groups Fluoride/Clearfil S3 (8.38±1.59 MPa) and Saliva/Clearfil S3 (6.83±1.87MPa) presented the same means of bond strength.

To evaluate the interaction between the enamel treatment and the dentin-adhesive bond system the data was submitted to two-way ANOVA for all the evaluated factors: Adhesive system, enamel treatment and the interaction between treatment × adhesive (α= 5%). Statistically significant differences were found among all the evaluated groups.

Table 3 illustrate the results of the Tukey test (5%) for the "enamel treatment" factor. Control and the Icon groups presented statistically significant higher means when compared to the other groups.

The Tukey test for the factor "Adhesive system" showed that the Single Bond Universal (17.30±7.64MPa)had statistically significant ($p<0.05$)higher values of bond strength, superior when compared to Clearfil S3 Bond(14.20±6.45MPa).

Table 2 - Descriptive analysis for the different groups in bond strength (MPa) decreasing order

Enamel treatment	Adhesive	Mean(Mpa)	D-P
ICON	Clearfil S3 Bond	27.00	±1.76
Control	Single Bond Universal	26.26	±3.19
ICON	Single Bond Universal	22.60	±2.29
Control	Clearfil S3 Bond	17.17	±3.52
Fluoride	Single Bond Universal	15.63	±1.25
Demineralized	Clearfil S3 Bond	11.60	±0.74
Saliva	Single Bond Universal	11.05	±1.74
Demineralized	Single Bond Universal	10.94	±2.00
Fluoride	Clearfil S3 Bond	8.38	±1.59
Saliva	Clearfil S3 Bond	6.83	±1.87

Table 3 - Tukey test results for the “enamel treatment” factor

Enamel treatment	Mean (± SD) (MPa)	Homogeneous groups*
Control	21.71±7.54	A
Demineralized	11.27±4.25	B
Saliva	9.72±1.76	B
Fluor	11.23±2.52	B
ICON	24.80±2.35	A

* Mean followed by the same letter are not significantly different (p>0,05).

DISCUSSION

According to the results, the group infiltrated with the infiltrantresin (Icon, DMG) was similar to the control group which shows that the bond strength was not affected when Icon (DMG) was used, probably as a result of the affinity between the resin infiltrant monomer (Icon, DMG) and the resin monomers from the adhesive system for both evaluated adhesive systems. These results are in agreement with Wiegand [20] that report that the use of a caries infiltrant material before the conventional adhesive application does not interfere with the bond [21]. Therefore, it can be noticed that the use of an infiltrant material before the adhesive system application does not interfere on the bond strength to the enamel.

Groups that were demineralized and remineralized later with artificial saliva (B2) and fluoride (B3), presented the worst results, with the group remineralized with fluorideiscapable of hypercalcification of the enamel, which inhibitsthe self-etching adhesive performance. Both showed statistically lower result values of bond strength when compared to the enamel infiltration with Icon (DMG).

In previous studies, the remineralization actions with highly concentrated fluorides, similar to the ones found in commercial mouth rinses, were observed and proven to prevent the incipient carious lesion progression. Nonetheless, this remineralization seems to be superficial. The internal portion of the enamel lesion is more susceptible to demineralization as a result of the gradient on the enamel solubility, with the internal enamel being more soluble compared to the enamel external portion [23-24].

Higher concentration of fluorides can result in a rapid mineral precipitation on the enamel surface and the enamel porous obliteration, which connects with the base of the demineralized lesion.This process can restrict even more the superficial enamel remineralization. Ideally, a remineralization should improve the mineral gain on the surface despite being deposited only on the superficial layer [25].

With respect to the type of adhesive, we could observe that the system that used phosphoric acid presented results statistically higher when compared to the self-etchingsystem. A possible explanation for that is the acid etching applied on the enamel surface can promote micro-retentions, which enlarges the contact surface, despite improving the surface energy, which promotes the wettability of the adhesive by means of reducing the contact angle between the adhesive and the etched surface [26-28].

A self-etching adhesive system has a weak acid and does not significantly infiltratethe enamel surface. As a result, less micro-porosityinhibits the adhesive action and promotes lower bond strength.

According to the results, the restorative treatment can be applied on the treated surface with the resin-based infiltrant material (Icon®–DMG), which does not negatively interfere on the bond between resin composite and enamel, it showed to be statistically superior to the other groups that were submitted to demineralization.

CONCLUSIONS

It was concluded that the surface infiltrated by Icon (DMG) did not interfere negatively on the bond strength to the resin composite. The groups demineralized and remineralized with fluoride or artificial saliva presented statistically lower results of bond strength.

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César Rogério Pucci
(Corresponding address)
Av. Eng. Francisco José Longo, 777 - Jd. São Dimas
São José dos Campos/ SP/BRAZIL, CEP 12245-000
Phone: 55 (12) 3947-9377
E-mail: cesar@fosjc.unesp.br

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