Simulating non-carious cervical lesions by chemical and mechanical alteration on dentin: Bond strength of universal adhesive systems

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ABSTRACT
Introduction: Non-carious cervical lesions (NCCL) is a very common dental pathology frequently demanding resin composite restoration.
Objective: This in vitro study had the objective to test the effect of altered dentin substrate by erosion and erosion/abrasion comparing it to sound dentin on the microtensile bond strength (µTBS) of All-Bond Universal (ABU) and Scotchbond Universal (SBU).
Materials and methods: Seventy-two teeth were randomly divided in twelve experimental groups according to the adhesive system – ABU and SBU; approaching – self-etch (SE) and Etch-and-rinse (ER); and dentin condition – sound (S), eroded (E) and eroded/abraded (EA) dentin. The teeth/restorations were reduced to sticks and the µTBS was performed after 24 hours and 12 months of storage in water at 37°C.
Results: Fractured specimens were analyzed under stereomicroscope to determine the failure patterns in adhesive/mixed or cohesive. µTBS data was analyzed through three-way ANOVA and Tukey's test (α=5%). It was demonstrated that the adhesive system and the substrate influenced on the results (p=0.00), but aging did not (p=0.81). Regardless of time, SBU SE performed higher values of bond strength on E than the EA and S.
Conclusion: EA dentin simulating NCCL did not impair the bond strength and presented similar behavior to sound dentin.

Keywords: Dental materials, Tooth abrasion, Tooth erosion, Tensile strength.

1 INTRODUCTION

Non-carious cervical lesions (NCCLs) are a pathology that affects mineralized tooth structures close to the cement-enamel junction. Such pathology is characterized by mineral loss frequently generating a wedge-shaped cavity or a concave and smooth one.[1,2] NCCLs commonly result from the synergy between erosion and abrasion processes where the dental substrate suffers an erosive attack caused by acidic diet and, in sequence, excessive brushing accelerates the cavity formation.[3] Its multifactorial etiology is widely accepted, which includes erosion, abrasion, attrition and abfraction.[4]

NCCLs frequently require adhesive restoration to reduce hypersensitivity, to prevent the loss of tooth structure or the increase of such loss, besides providing esthetics.[5] Resin composites are the first choice as restorative material and it is frequently suggested in association with universal adhesives on different approaches such as: etch-and-rinse, self-etch or selective etch, according to the clinician's choice.[6,7] The best adhesive approaching to be employed in resin restorations of such lesions is frequently discussed,[7,8] as well as erosive and abrasive tissues characteristics[9,10] involving different processes concerning the substrate that may affect the adhesive systems performance.

This *in vitro* study had the objective to compare the bond strength of two universal adhesives applied over sound dentin versus chemically and mechanically altered substrates by erosion and by erosion/abrasion. The tests were performed after 24h and after 12m. The null hypothesis tested was that the altered dentin substrates do not influence on the bond strength, regardless of time.

2 MATERIALS AND METHODS

2.1 TEETH SELECTION AND PREPARATION

Seventy-two extracted caries-free human third molars donated by the institutional permanent human teeth bank were used in the present study. The study protocol was reviewed and approved by the Research Ethics Committee. All teeth were inspected to verify their integrity and to discard the ones that presented enamel defects, cracks or restorations. The selected teeth were cleaned, disinfected in 0.5% chloramine-T solution for seven days.[11] The occlusal surface was cut in a cutting machine to obtain a flat dentin surface (Labcut 1010, Extec Co., Enfield, USA). The smear layer was standardized with 600 silicon carbon sandpaper in a circular mechanical polishing machine under running water for 60 seconds.

2.2 MATERIALS

Materials composition, manufacturers and batch numbers are shown in Table 1.

Table 1. Materials composition and manufacturer.

	All-Bond Universal (ABU)	**Scotchbond Universal (SBU)**	**Filtek Z350 XT**	**37% Phosphoric acid**
Manufacturer	BISCO, Schaunburg, IL, USA	3M ESPE, St. Paul, MN, USA	3M ESPE, St. Paul, MN, USA	2, Joinville, SC, Brazil
Composition	MDP phosphate monomer, bis-GMA, HEMA, ethanol, water, initiators	MDP phosphate monomer, UDMA, HEMA, Vitrebond copolymer, water, ethanol, initiators, silane	Silica 20nm, zirconia 4-11nm, bis-GMA, bis-MOM, UDMA, PEGDMA,TEGDM A	37% Phosphoric acid, aqueous gel base
Batch number	1200002617	1926600462	591639	281019
10-MDP: 10-methacryloyloxydecyl dihydrogen phosphate; Bis-GMA: bisphenol A glycidyl methacrylate; HEDMA:1,6-hexanediol dimethacrylate; HEMA: 2-hydroxyethyl methacrylate; PENTA: dipentaerythritol penta acrylate monophosphate.				

2.3 EXPERIMENTAL DESIGN

Seventy-two teeth, which were included in this study, were randomly divided in twelve experimental groups according to the adhesive system and approaching (4) - All-Bond Universal (ABU) and Scotchbond Universal (SBU), both in self-etching (SE) and Etch-and-rinse (ER) approaching. Besides the adhesive system, they were separated in groups according to their dentin condition (3) - sound dentin (S), as control group; eroded dentin (E) where the dentin was chemically demineralized and finally, eroded/abraded dentin (EA) - where the dentin suffered a combined action of chemical demineralization and mechanical abrasion, as it is explained below:

2.3.1 Dentin condition - applied protocols

(1) Sound dentin, (n=24): immersion in artificial saliva (1.5 mM Ca $(NO_3)_2 \cdot 4H_2O$, 0.9 mM $NaH_2PO_4 \cdot 2H_2O$, 150 mM KCl, 0.1 M Tris buffer, 0.03 ppm fluoride, pH 7.0) during the experimental period.

(2) Eroded dentin, (n=24): following the pH-cycling model:

3 daily pH-cycles of 5 minutes of agitated immersion in cola drink (Coca-Cola [pH: 2.6, phosphate: 5.43 mM Pi, calcium: 0.84 mM Ca^{2+}, fluoride: 0.13 ppm F, titratable acid: 40.0 mmol/l OH^- to pH 5.5 and 83.6 mmol/l OH^- at pH 7.0], Spal; Porto Real, Brazil) were performed with an interval of 6 hours between each cycle.[8]

(3) Eroded/abraded dentin, (n=24): combined erosion challenge and mechanical demineralization.

Eroded cycle according to what was mentioned in protocol (2) associated to mechanical abrasion where the dentin surface was brushed with a standardized load of 200g for 2 minutes associated to the Colgate Total 12 dentifrice and artificial saliva.[12]

After applying the protocols, the teeth were washed in distilled water and immersed in artificial saliva solution until the next cycle.

2.4 RESTORATIVE PROTOCOL

Adhesive systems were applied on previous prepared dentin surface, which was performed with a trained and calibrated operator according to the adhesive approaching and the manufacturer's recommendations (Table 2). After applying the adhesive system, a resin composite restoration of 5.0 mm high was built. The composite was applied in increments of 1.0 mm and photoactivated for 20 seconds with a photocuring unit (Emiter C, Schuster Eqto Odontol Ltd, Santa Maria, Brazil) with the monitored irradiance of 1000mw/cm2.

Half of the specimens of each group were kept in distilled water and tested after 24 hours. The other half was stored in distilled water - weekly changed - for 12 months in a microbiological incubator with controlled temperature of 37°C. To perform the test, the teeth were longitudinally sectioned in two perpendicular axes with diamond saw discs in a cutting machine (Labcut 1010, Extec Co, Enfield, USA) to obtain specimens with a cross-sectional area of approximately 0.8mm².

Table 2. Adhesive system approaching (according to manufacturers' instructions).

Approaching	**All-Bond Universal (ABU)**	**Scotchbond Universal (SBU)**
	a. Self-etch 1. Apply two separate adhesive coats with agitation for 10 -15s per coat.2. Evaporate the solvent completely by air drying using an air syringe for at least 10 s. It should be no visible movement of the adhesive.3. The surface should have a uniform glossy appearance. If not, steps 1 and 2 need to be repeated.4. Light cure for 10 s.	a. Self-etch 1. Apply the adhesive or adhesive mixture to the prepared tooth and rub it on the tooth's surface for 20 s. 2. Gently air-dry the adhesive for approximately 5s to make the solvent evaporate.3. Light cure for 10s.
	b. Etch-and-Rinse 1. Etch for 15s.2. Rinse thoroughly.3. Remove the excess of water by blotting the surface with an absorbent pellet or high volume evacuation for 1-2 s, leaving the preparation with visible moisture.4. Apply the adhesive according to the self-etch mode.	b. Etch-and-rinse 1. Apply the etchant for 15s.2. Rinse thoroughly with water and dry with water-free and oil-free air or with cotton pellets; do not overdry.3. Apply the adhesive according to the self-etch mode.

2.5 MICROTENSILE BOND STRENGTH (μTBS)

The specimens were fixed in a μTBS testing device with cyanoacrylate-based adhesive gel (Three Bond Super Gel, ThreeBond Brazil Ind. Com. Ltda., Diadema, SP, Brazil). In sequence, they were submitted to a tensile force in a universal test machine (EMIC DL 1000 – Instron Brazil Ltda. São José dos Pinhais, PR - Brazil) with a 10KgF load cell at a crosshead speed of 1mm/min until they fractured. The experimental unit was the tooth, this way, the average of the results (in MPa) of each tooth stick was used for statistical analysis.

2.6 FAILURE MODE ANALYSIS

After the microtensile test, the specimens were analyzed in a stereomicroscopy (Discovery V20, Carl-Zeiss, Oberkochen, Germany) with 10x magnification to determine the type of fracture and they were classified as adhesive/mixed (interface failure or mixed with cohesive failure) or cohesive (dentin or composite resin failure).

2.7 STATISTICAL ANALYSIS

The Shapiro-Wilk test was used to verify data normality. As data was normally distributed, a three-way ANOVA test was performed to analyze the differences between the groups. Tukey's *post* *hoc* test was applied to multiple comparisons between groups. All statistical tests were performed in the IBS SPSS Statistics 25 software (IBM Corp. Chicago, USA).

3 RESULTS

It was demonstrated by the three-way ANOVA test that the adhesive system (p=0.00) and substrate (p=0.00) influenced in the results, but aging, did not (p=0.81). Besides, the triple interaction (adhesive x substrate x aging) was not significant (p=0.11). ABU showed significant difference in the E group between the SE and ER after 24h. The E group showed higher values on the bond strength than the EA and S in the SBU SE at any time. The microtensile bond strength (μTBS) values of the tested groups are shown in table 3.

All types of failures were observed in all groups where adhesive failures were predominant (figure 1 and 2).

Table 3. Microtensile Bond Strength (μTBS) means (standard deviation) in MPa of different experimental groups.

	Eroded dentin (E)		**Eroded + abraded dentin (EA)**		**Sound dentin (S)**	
	24h	12m	24h	12m	24h	12m
ABU SE	51.92 (15.09) Ab	50.84 (8.01) Ab	49.52 (21.54)	47.51 (14.23) Ab	43.40 (14.07) This	45.09 (8.29)
ABU ER	71.06 (12.30)	69.73 (12.85) Ab	65.64 (7.15) ABa	68.42 (10.03)	56.44(16.70) ABa	48.27(15.05) or
SBU SE	71.32 (14.94)	75.59 (13.88)	49.52 (15.12) or	42.26 (19.35) Bb	46.12(9.95) or	51.53(8.30) or
SBU ER	64.20 (8.51) This	67.31 (17.19) Ab	48.32 (9.07)	62.51 (10.20)	57.88(7.70) ABa	52.62(7.25) ABa
ABU: All-Bond Universal; SBU: Scotchbond Universal; SE: Self-etch; ER: Etch-and-rinse. Means with the similar uppercase (lines) and lowercase (columns) letters indicate no significant difference (p > 0.05).						

Figure 1 – Fracture pattern – samples tested in 24 h – adhesive system/approaching.

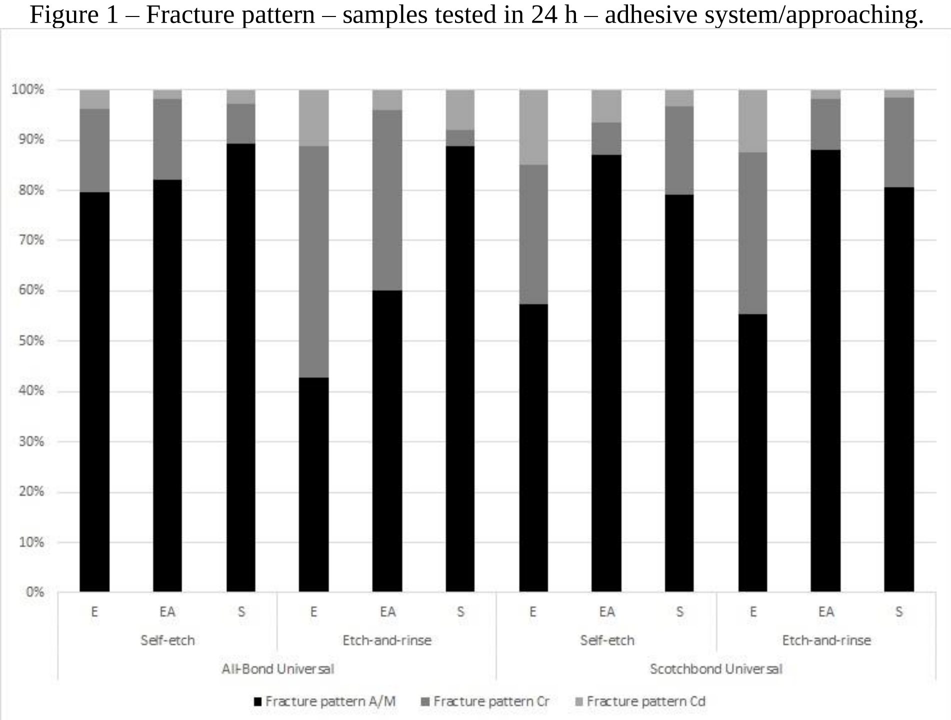
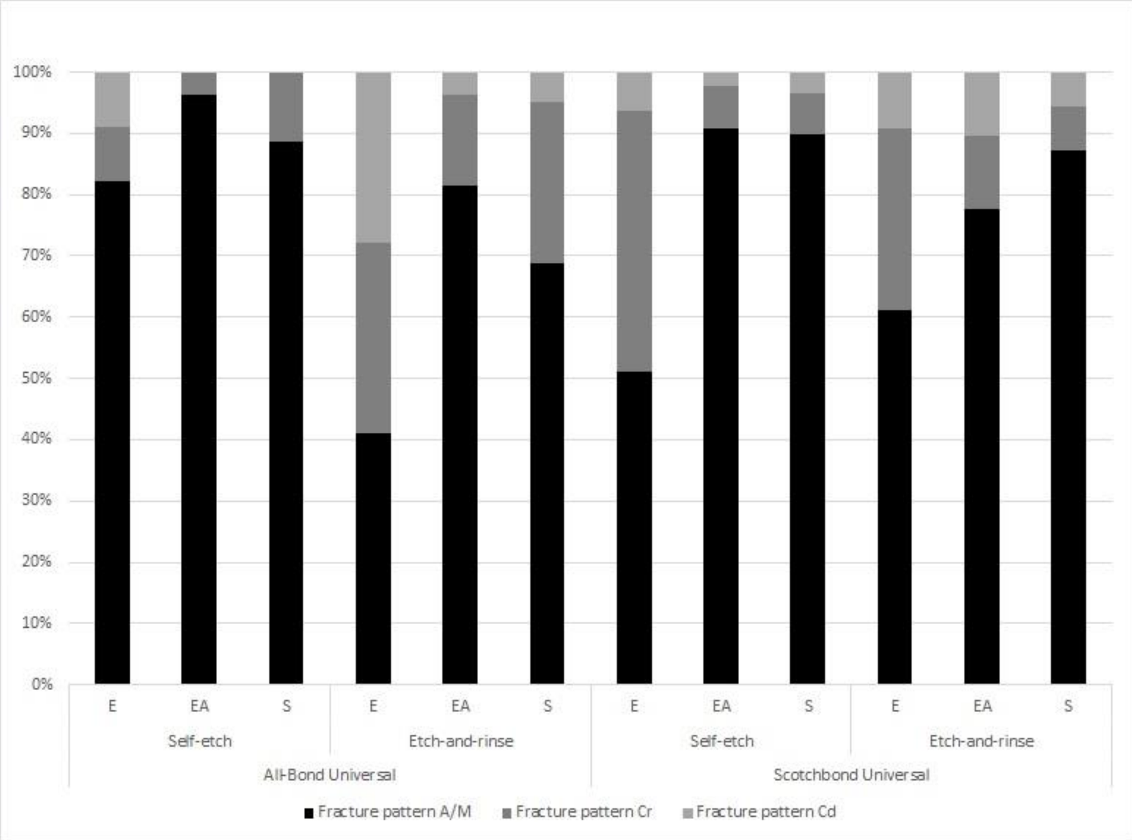


Figure 2 – Fracture pattern – samples tested in 12 m – adhesive system/approaching.



4 DISCUSSION

Concerning dentin substrates submitted to an erosive and abrasive process, laboratory studies have investigated the influence of the cited conditions on bond strength using different adhesive systems.[8,13,14] Clinically, these alterations usually involve physiological processes with dentin responses to external stimuli. These pathological processes result in irreversible loss of mineral caused by well-defined and diverse etiological factors.[15]

This study simulated a clinical condition of erosion and abrasion processes. The EA group simulates dental wear that clinically occurs and may alter the dentin in a different way from that occurring in the erosive process only. The protocol to simulate EA was a combination of two laboratory protocols to first simulate an erosion[8] followed by a simulating abrasion[12] that seems to occur when the patient brushes the teeth immediately after dietary acid defy.[4]

Universal adhesives are widely used for NCCLs treatment because they are versatile, easy to handle and may exclude the step of etch-and-rinse in some situations.[16] This study rejected the null hypothesis since our findings suggest that dentin substrate treated to simulate NCCLs conditions affect the bond strength. ABU ER bond strength of E dentin groups was higher than ABU SE after 24h. It was clear that the dental erosion positively influenced on the bond strength. The artificial erosion promoted by using this proposed method, probably made the dentin substrate seems like the phosphoric acid effect on regular bonding process since the smear layer is removed and superficial dentine has its collagen fibrils exposed due to the demineralization process that may promote the formation of a very adhesive impregnated hybrid layer.[14]

In the SBU SE, the E group showed higher μTBS values when compared to the EA and S at any time tested. This can be explained by the eroded dentin morphological alteration, which leads to the removal of dentinal buffers and intertubular dentin, as well as the increase in tubule diameter and collagen fiber exposure.[8,14] In addition, the results to EA groups showed no statistical differences in relation to the S groups for both adhesives systems at any time tested. It could be explained by the fact that, after the erosive process, collagen fibrils were exposed and the dentin tubules diameter was increased,[10] then the subsequent abrasive attack may have disorganized the collagen fibrils generating a smooth surface, similar to the sound substrate[4] justifying the similar results of bond strength to the sound substrate.

Regardless of adhesive or approaching used, our findings showed that the aging in water did not influence on bond strength values, what is in agreement with others studies.[17] The presence of MDP as a functional monomer in the composition of the both adhesives may explain the similar results. Since the 10-MDP monomer chemically binds to the dentin, it could benefit the formation of a stable calcium-phosphate structure by the chemical adhesion with the hydroxyapatite calcium.[8] Thus, this monomer chemical structure and mechanism of bonding is considered a contributing factor to adhesion durability,[7,18] resulting in a more hydrolytically stable adhesion to the dental substrate.

5 CONCLUSION

In this study it was clear that generally the E dentin favored the bond strength in 24h and 12m and EA dentin presented similar behavior to S dentin.

- E dentin shows that in 24h the ABU SE group performed lower bond strength than the others;
- EA dentin performed similar results of bond strength for all adhesives and approaching in 24h but in 12m SBU SE performed lower results than ER approaching;
- S dentin performed similar results for all adhesive, approaching and tested times.

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