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Bruna Dias Ilha

ESTABILIDADE DA UNIÃO DE UM ADESIVO UNIVERSAL AO ESMALTE HÍGIDO, ERODIDO E DESMINERALIZADO

Santa Maria, RS
2017

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Dissertação apresentada ao Curso de Mestrado do Programa de Pós Graduação em Ciências odontológicas, Área de Concentração em Odontologia, ênfase em Materiais Dentários, da Universidade Federal de Santa Maria (UFSM, RS) como requisito parcial para obtenção do grau de **Mestre em Ciências Odontológicas**.

Orientador: Prof. Dr. Fábio Zovico Maxnuck Soares

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RESUMO

ESTABILIDADE DA UNIÃO DE UM ADESIVO UNIVERSAL AO ESMALTE HÍGIDO, ERODIDO E DESMINERALIZADO

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Recentemente, novos sistemas adesivos autocondicionantes de passo único vêm ganhando espaço no mercado com a proposta de versatilidade, chamados “adesivos universais” ou “multi-modo”. Tais adesivos permitem sua aplicação sob diferentes estratégias, com ou sem condicionamento prévio com ácido fosfórico. Na prática diária, os profissionais comumente se deparam com substratos em condições diferentes das quais os materiais foram originalmente desenvolvidos. Considerando que a adesão ao esmalte desempenha papel importante na longevidade da união resina/dentina, a presença de esmalte com perda mineral nas margens de preparos conservadores pode comprometer as restaurações. O desempenho dos adesivos universais em esmalte desmineralizado e erodido ainda foi pouco estudado, apesar da relevância desses substratos. O objetivo deste estudo foi comparar a resistência de união (RU), imediata e após seis meses, do sistema adesivo Scotchbond universal (3M-Espe), aplicado nas duas estratégias de condicionamento, ao esmalte hígido, desmineralizado e erodido. Foram utilizados 60 incisivos bovinos hígidos recém extraídos, divididos aleatoriamente em 6 grupos (n=10) de acordo com: a) a condição do substrato: hígido (HIG), desmineralizado (DES – ciclagem de pH) e erodido (ERO - imersão em refrigerante de cola); e b) estratégia de aplicação: com condicionamento ácido (ER) ou autocondicionante (SE). O sistema adesivo foi aplicado sobre as superfícies de esmalte de acordo com as recomendações do fabricante e então cilindros de resina composta foram obtidos a partir do preenchimento de tubos de amido (diâmetro interno - 0,96mm) posicionados sobre o adesivo. Seis cilindros de resina composta (corpos de prova) foram obtidos em cada dente (espécime – unidade experimental). Os corpos de prova foram armazenados em água destilada em estufa a 37°C. Dois cilindros de cada espécime foram submetidos ao ensaio de microcisalhamento imediatamente, e dois após seis meses, os dois cilindros restantes serão testados após um ano de envelhecimento. Os valores de RU (MPa) foram submetidos à ANOVA-3 fatores (substrato, estratégia e tempo) e teste Tukey (α=0,05). A interação tripla não foi estatisticamente significante. Os fatores isolados “substrato” (p=0,000) e “estratégia” (p=0,021), bem como sua interação (p=0,008), foram significantes. Os valores de RU apresentaram a seguinte ordem: ERO>HIG>DES tendo a estratégia ER apresentado valores significantemente maiores em HIG. O esmalte desmineralizado reduz a adesão do sistema adesivo Scotchbond Universal, já o erodido não compromete a adesão. O condicionamento ácido prévio à aplicação do adesivo resulta em maior adesão ao esmalte hígido.

Palavras-Chave: Colagem dentária. Esmalte dentário. Resistência ao cisalhamento.

ABSTRACT

BOND STRENGTH STABILITY OF A UNIVERSAL ADHESIVE TO SOUND, ERODED AND DEMINERALIZED ENAMEL

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Recently, new single-step self-etching adhesive systems have been getting space in the market with more versatility proposal, the so called "universal adhesive" or "multi-mode" adhesive systems. These adhesives enable the clinician to select the most appropriate protocol for each clinical situation, allowing its application in different adhesion strategies, with or without prior acid etching. In daily practice, professionals commonly deal with substrates in different conditions from which the materials were originally developed. Considering that adhesion to enamel plays an important role in the longevity of resin/dentin bonds, the presence of enamel with mineral loss in the margins of conservative tooth preparations can compromise the restorations. Performance of the universal adhesives in demineralized and eroded enamel was still little studied, despite the relevance of these substrates. The aim of this study was to compare the immediate and six month shear bond strength (SBS) of Scotchbond universal adhesive systems applied in both etching strategies, to sound, demineralized and eroded enamel. Sixty sound permanent bovine incisors will be randomly divided into 6 groups (n=10) according to: a) substrate condition: sound (SND), demineralized (DEM - pH cycling) and eroded (ERO - immersion in cola drink); and b) etching strategy: etch-and-rinse (ER) or self-etching (SE); The adhesive system was applied on enamel surfaces according to manufacturer's recommendations and then cylinders of composite resin were obtained from filling starch tubes (internal diameter of 0,96mm) positioned over the adhesive. Six composite resin cylinders were obtained for each tooth (specimen - experimental unit). The teeth were stored in distilled water at 37 ° C. Two cylinders of each tooth were submitted to microshear test immediately and two after six months, the two remaining cylinders will be tested after one year of aging. The SBS values (MPa) were submitted to three-way ANOVA (substrate, strategy and time) and Tukey test ($\alpha = 0.05$). The triple interaction was not statistically significant. The main factors "substrate" ($p = 0.000$) and "strategy" ($p = 0.021$), as well as their interaction ($p = 0.008$), were significant. The SBS values presented the following order: ERO> SND> DEM and the ER strategy presented significantly higher values in SND. Demineralized enamel reduces the bond strength of Scotchbond universal adhesive system, while the eroded one does not compromise the adhesive performance. Prior acid etching results in greater adhesion to sound enamel.

Key words: Bonding, Dental. Enamel, Dental. Strength, Shear.

SUMÁRIO

1 INTRODUÇÃO

Os sistemas adesivos disponíveis atualmente têm sido classificados de acordo com o número de passos clínicos necessários para sua aplicação e, principalmente, pela estratégia utilizada, dividindo-os em dois grandes grupos: autocondicionantes (*self-etch*) e com condicionamento ácido (*etch-and-rinse*) (PASHLEY et al., 2011; VAN MEERBEEK et al., 2003, 2011). No intuito de reduzir o tempo de aplicação e oferecer versatilidade, recentemente, sistemas adesivos autocondicionantes de passo único tem sido formulados de forma a permitir sua aplicação nas duas estratégias, com ou sem condicionamento prévio com ácido fosfórico (DE GOES; SHINOHARA; FREITAS, 2014; HANABUSA et al., 2012). Tais sistemas tem sido classificados como “adesivos universais” ou “multi-modo” por permitirem ao profissional optar pelo protocolo mais adequado para cada situação clínica (CARDENAS et al., 2016; HANABUSA et al., 2012; MUÑOZ et al., 2013; WAGNER et al., 2014).

O condicionamento ácido prévio promove adesão eficiente e estável ao esmalte por meio da dissolução seletiva dos cristais de hidroxiapatita, produzindo microporos profundos a serem ocupados pelo adesivo por capilaridade, possibilitando a formação de tags (VAN MEERBEEK et al., 2003). Ao passo que os adesivos auto-condicionantes não requerem um passo exclusivo para o condicionamento, pois possuem em sua composição monômeros acídicos que condicionam a superfície do esmalte e infiltram no espaço simultaneamente (VAN MEERBEEK et al., 2011). Esses materiais são também classificados de acordo com a acidez, o que influencia diretamente na extensão e profundidade da desmineralização (ROTTA et al., 2007).

A técnica de condicionamento ácido prévio ainda é a primeira escolha dos clínicos quando se trata de esmalte (ERICKSON; BARKMEIER; KIMMES, 2009; ROTTA et al., 2007; VAN MEERBEEK et al., 2011) e embora estudos recentes mostrem maior efetividade na adesão de adesivos universais a esse substrato quando aplicados sob tal estratégia (ANTONIAZI et al.,2016;DE GOES; SHINOHARA; FREITAS, 2014; HANABUSA et al., 2012; LOGUERCIO et al., 2015a; MCLEAN et al., 2015; PERDIGÃO; LOGUERCIO, 2014; ROSA; PIVA; SILVA, 2015), estudos clínicos que avaliaram o uso de adesivos universais aplicados sob diferentes estratégias em lesões não cariosas não encontraram diferenças significativas relacionadas à retenção das restaurações, mostrando que o desempenho clínico dos adesivos universais parece não depende da forma de aplicação (LOGUERCIO et al., 2015b; MENA-SERRANO et al., 2013; PERDIGAO; TAY, 2013).

Os procedimentos de adesão ao esmalte desempenham um papel importante tanto na odontologia restauradora quanto preventiva (LENZI et al., 2013a), considerando a necessidade de um bom selamento para reduzir os riscos de manchamento e lesão de cárie secundária (HEINTZE, 2013) e tendo em vista que a longevidade da união resina/dentina está associada à união ao esmalte circundante (HEINTZE, 2013; TORKABADI et al., 2009) e que com o crescimento da odontologia minimamente invasiva, certa quantidade de esmalte com perda mineral poderia permanecer nos contornos da cavidade, a possível presença desse substrato poderia comprometer a durabilidade da restauração.

Ademais, maneira como os ácidos da dieta são introduzidos é determinante na localização e na duração do ataque ácido nas superfícies dentárias (JOHANSON et al., 2004) podendo gerar lesões não cariosas que têm sido recentemente classificadas como erosão dental, lesões de causa multifatorial de difícil diagnóstico em fases iniciais (LUSSI et al., 2011) mas que resultam de uma perda de estrutura dentária patológica, crônica e localizada, causada por ácidos, mas sem o envolvimento de bactérias (LINNETT; SEOW, 2001; TEN CATE; IMFELD, 1996) diferentemente do desafio cariogênico.

O desempenho dos adesivos universais em esmalte desmineralizado e erodido ainda foi pouco estudado, apesar da relevância desses substratos, dado que a prevalência de lesões não cariosas vem crescendo (GAEGGI; LUSSI 2014) em função de mudanças no estilo de vida e padrão de alimentação, além de um aparente aumento na prevalência de desordens gastrointestinais (COSTENOBLE et al., 2016; LUSSI; JAEGGI, 2008; LUSSI et al., 2011) e ainda, que os conceitos atuais de odontologia restauradora preconizam tratamentos o mais conservadores possíveis.

Estudos recentes demonstram menores valores de resistência de união de sistemas adesivos ao esmalte com perda mineral, quando comparados ao esmalte hígido (ANTONIAZI et al.,2016;MOBARAK; ALI; DAIFALLA, 2015; TEDESCO et al., 2014), ao contrário do que parece acontecer no esmalte erodido, para o qual foram encontrados valores de resistência de união superiores, quando comparado ao esmalte hígido (FRATTES et al.,2017; LENZI et al., 2013a).

Neste sentido, a obtenção de união uniforme, eficaz e estável aos tecidos dentários, tanto esmalte quanto dentina, mesmo em possíveis condições de perda mineral tem sido um desafio (CARDOSO et al., 2011; VAN MEERBEEK et al., 2011). Embora a união ao esmalte seja atualmente bastante previsível, especialmente quando se usa sistemas adesivos com condicionamento ácido prévio (CARDOSO et al., 2011; ERICKSON; BARKMEIER; KIMMES, 2009; LENZI et al., 2013b; ROSA; PIVA; SILVA, 2015; TAKAMIZAWA et al., 2015), é necessário considerar que na prática diária os profissionais comumente se deparam com substratos em condições diferentes das quais os materiais foram originalmente desenvolvidos (TEDESCO et al., 2014) gerando incertezas em relação à estabilidade da união em tais substratos com estrutura alterada (CRUZ et al., 2015). Além disso, tais sistemas adesivos apresentam misturas complexas de componentes hidrofóbicos e hidrofílicos em um mesmo frasco (VAN MEERBEEK et al., 2011), essa natureza os torna sujeitos à sorção de água e consequentemente mais sucetíveis à degradação (CARDOSO et al., 2011) o que reforça a necessidade de avaliação da resistência de união a longo prazo.

Levando em consideração os argumentos citados, o objetivo deste trabalho foi avaliar a resistência de união imediata e após seis meses, de um sistema adesivo universal, ao esmalte em diferentes condições e sob diferentes estratégias de aplicação.

2 ARTIGO – BONDING WITH A UNIVERSAL ADHESIVE TO SOUND, DEMINERALIZED AND ERODED ENAMEL

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Title Page

Title: Bonding with a universal adhesive to sound, demineralized and eroded enamel

Short Title: Enamel condition influences bonding of a universal adhesive

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Keywords: Dental Bonding, Shear Strength, Eroded Enamel, Demineralized Enamel, Multi-Mode Adhesive.

Bonding with a universal adhesive to sound, demineralized and eroded enamel

Abstract

Objectives: To evaluate the immediate and six-month microshear bond strength of Scotchbond Universal Adhesive (SBU - 3M-Espe) to sound, demineralized and eroded enamel using both adhesion strategies, etch-and-rinse and self-etch. **Methods:** Buccal flat enamel surfaces of sixty sound bovine incisors were randomly assigned to 6 groups (n=10) according to: substrate condition (sound enamel - SND, demineralized enamel - pH cycling for 14 days – DEM and eroded enamel – immersion in cola based drink – ERO) and etching modes (etch-and-rinse – ER and self-etch – SE). The adhesive system was applied following the manufacturer's recommendations. Starch tubes (internal diameter of 0,96mm) were positioned in the surface and light cured. The tubes were completely filled with resin composite. After storage in distilled water at 37° C for 24h, starch tubes were removed. Two specimens for each tooth were submitted immediately, and two after six months of storage, to microshear bond strength test (μSBS). Bond strength values (MPa) were analyzed by three-way ANOVA and post-hoc Tukey test (α=0,05). **Results:** The triple interaction was not statistically significant. The main factors "enamel condition" (p=0,000) and "strategy" (p=0,021), as well as, cross-product interaction "enamel condition vs strategy" were statistically significant (p=0,008). The bond strength values presented followed order ERO>SND>DEM. SBU in ER produced higher values in SND. **Conclusion:** Demineralized enamel decrease the bond strength of Scotchbond Universal adhesive while eroded enamel does not jeopardize the adhesive performance. Prior acid etching results in better adhesion to enamel.

Clinical Significance: When bonding to enamel with Scotchbond Universal adhesive, clinicians should opt to etch-and rinse mode. In addition, if demineralized enamel remains in cavity preparation, bond strength will be reduced

Keywords: Dental Bonding, Shear Strength, Eroded Enamel, Demineralized Enamel, Multi-Mode Adhesive.

Introduction

The lastest development in adhesive dentistry seeking to promote stable adhesion to dental substrates with simplified and fast procedures led to the development of new adhesive systems known as “universal” or “multi-mode” which are designed with the “all-in-one” concept adding the versatility of being adaptable to different clinical situations in both adhesion strategies [1–4], either self-etch mode, etch-and-rinse or even selective enamel etching.

Although enamel is considered a simple and predictable substrate for bonding [5,6], it may present a challenge when this substrate is altered, such as demineralized or eroded. Moreover, there is concern when self-etch adhesives are used in enamel. Self-etch adhesives have in their composition acidic monomers that condition the enamel surface and infiltrate the spaces simultaneously [7], these systems are classified according to acidity, which directly influences the extent and depth of demineralization [8]. The increase in the surface area generated by self-etching adhesives in enamel is smaller than that achieved with phosphoric acid and this effect is dependent on the pH of the adhesive [9].

Currently, dental erosion is a common problem of a multifactorial cause and difficult diagnosis in the early stages [10]. It is defined as dental mineral loss attributed to a chemical process of dissolution caused by acids without involving microorganisms [11,12]. Sources of acids can be attributed both extrinsic and intrinsic ways, and it increases owing to changes in lifestyle and eating patterns, as well as an apparent increase of gastrointestinal disorders [10,13,14]. Most reports related a later intervention, when dentin is exposed, in the presence hypersensitivity and more complex restorative needs. However, bonding to eroded enamel may be necessary when restorative procedures using few or no preparation [15] are chosen to correct aesthetic problems associated with tooth shape, position, and color. In addition, the margins of class V erosion lesions are commonly presented in enamel. There is a lack of research available on bonding to eroded enamel.

Demineralized enamel is different from sound enamel, which may lead to an unsuitable etching pattern and deficient infiltration of monomers, resulting in decreased bond strength [16–18]. Whereas the minimally invasive approach is becoming routine, some demineralized enamel may be left after selective caries removal. Considering this, and that the longevity of the resin/dentin bonds is associated with adhesion to the surrounding enamel [19,20], the investigation on bonding to this substrate under different conditions is relevant. Thus, this investigation aimed to evaluate the influence of the enamel condition – sound, demineralized and eroded on the bond strength of a universal adhesive using two etching strategies after 24-h and 6-mo water storage. The null hypothesis was that the enamel condition has no influence on the bond strength values of universal adhesive, regardless the etching approaches and storage time.

Materials and Methods

Selection and Tooth Preparation

Sixty freshly extracted bovine incisors were selected. Teeth were cleaned and disinfected in 0.5% aqueous chloramine and stored in distilled water at 4 °C until use. The roots were removed using diamond saw in a hand piece under low speed. Crowns were partially embedded in self-curing acrylic resin inside PVC rings (JET clássico, São Paulo, SP, Brazil), leaving the buccal surfaces exposed. The exposed buccal surfaces were ground with 180-grit SiC paper, under running water, to create flat enamel surfaces. Then, enamel surfaces were ground for 60-s with 600-grit SiC paper, under running water, for standardization of surface smoothness.

Experimental design

Specimens (embedded crowns with an enamel surface exposed) were randomly allocated (RANDOM.ORG) to 6 groups (n=10), according to the enamel condition – sound, artificially eroded and demineralized and adhesion strategy of Scotchbond Universal Adhesive (3M ESPE, St. Paul, MN, USA) – self-etch and etch-and-rinse modes. In each specimen the cylinders obtained were divided in two for each storage time – 24-h and 6-mo.

Artificially induction of Demineralized Enamel

Twenty specimens were subject to pH cycling prior to restorative procedures. Each specimen was immersed individually in 10 ml of demineralizing solution for 8h

(2,2 mM de $CaCl_2$, 2,2 mM de NaH_2PO_4, 0,05 M acetic acid adjusted to pH4,5 with 1M de KOH) and in 10 ml of remineralizing solution for 16h (1,5 mM de $CaCl_2$, 0,9 mM deNaH_2PO_4, 0,15 mM de KCl adjusted to pH 7,0) [21]. This procedure was carried out for 14 days. Between the cycles specimens were washed with deionized water, dried with absorbent paper and the solutions were changed. Solutions were periodically measured under a pH meter.

Artificially induction of Eroded Enamel

Twenty specimens were subject to erosive challenge through immersion three times a day for seven days. In each of them, teeth were immersed individually in a cola drink (Coca-Cola, [pH—2.6, phosphate—5.43mM 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^- to pH 7.0], Spal, Porto Real, RJ, Brazil) for 5 minutes under agitation and kept in artificial saliva at room temperature (1.5 mmol/l–1 $Ca[NO_3]_2.4H_2O$, 0.9 mmol/l–1 $NaH_2PO_4.2\ H_2O$, 150 mmol/l–1 KCl, 0.1 mol/l–1 Tris buffer, 0.03 ppm F, pH 7.0) in the remaining time [22]. Between the cycles, specimens were washed with deionized water, dried with absorbent paper and saliva was changed.

Bonding and Restorative Procedures

Scotchbond Universal Adhesive (SBU) was used in self-etch (SE) and etch-and-rinse (ER) strategies. The composition and adhesion strategy are described in Table1. The adhesive was applied following the manufacturer's recommendations, by a single operator, on six areas of each enamel surface. Starch tubes [23] (0,96mm internal diameter x 1mm height) were carefully positioned before light curing (EMITTER C, SCHUSTER, Santa Maria, RS, Brazil). The tubes were completely filled with resin composite (Z250, 3M ESPE, St. Paul, MN, USA, shade A3) and light cured for 20 s. For each specimen were obtained 6 cylinders of composite resin.

Microshear test (µSBS): Bond Strength and stability

After 24h storage (distilled water,37° C), starch tubes were removed using air/water spray. Two random cylinders of each specimen were submitted immediately and other two after additional six months of water storage to michoshear bond strength test in a universal machine (Emic DL 1000, Equipment and Systems; São José dos Pinhais, PR, Brazil) using a stainless-steel wire loop (0,2mm in diameter) placed as close as possible to the resin/enamel interface. Shear load was applied at a crosshead speed of 1mm/min until failure. The remaining two cylinders will be evaluated after one year of storage. For cylinders that failed prior to the test a value of 2.8 MPa was assigned which were considered in the statistical analysis (minimum load cell sensitivity value). All tests were performed by a single blinded operator.

Failure Mode

The fractured specimens were examined under a stereomicroscope at 40X magnification to determine the failure mode and categorized as mixed/adhesive, cohesive in enamel, or cohesive in resin composite [16].

SEM preparation

For each substrate one additional tooth was prepared for evaluation under scanning electron microscope (SEM). Specimens were dehydrated in ascending degrees of ethanol (50, 75 and 95% for 5 min each, and 100% for 3 h) [24], kept in a desiccation chamber to remove any remaining water and gold sputter coated for SEM (VEGA3, TESCAN) observation, operated in secondary electron mode with 5.0kV voltage.

Statistical Analysis

The experimental unit in the study was the tooth. Thus, the means of µSBS (MPa) values of cylinders from each tooth in each storage time (24h and 6mo) were averaged for statistical purposes. The sample size was determined considering a coefficient of variation of the pool data of a pilot study assuming a standard error of 20% a minimum of 8 teeth per group was required to achieve a power of 0.8 and and α-error probability of 5%. Taking into account possible losses not related to the considered outcome, the n value was set to 10.

Three factors were considered for statistical analysis: enamel condition (sound, demineralized and eroded), strategy (i.e. SBU applied in etch-and-rinse or self-etch strategies) and time (24h and 6mo).

A normal distribution of the data was confirmed by Kolmogorov-Smirnov test. Data were analyzed by three-way repeated measures Analysis of Variance (ANOVA) and a post-hoc Tukey at a significance level of 0.05, using a statistical software package (Minitab, Minitab Inc., State College, PA, USA).

Results

Descriptive statistics of μSBS, including means (MPa), standard deviations and total of tested specimens/premature failures for all groups are presented in Table 2. The triple interaction was not statistically significant. The main factors “enamel condition” ($p=0.000$) and “strategy” ($p=0.021$) (Table 3), as well as, cross product interaction “enamel condition vs strategy” (Table 4) were statistically significant ($p=0.008$). Artificially eroded enamel produced higher values compared to the other substrates while artificially demineralized enamel presented the lowest values of μSBS.

Considering the ‘strategy’ factor, SBU applied at an etch-and-rinse mode resulted in higher bond strength values than in a self-etch mode.

The μSBS of SBU, regardless the strategy, in eroded enamel was similar to that obtained in sound enamel, using the etch-and-rinse mode, which were superior to the other groups.

In sound enamel, SBU in etch-and-rinse mode produced higher values. Demineralized enamel negatively affected the microshear bond strength, while eroded enamel produced higher bond strength values compared to the other substrates (table 4).

Storage time factor was not significant ($p=0.774$) as well as its interactions with enamel condition and strategy (0.600 and 0.343 respectively).

With regard to the failure mode, only mixed/adhesive failures were found in all conditions.

SEM images revealed a regular surface for SND (Fig. 1-A) compared to ERO (Fig. 1-B) that showed rough surface with distributed erosion areas and DEM (Fig. 1-C) that presented a disorganized demineralization pattern.

Discussion

Although enamel is considered a reliable substrate that produces stable and strong adhesion, changes in the structure that might be present on clinical conditions may compromise the bonding. In this study, demineralized enamel presented the lowest µSBS values, irrespective of the etching modes. These findings may be attributed to the lower amount of minerals and higher porosity of the surface [25], which leads to consider demineralized enamel a poor and improper substrate for bonding [17]. These results corroborates with previous studies [16–18] and are a relevant outcome considering the risk that some demineralized enamel may be accidentally and unnoticeably left around the cavity after preparation, since that the enamel bond procedures play an important role in restorative dentistry [26] as that enamel seal protects resin/dentin bonds against degradation [20,27] and reduce the risks of marginal staining and marginal caries [19]. The *in vitro* cariogenic challenge used in this study causes similar mineral loss, albeit not as deep as natural lesions [28] because the variation of pH in the oral environment are more acute and occur for longer periods [16], thus, it may be suggested that clinical behavior may be even worse.

Conversely, an opposite trend was observed in eroded enamel, which values were significantly higher than other substrates, including sound enamel. There is few evidence reported on bonding to this substrate, even considering its relevance, as the prevalence of non-carious cervical lesions has been increasing [29] and even when involving dentin, the margins are frequently in enamel. Moreover, the adhesive restoration of non-carious cervical lesions with different etiological factors can improve structural integrity and biomechanical function around the lesion almost to the levels of healthy teeth [30–32]. The erosive pH cycling model employed in this study used cola drink, because this beverage has low pH and low fluoride and calcium concentration [22] thus having a high erosive potential [33]. In the present study, eroded enamel showed the highest bond strength values, in accordance with previous researches [26,34]. Rougher enamel surfaces, as clearly seen in SEM image (Figure 1-B), as a result of erosive challenge, seems to play a relevant role in the adhesion mechanism, as it promotes more intense interlocking to enamel [35]. These results differ from those found by Wang et al [36] and Casas-apayco et al [37] where the eroded enamel impaired and had no interference in the bond strength respectively. Divergent results may be due the differences in acid challenge and materials tested. Furthermore, these studies used microtensile test which use for enamel evaluation have been questioned [38] due to its brittle charactheristic [39].

The SEM representative images revealed for SND (Fig. 1-A) a regular surface compared with ERO (Fig. 1-B) and DEM (Fig. 1-C). ERO observation shows a rough surface, with distributed areas of erosion, which increases wettability and can promote better interlocking with enamel [35], whereas DEM indicated a rougher surface but with a disorganized demineralization pattern.

Despite SBU's manufacturer recommends the use in either self-etch or etch-and-rinse mode even in enamel, this in vitro study found that the etch-and-rinse approach is more effective in this substrate. This application method significantly increased the shear bond strength for SBU in SND, in agreement with previous studies [2,3,18,40–42]. Thus, the versatility claimed for the "multi-mode" adhesives is not supported, at least for enamel, by the literature findings. This can be attributed to an improved micromechanical bond produced by phosphoric acid, which increases the bonding area and the wettability of the surface [43]. In this context, universal adhesives have less acidic composition compared with phosphoric acid, reducing their potential to demineralize enamel and create appropriate retentive pattern [5].

This in vitro study demonstrated that six month of water storage did not influence the bond strength of SBU, regardless of application strategy used and the substrate. Differences in µSBS values between 24 hours and 6 months ageing were not significant, which corroborates previous findings [40]. Six months may represent a short water storage time, since even in DEM and ERO substrates no degradation on adhesive interface were observed. This was expected for SND enamel whereas its degradation does not occur so quickly [44].

It is worth to note that bovine incisors were used, as it have been used, especially in adhesions tests due to easier obtaining and standardization on tooth age, additionally it produces similar bond strength results to human teeth [45]. One should note the limitation that erosive challenge employed simulates chemical alteration on superficial enamel but without mechanical abrasive effect of tooth brushing that occurs in oral environment.

From the results of this study, we partially rejected the null hypothesis as enamel condition produced different outcomes, application strategies differed only on SND and storage time had no influence in bond strength values. Further studies evaluating longer storage times and clinical performance should be encouraged.

Conclusion

Demineralized enamel reduces bonding with Scotchbond Universal Adhesive, regardless the etching mode. Conversely, eroded enamel does not compromise the adhesive performance. Prior acid etching step improves the bond strength to sound enamel.

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Tables

Table 1: Scotchbond Universal composition and application mode

Mode	Self-etching	Etch-and-rinse
Application mode*	Apply the adhesive for 20 s with vigorous agitation Gently air thin for 5 s Light cure for 10 s	Apply etchant for 15 s Rinse for 10 s Air dry to remove excess water Apply the adhesive as for the self-etching mode
Components (pH 2,7)	Etchant: 34% phosphoric acid, water, synthetic amorphous silica, polyethylene glycol, aluminium oxide Adhesive: MDP phosphate monomer, dimethacrylate resins, HEMA, methacrylat emodified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane	

*According to the manufactures' instructions.

Table 2. Descriptive statistics for µSBS means, standard deviations (MPa) and number of total specimens/premature failures for all experimental groups.

Strategy	Time	Sound Enamel	Demineralized Enamel	Eroded Enamel
SBU ER	24h	19,9 (5,6) 10/0	12,5 (5,1) 10/2	21,7 (4,3)10/0
	6m	20,5 (4,0) 10/1	14,3 (3,8) 10/0	22,1 (2,7) 10/0
SBU SE	24h	16,4 (5,1) 10/0	14,6 (5,5) 10/1	19,8 (4,3) 10/1
	6m	13,9 (3,3) 10/0	14,8 (5,0) 10/1	20,4 (4,7) 10/0

SBU: Scotchbond Universal Adhesive; ER: etch-and-rinse; SE: Self-etching.

Table 3. μSBS (MPa) means (standard deviations) for the main factors enamel condition and etching mode

Factor		**MPa**
Enamel condition	Eroded	21.0 (4.0)^{A}
	Sound	17.7(5.2)^{B}
	Demineralized	14.1 (4.8)^{C}
Strategy	SBU ER	18.5 (5.6)^{A}
	SBU SE	16.6 (5.2)^{B}

Different superscript letters indicate statistically significant differences ($p<0.05$).
SBU: Scotchbond Universal Adhesive; ER: etch-and-rinse; SE: Self-etching.

Table 4. µSBS means (standard deviations) (MPa) for the interaction 'enamel condition *vs* strategy'

	Enamel condition		
Strategy	Sound	Demineralized	Eroded
SBU ER	20.2 (4.7)^{A}	13.4 (4.5)^{B}	21.9 (3.5)^{A}
SBU SE	15.1 (4.4)^{B}	14.7 (5.1)^{B}	20.1 (4.4)^{A}

Different superscript letters indicate statistically significant differences (p<0.05).
SBU: Scotchbond Universal Adhesive; ER: etch-and-rinse; SE: Self-etching.

Figures

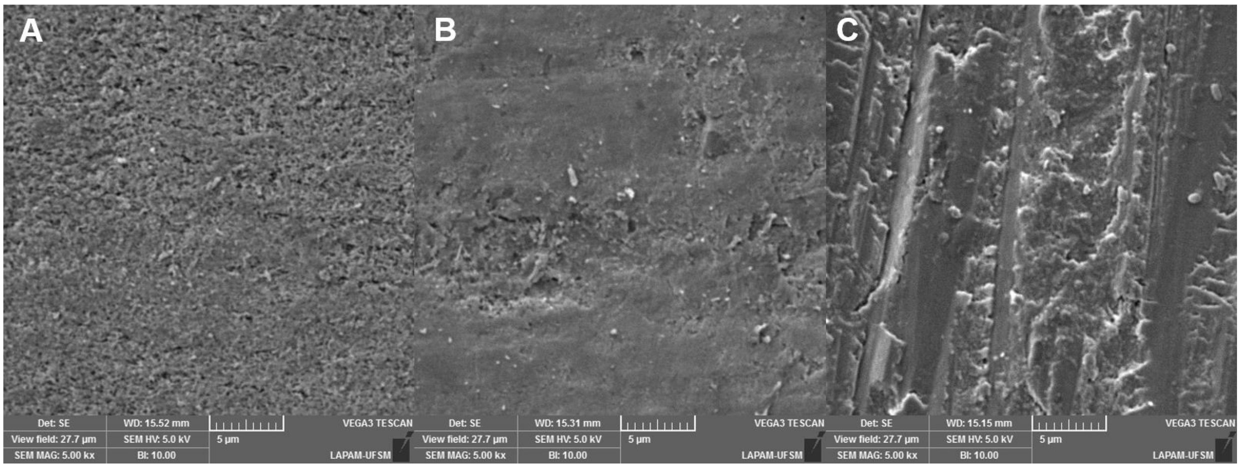

Figure 1: SEM micrograph of SND enamel (A), ERO enamel (B) and DEM enamel (C) surfaces

3 CONCLUSÃO

Esta dissertação analisou o desempenho de um sistema adesivo universal, aplicado sob diferentes estratégias de condicionamento em três condições de substrato, imediatamente e após seis meses de armazenamento.

Os adesivos universais têm como principal proposta a versatilidade, sobretudo quanto a estratégia de condicionamento, pois podem ser utilizados com condicionamento ácido prévio, no modo autocondicionante, ou ainda com condicionamento seletivo do esmalte.

Tendo em vista o crescimento da odontologia minimamente invasiva, onde os tratamentos apresentam-se de forma mais conservadora possível, os profissionais acabam se deparando com substratos em diferentes condições. Esmalte com perda mineral devido ao ataque ácido, causado por bactérias ou não, apresenta comportamento diferente de esmalte hígido frente aos materiais adesivos. Logo, as incertezas em relação à estabilidade da união de novos sistemas adesivos a tais substratos permanecem.

O presente trabalho mostra, com as limitações de um estudo *in vitro,* que em esmalte hígido o adesivo Scotchbond universal apresenta melhor desempenho na estratégia de condicionamento ácido prévio. Considerando a adesão aos diferentes substratos, o esmalte desmineralizado prejudica a resistência de união do material estudado, enquanto o esmalte erodido não compromete o comportamento do adesivo. A estabilidade da união se manteve ao longo dos seis meses de avaliação, independentemente da condição do substrato ou do modo de aplicação.

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ANEXO A - NORMAS PARA PUBLICAÇÃO NO PERIÓDICO *JOURNAL OF DENTISTRY*

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