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Marcos Paulo Marchiori Carvalho

USO DA ACRILAMIDA E DIFERENTES PROTOCOLOS DE APLICAÇÃO DE UM SISTEMA ADESIVO UNIVERSAL NA RESISTÊNCIA DE UNIÃO À DENTINA

Santa Maria, RS
2017

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Tese de doutorado apresentado ao Curso de Doutorado do Programa de Pós-Graduação em Ciências Odontológicas, Área de Concentração em Odontologia, ênfase em Dentística, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Doutor em Ciências Odontológicas**.

Orientador: Prof. Dr. Alexandre Henrique Susin

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USO DA ACRILAMIDA E DIFERENTES PROTOCOLOS DE APLICAÇÃO NO AUMENTO DA RESISTÊNCIA DE UNIÃO À DENTINA DE UM ADESIVO UNIVERSAL

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Santa Maria, RS
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RESUMO

USO DA ACRILAMIDA E DIFERENTES PROTOCOLOS DE APLICAÇÃO DE UM SISTEMA ADESIVO UNIVERSAL NA RESISTÊNCIA DE UNIÃO À DENTINA

AUTOR: Marcos Paulo Marchiori Carvalho
ORIENTADOR: Alexandre Henrique Susin

Este trabalho tem o propósito de testar diferentes protocolos adesivos, bem como influência da alteração de composição do sistema adesivo universal Single Bond Universal com o intuito de verificar o efeito obtido na resistência de união e estabilidade da camada híbrida. Cento e quarenta e quatro dentes foram obtidos através do Banco de dentes permanentes Humanos da UFSM e distribuídos aleatoriamente para cada grupo, divididos de acordo com o protocolo de aplicação (Controle, Clorexidina, Ar Quente, Dupla aplicação do adesivo, Fotoativação por 40 segundos e “All in One”), pelo tipo de adesivo (Single Bond Universal e Single Bond Universal modificado) e pelo tempo de armazenamento (24 horas e 06 meses). Para testar os efeitos de protocolo versus modificação da composição, será usado um único adesivo universal a base de etanol (Single Bond Universal) para isolar o fator composição química. As alterações na composição química do sistema adesivo foram realizadas pela incorporação de uma acrilamida. A adição do polímero foi realizada em uma concentração de 0,05ml para cada 1ml do adesivo. Para analisar as diferenças entre as versões do adesivo, foi realizado ensaio de ultimate bond strength. Para verificar diferenças na camada híbrida e na resistência de união entre os adesivos e os diferentes protocolos de aplicação, foram realizado testes de microtração. A hipótese nula é de que alterações no protocolo e na viscosidade do adesivo não resultariam em melhoras significativas nos desfechos estudados. Os resultados observados nos dois artigos mostrou um aumento na resistência de união à dentina para o adesivo com adição de acrilamida, bem como um aumento na resistência intrínseca do adesivo verificado pelos ensaios de ultimate em ambos os trabalhos. Entre os protocolos, o grupo “All-in-One” obteve os melhores resultados mas sem diferença estatistica em relação aos grupos Clorexidina, Ar Quente e Dupla aplicação. O grupo Controle apresentou o pior resultado porém não foi estatisticamente pior que o grupo 40s. A adição de acrilamida a um adesivo universal a base de etanol aumentou a resistência intrínseca do adesivo bem como a resistência de união desse adesivo à dentina. Algumas mudanças no protocolo de aplicação de um adesivo universal também aumentou a resistência de união à dentina.

Palavras-chave: Adesivos dentinários. Dentina. Resistência à tração. Adesão.

ABSTRACT

USE OF ACRYLAMIDE AND DIFFERENT APPLICATION PROTOCOLS ON THE DENTIN BOND STRENGTH OF A MULTI-MODE ADHESIVE SYSTEM

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This study aims to test different adhesive protocols, as well as influence of chemical changes of Single Bond Universal to verify improvement in bond strength and stability of the hybrid layer. One hundred and forty-four teeth was obtained through the Bank of Human permanent teeth of UFSM and randomly assigned to each group, divided by the type of application protocol (control, Chlorhexidine, Warm Air, Double adhesive application, Photoactivation for 40 seconds and "All in One"), the type of adhesive (Single Bond Universal and Single Bond Universal modified) and storage time (24 hours and 06 months). To test the effects of the chemical change versus protocol was used a single multi-mode ethanol-based adhesive (Single Bond Universal) to isolate the chemical composition factor. Changes in chemical composition were held by incorporating the adhesive of a acrylamide of pharmaceutical use. The addition of the polymer will occur in a concentration of 1ml/0,05ml the adhesive to generate a different versions to be tested. To analyze the differences between the two adhesives, ultimate bond strength analyzes was made to compare properties of the universal single bond with the universal single bond modified. To verify differences in the hybrid layer and the bond strength between the adhesive and the different application protocols, microtensile tests was done. The conceptual hypothesis of this study is that altered viscosity and different application protocols will improve adhesive properties and long-term bond strength. The null hypothesis is that changes in the protocol and viscosity of the adhesive do not result in significant improvements in outcomes studied. The results observed in the two articles showed an increase in dentin bond strength when an adhesive with addition of acrylamide was used, as well as an increase in its intrinsic resistance verified by ultimate tests in both articles. Among the protocols, the group "all-in-One" obtained the best results but without statistical difference compared to Chlorhexidine, Warm air and Double Application. The Control group presented the worst result however was not statistically worse than the 40s Group. The addition of acrylamide in a universal ethanol based adhesive increased its intrinsic resistance as well as the dentin bond strength. Some changes in the adhesive´s protocol of a universal adhesive also increased the dentin bond strength.

Keywords: Dentin. Dentin-bonding agents. Tensile strength. Bond strength.

SUMÁRIO

1 INTRODUÇÃO

A adesão na estrutura dental tem tido grandes avanços desde que o mecanismo do processo de hibridação foi relatado por Nakabayashi. (NAKABAYASHI, 1982)

Os atuais sistemas adesivos permitem que os profissionais realizem procedimentos restauradores de alta qualidade com mais segurança. Sistemas adesivos foram desenvolvidos em suas formulações químicas e propriedades mecânicas com introdução de novos monômeros e novos balanços químicos. (LANDUYT et al 2007; VAIDYANATHAN. VAIDYANATHAN. 2009; SCHERRER; CESAR; SWAIN. 2010). Dentro desse contexto, sistema adesivo universal como o Single Bond Universal é a geração de adesivos mais recente onde é possível sua aplicação tanto no modo total-etching quanto no self-etching. Porém, alguns estudos já demonstraram que o modo self-etching é melhor em dentina por promover maior estabilidade da camada híbrida e menor degradação (ROSA et al 2015)

Apesar dos sistemas adesivos e dos procedimentos adesivos atuais terem promovido mudanças revolucionárias na odontologia nos últimos anos, estes, ainda não são suficientes para garantir uma interface adesiva estável ao longo do tempo (VAIDYANATHAN; VAIDYANATHAN; 2009), mesmo apresentando melhores resultados em testes mecânicos, realizados in vitro, e melhor desempenho in vivo (SCHERRER; CESAR; SWAIN, 2010; HAHN et al. 2008; CHERSONI et al. 2004; TAKAHASHI et al. 2002; CARDOSO et al. 2011, ZHANG et al. 2005)

Para mensurar resultados de testes mecânicos, como a resistência de união da interface adesiva, ensaios de microtração ou microcisalhamento são comumente utilizados (CARDOSO et al. 2011; DE MUNCK et al. 2005) (NAVARRA et al. 2012) para inferir sua predição ao longo do tempo (DE MUNCK et al. 2005; TAKAHASHI et al. 2011; MARTINS et al. 2009). O teste de microtração foi adotado nesses trabalhos por ser um teste confiável, amplamente utilizado e que pode ser replicado por outros em outros estudos, além de serem adequados para predizer desfechos clínicos, com relativa segurança. (ROEDER et al. 2011). Quando submetidos ao envelhecimento dos espécimes, o ensaio de microtração é o método mais indicado para aferir dados de retenção de restaurações adesivas (VAN MEERBEEK et al. 2010). Os testes realizados para avaliar envelhecimento podem acessar fatores diretamente ligados à interface adesiva, comparando resultados de integridade marginal, micro e nano infiltração e selamento, associados ou não a testes de resistência de união (HASHIMOTO et al. 2003; HEINTZE. 2013; MUTLUAY et al. 2013; MIYAZAKI et al. 2012; HASHIMOTO et al. 2011).

Adesão à dentina depende diretamente da interdifusão e embricamento micromecânico do adesivo no substrato para a formação da interface adesiva (NAKABAYASHI, 1982, MIYAZAKI et al. 2012) e o padrão de biodegradação da interface adesiva é influenciado por uma série de fatores químicos e fisiológicos. (HASHIMOTO et al. 2011).

Na tentativa de minimizar a degradação ao longo do tempo, fabricantes e pesquisadores desenvolveram formas de interferir positivamente na estabilidade da camada híbrida, visando maior longevidade do procedimento restaurador. (REIS et al. 2010; MUÑOZ et al. 2013; PERDIGÃO et al. 2014; SADEK et al. 2010; BRESCHI et al. 2008; MENA-SERRANO et al. 2013). Além das alterações químicas dos sistemas adesivos, tais como a adição de monômeros que promovem a formação de uma camada híbrida mais estável e mudanças nos protocolos adesivos aumentando a evaporação do solvente e aquecimento o sistema adesivo, alguns autores têm relatado melhores resultados em ensaios mecânicos a longo prazo, utilizando digluconato ou diacetato de clorexidina, porém isso não foi observado clinicamente. (REIS et al. 2010; MENA-SERRANO et al. 2013; HASS et al. 2013; MATSUI et al. 2015; IWAI et al. 2013).

Uma vez que os procedimentos técnicos na adesão são modificados para aumentar o desempenho do adesivo, espera-se que as características mecânicas da interface adesiva sejam melhoradas. Testes como nanoinfiltração e nanodureza possibilitam avaliar as características mecân/icas da camada híbrida. Nanoinfiltração é um teste utilizado na base e interior da camada híbrida para avaliar a infiltração de agentes estranhos à interface original e está diretamente associada à degradação hidrolítica da camada híbrida. Comumente valores de nanoinfiltração são avaliados por meio de marcador de nitrato de prata submetidos à solução reveladora fotográfica e observados em microscopia eletrônica (HASS et al. 2013). A Nanodureza, na camada híbrida, é obtida a partir de testesde *modulo de young* por meio de nanoindentação, numa escala pequena como a camada híbrida, linha adesiva ou numa região mais específica como dentina intertubular ou peritubular (VAN MEERBEEK et al. 1993; HIGASHI et al. 2009; JOVES et al. 2014).

Este estudo introduziu uma alteração físico-química no adesivo relacionado a sua melhor estabilidade e alteração de sua viscosidade. O uso de acrilamidas que podem ser solúveis ao etanol ou acetona, adicionadas aos sistemas adesivos, pode produzir ganhos em qualidade da interface adesiva uma vez que as acrilamidas são mais resistentes à degradação hidrolítica do que os acrilatos e metacrilatos utilizados comumente, e esta característica pode ser transferida à interface restauradora. (MONZSNER et al. 2005; SALZ et al. 2005, GREGOIRE et al. 2013)

O conceito de viscosidade é a medida da resistência de uma solução ao escoamento. Baixas concentrações de acrilatos como polímero à base de poliacrilamida aumentam a viscosidade adesiva e podem ser associados com os protocolos de alterações, especialmente na aplicação do adesivo por meio de agitação, sem a necessidade de um passo extra ou de um dispositivo específico para realização da alteração do adesivo.

Executando um aumento controlado da viscosidade da solução adesiva, é possível conseguir uma melhor resistência de união também pelo maior controle para a aplicação em agitação, com uma melhor distribuição do adesivo sobre a superfície a ser aderida. Assim, pode ser especulado que a presença de um polímero de espessamento promoveria alteração no modulo de elasticidade e aumento no grau de conversão devido ao isolamento parcial de oxigênio do monômero.

Sabe-se que ainda não está esclarecida a ação das alterações de protocolo na hibridização dos tecidos dentais, bem como qualquer efeito sinergético proporcionado pela aplicação simultânea das alterações de protocolo associado à alterações químicas do adesivo. Os autores desse trabalho desconhecem a existência de estudos comparando protocolos de aplicação, entre si ou combinados, no processo de hibridização, bem como estudos com alteração do sistema adesivo com uso de uma acrilamida e seu efeito no processo de hibridização.

Assim, a associação de alteração química do sistema adesivo e alterações no protocolo adesivo foi apresentado nestes estudos para ajudar a esclarecer os efeitos dessas alterações para melhorar o desempenho de um sistema adesivo universal. A hipótese nula 1 é de que as alterações no protocolo adesivo não repercutem em melhores resultados nos ensaios mecânicos a serem realizados. A segunda hipótese nula é de que a adição de acrilamida não repercute em melhoras nos resultados dos ensaios de microtração realizados.

2 ARTIGO 1 – USE OF ACRYLAMIDE AS MODIFIER OF A UNIVERSAL ADHESIVE TO PRESERVE DENTIN ADHESIVE BOND STRENGTH ON THE LONG TERM

Este artigo será submetido ao periódico *Journal of Adhesive Dentistry*, *Quintessence*, ISSN (print): 1461-5185, ISSN (online): 1757-9988, Fator de impacto = 1.594; Qualis A2. As normas para publicação estão descritas no Anexo A

Use of acrylamide as modifier of a universal adhesive to preserve dentin adhesive bond strength on the long term

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Contributed substantially to material and methods, proofread manuscript and wrote manuscript

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Developed experimental design and methodology, performed statistical analysis and wrote manuscript

Key words: acrylamide, hybrid layer, microtensile bond strength, hydrolytic degradation

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Abstract

Objective: The purpose of this study was to evaluate the effect of adding an acrylamide to the chemical composition of a universal adhesive on immediate and long term (6 MESES) bond strength on dentin. TIRAR EARLY E DEIXAR 24H OU IMMEDIATE
Material and Method: FALAR DO ULTIMATE. The dentin surfaces of 24 human third molars were sectioned to remove the enamel from the mid third. The teeth were assigned to two groups: The unmodified (V_0) and with 0.05 µL of acrylamide to each 1.0 ml of adhesive modified (V_1) universal adhesive (Scotchbond Universal, 3M ESPE, ST Paul, MN, USA). Composite resin restorations were built and after 24 h sectioned to produce 0.8 mm^2/cross-section sticks Microtensile bond strength (µTBS) tests (EMIC DL 1000, Instron do Brazil, S.J. Pinhais, PR, Brazil) were performed on one half of the samples at 24 and the other half after long term storage. Results of µTBS were analyzed by two-way ANOVA and Tukey's test. Ultimate tensile strength (UTS) was analyzed by one-way ANOVA and Bonferroni's test.
Results: In respect to µTBS, significant differences between the two groups ($p<0.01$) were revealed but no significant differences between storage times ($p=0.10$) nor in the interactions between storage and groups ($p=0.98$) were detected. V_0 group presented 26.55 MPa early bond strengths and 23.76 MPa after 6 months, while V_1 group presented 35.14 MPa and 32.27 MPa respectively. Concerning UTS, V_0 presented 32.46 MPa and V_1, 44.52.
Conclusion: Addition of low concentration of acrylamide to the universal adhesive was effective to increase microtensile bond strength on the early.

Key words: Contact Angle, Ultimate Bond strength, acrylamide.

INTRODUCTION

Performance of adhesive systems has been frequently studied in laboratory tests.[6,16,19,22] Proper monomer impregnation of dentin substrate improves quality of the hybrid layer and often resulting in higher bond strength.[6] Use of conditioners such as phosphoric acid in etch-and-rinse adhesives or of acidic primers in self-etch adhesives should promote adequate conditions for hydrophilic monomers to penetrate into and wet the prepared dentin surface.[12,28,30,31,41]

Exposed collagen fibrils on the top of treated dentin are impregnated by polymerizable monomers that protect them against hydrolytic degradation.[14] It has been demonstrated that the depth of demineralized dentin and the monomer impregnation are not necessarily in tune, leaving a non-impregnated and unprotected zone of collagen fibrils at the base of the hybrid layer.[3] Bonding durability depends on the integrity of the hybrid layer because its degradation process irreversibly compromises bond strength which leads to debonding and finally may have the loss of the restoration as a consequence.[2,37]

Protocols of adhesive application may be modified to improve the quality and stability of the hybrid layer, such as application of multiple layers of adhesive, prolonged application time or warming up.[15,23,32,34,38] However, modifications of the application protocol have the drawback of additional time and/or use of special devices. Agitation the adhesive during application may be an interesting way to improve the bond and consequently to obtain better bond strength. For this purpose a micro-brush gently introduces the adhesive monomers into the mesh of dentin to achieve optimal penetration.[8] Due to the low viscosity of some adhesives, and due to an excess of solvent such as acetone or ethanol, agitation technique is not easy to realize because of difficult control of the amount of adhesive applied. In such a case the active application may not be effective.

Acrylamides in their polymer representation in the form of as polyacrylamides are widely used as stabilizers, thickeners and suspending agents in pharmacological formulations. Low concentrations of acrylamides added to the adhesive increase its viscosity and may represent a way to achieve better stability of the adhesive solution allowing for a better control of application thus potentially improving the adhesive bond.

The purpose of this study was to evaluate the effects of polyacrylamide addition to a one bottle adhesive system on its early and long term bond strength when applied with the agitation technique. The hypothesis tested was that the addition of polyacrylamide to the one component adhesive will significantly improve both early and long term bond strengths.

MATERIAL AND METHODS

The protocol of the study was approved by the local Ethical Committee. Twenty four caries free third molars were selected from the Institutional bank of teeth. The teeth were stored in aqueous solution 1% of chloramine T until the use in this study. The crowns of the teeth were sectioned in order to expose the mid coronal dentin surface and a 600-grit silicon carbide paper under running water for 20 s was applied to standardize smear layer.

Sample size was calculated to the power of 0.8 and alpha 0.05 by using the software OriginPro (OriginLab Co, Northampton, MA, USA) and was stablished N=6.

Adhesive Preparation

Scotchbond Universal (SBU) (3M ESPE, St Paul, MN, USA) was used in the two experimental groups SBU V_0 and SBU V_1 to perform the tests. V_0, was the original adhesive (no modification) and V_1 corresponded to altered viscosity with 0.05 µL/ml of an acrylamide (polyacrylamide C13-14 Isoparaffin Laureth-7, Farmatox, UFSM, Santa Maria, RS). To prepare adhesive with viscosity V_1, 0.05 µL of polyacrylamide was vigorously mixed in each 1.0 ml of adhesive in a dark room using a glass grail and pistille for 1minute.

The contact angle between adhesive and dentin surface, based on Young's equation (Figure 1), was measured five times for each viscosity using slice of teeth embedded in acrylic resin where the axial dentin was exposed to receive the adhesive to be calculated the contact angle. Dentin surfaces prepared according to the above mentioned protocol were dried with oil-free compressed air for 5 s and then a drop of 10 µL of adhesive was deposited on top of the samples to measure the contact angle with a drop shape analyzer (DSA 100, Krüss Ltd, Hamburg, Germany). The contact angle was taken 5 s and 20 s after application and the averages from measures for both viscosities and for both time intervals was recorded as contact angle.

$$\gamma_{LV} \cos\theta = \gamma_{SV} - \gamma_{SL}$$

$\gamma_{LV} = Liquid - vapor\ interfacial\ tension\ or\ surface\ tension$

$\gamma_{SV} = Solid - vapor\ interfacial\ tension$

$\gamma_{SL} = solid - liquid\ interfacial\ tension$

$\theta = contact\ angle\ (angle\ adhesive\ makes\ with\ dentin\ suface)$

Figure 1.

Bonding and restorative procedures

24 extracted human teeth were randomly assigned into 2 equal groups according to the bonding procedure (Table 1). Adhesive system, composite resin, manufacturer, composition and batch number are listed in Table 2.

Table 1.

Table 2.

The adhesives were applied in the self-etching mode by a trained operator according to the recommendations of the manufacturer. Light-curing unit used was a LED (Bluephase, IvoclarVivadent Corp. Schaan, Liechtenstein) at 1200 mW/cm2 of intensity, verified with a Gnatus Radiometer Unit (Gnatus Eq Med Odontol, Ribeirão Preto, SP, Brazil).

Composite resin restorations (Filtek Z350, shade A2E, 3M ESPE, St Paul, MN, USA) of 6-mm height were built in three layers of 2.0 mm each. The restored teeth were stored in distilled water for 24 h before being cut longitudinally in sticks of 0.8 mm^2 cross-section with a diamond saw disc at 250 rpm in a Labcut 1010 (Extec Corp. Enfield, CT, USA) under copious water cooling.

Microtensile bond strength test (μTBS)

One half of the samples were tested after 24 hrs (early bond strength) and the other half after 6 months (long term bond strength). The tests were performed in an EMIC DL 1000 (Instron Brazil Ltd, S.J. Pinhais, Brazil), at 1 mm/m of velocity by a blinded operator.

For the early bond strength test, samples were kept in distilled water at 37°C for 24 h.

For the long term test after 6 months, samples were stored in distilled water at 37°C for 6 months changing the water every week.

Following disruption, the failures were analyzed at 30× magnification at stereomicroscopy (ZEISS) and classified as adhesive/mixed, cohesive in resin, or cohesive in dentin.

Ultimate Tensile Strength test (UTS)

To measure the immediate ultimate tensile strength, six specimens were prepared for each tested adhesive. Starch matrix with 10.0 mm in length and with a longitudinal orifice of 1.0 mm in diameter was employed to build up the specimens in the form of cylindrical bats. 0.1 ml of the adhesive was collected from the bottle with a syringe and carefully disposed in the orifice to complete filling. The adhesive was light-cured for 60 s with a light-curing unit LED (Blue Phase). The starch matrix was dissolved by immersion in water and the samples were stored in distilled water at 37°C for 24 h before testing in an EMIC DL 1000 (Instron Brazil Ltd, S.J. Pinhais, Brazil), at 1 mm/m of velocity by a blinded operator.

Statistical Analysis.

Statistical analyses were performed with OriginPro Software (OriginLab Co, Northampton, MA, USA) at confidence level of 95%.

Results of μTBS were analyzed by two-way ANOVA. Tukey's Test was applied for multiple comparisons in order to examine the effect of bonding techniques (REMOVER), adhesive viscosities, time of storage and interactions. Results of UTS were analyzed by one way ANOVA and Bonferroni's Test.

RESULTS

Microtensile test results:

Concerning µTBS the two-way ANOVA revealed significant differences between the two types of the adhesive ($p>0.001$) but nor between storage times ($p=0.10$) nor for the interaction between storage and adhesive types ($p=0.98$).

Both adhesives V_0 and V_1 showed no significant difference between bond strength at 24h and 6 months of storage. V_1 presented significantly better results than V_0 both after 24h and after 6 months of storage. (Table 3 and Figure 2) Most the encountered failures were adhesive (75%) following by mixed failures (25%). No cohesive failures were observed.

Table 3.

Figure 2.

Ultimate tensile strength

One-way ANOVA examined results of UTS after 24h and revealed significant differences between both groups ($p=0.01$). (Table 4 and Figure 3).

Table 4. UTS means (sd) from regular (V_0) and modified adhesive (V_1).

Figure 3. Box-plot and whiskers from means of UTS of regular (V_0) and modified (V_1) adhesive.

Contact angle

Contact angle was measured on dentin surface 5s and 20s after application. The results confirmed that the addition of polyacrylamide made the consistency of SBU

V_1 thicker than SBU V_0. The average for each viscosity and time were: V_0, 5s 25° and 20s 22° / V_1, 5s 28° and 20s 24°. In both viscosities, the contact angle decreased with time (Figure 4)

Figure 4. A and B.

DISCUSSION

Bond strength tests performed at early and at long term are more representative of the adhesive performance than those at early term only, since at long term some deleterious mechanisms such as hydrolytic degradation of the hybrid layer may damage the bonded interface and jeopardizes adhesion. [2,27,33]

Changes in the adhesive protocols, composition and surface treatment can result in better bond results.[1,8,9,10] In order to have better understanding of bonding, researchers have opted to modify the treatments of the substrate, in order to promote micromorphologic alterations of dentin, modify the protocols of adhesives' application and chemical modifications of adhesives' composition. Mechanical tests like microtensile bond strength test (µTBS) have been widely used to test adhesive systems, but ultimate tensile strength tests (UTS) can may be performed to provide additional information about the intrinsic properties of the material such as mechanical modulus and cohesive strength.[10,17,24]

Based on the above, the present study tested microtensile bond strengths after 24h and after 6-months by introducing a modification in a universal adhesive system through the addition of a low concentration of an acrylamide polymer to produce a more stable resin-dentin interface. Acrylamide was used as modifier of the adhesive (0.05 µl per ml of adhesive) resulting in a more viscous solution. The interaction of an adhesive and a dental substrate is influenced by the contact angle formed between them and it is frequently considered as predictor of wettability.[11,13,20,21]

In this study, the measurement of contact angles of adhesives on dentin showed that acrylamide increased the contact angle (Figure 4), but it apparently did not reduce the wetting ability, most probably because the application of the adhesive under agitation may have neutralized the higher viscosity. The results might been different when the adhesive is applied with the "apply and let undisturbed" method.

Once the adhesive is agitated with a micro-brush, demineralization is initiated leading to the surface alteration, similar to the one provided by the application of phosphoric acid, used as conditioner.[11]

Longevity of bonded restorations is directly related to the quality of the hybrid layer which must preserve its morphological integrity and stability. Hydrolytic degradation has been reported as the main cause of adhesion loss.[26,33,36,37,39,40]

To increase bond strength and to retard the disintegration of the bonded interface, several studies evaluated bonding protocol modifications such as adhesive heating; multiplication of the number of adhesive layers; adhesive's application with agitation; addition of new substances into the chemical composition, and alteration of the treatment of the dentin surface. [1,4,18,19,23,25,26,36,39,40]

In this study, although the bond strength to V_0 and V_1 was not affected by storage time, the results of microtensile bond strength showed that the adhesive V_1 presented significantly higher microtensile bond strengths at both test intervals than V_0, (Table 3), rejecting the null hypothesis of this study. It is speculated that the effects of polyacrylamide on mechanical properties of the hybrid layer positively influences early bond strength by producing a more resilient interface, and at the long term, besides to reinforce and to stabilize the hybrid layer by altering its mechanical modulus, preserving the bonded interface by protecting the collagen fibrils from hydrolytic degradation. Acrylamides are more resistant to hydrolytic degradation than acrylates and it is speculated that their characteristics are transferred to the adhesive.[25,36]

Amides groups from acrylamide and their carboxyl groups interact with the amino acids of collagen fibrils, due to their similarities, forming more stable hydrogen bonds between acrylates monomers and carboxylic groups of collagen.[26,39] Amide monomers were used in the past in experimental adhesives to solve the problem of hydrolysis of monomers, until the advent of self-etch adhesives and synthetized of many others acrylamides theoretically overcame the problem.[25,40]

The UTS tests were performed only after 24hrs, since water storage does not alter results of UTS(25). The findings of UTS tests with V_0 and V_1 reinforce the speculation above and it establishes a coherent relation between μTBS and UTS, confirming significantly higher results of V_1 in comparison to V_0.

If manufacturers produce modified adhesives with low concentrations of acrylamides such as proposed in this study, then dentists do not need to spend extra-time or to use any special device to improve adhesion under the condition that they apply the agitation application method.

CONCLUSION

Under the conditions of the present study, low concentration of acrylamide polymer added to a universal adhesive was effective to improve bond strength both after 24hrs and at long term. However more studies are required to explain the mechanism of interaction between acrylamide-modified adhesives and dentin and to confirm the results in controlled prospective clinical studies.

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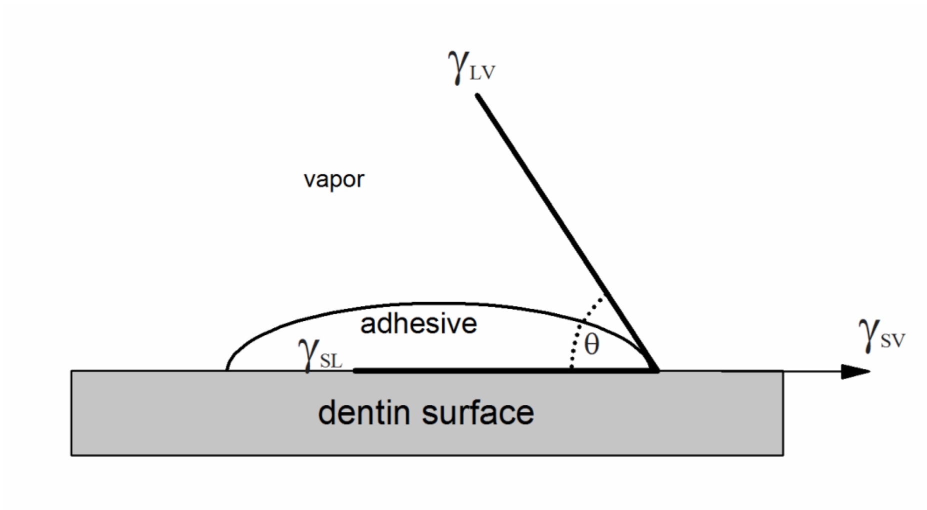

$$\gamma_{LV}\, cos\theta = \gamma_{SV} - \gamma_{SL}$$

$\gamma_{LV} = Liquid - vapor\ interfacial\ tension\ or\ surface\ tension$

$\gamma_{SV} = Solid - vapor\ interfacial\ tension$

$\gamma_{SL} = solid - liquid\ interfacial\ tension$

$\theta = contact\ angle\ (angle\ adhesive\ makes\ with\ dentin\ suface)$

Figure 1. Schematic view of contact angle and Young's Equation

Table 1. Tested groups, bonding procedures, n, and storage time for regular (V_0) and modified (V_1) adhesive.

Group	**Bonding procedure**	**24 h n**	**6 m n**
G1. SBU (V_0)	Application by agitating for 20 s; Gentle drying with compressed air for 5 s; Photo-curing for 10 s	6	6
G2. SBU (V_1)	Application by agitating for 20 s; Gentle drying with compressed air for 5 s; Photo-curing for 10 s	6	6

Abbreviations: SBU= Scotchbond Universal; V_0= Regular viscosity; V_1= Increased viscosity with acrylamide

Table 2. Materials used in this study.

Material	**Composition**	**Manufacturer**	**Batch No.**
Scotchbond Universal (Universal adhesive)	Dimethacrylate resins, MDP phosphate monomer, HEMA, Vitrebond copolymer, silane, ethanol, water and initiators	3M ESPE, St Paul, MN, USA	582957
Filtek Z350 (nanofilled composite resin)	Bis-GMA, UDMA, TEGDMA, Bis-HEMA, Silica and Zirconia	3M ESPE, St Paul, MN, USA	N348519BR

Abbreviations: MDP: 10-Methacryloyloxydecyl Dihydrogen Phosphate; Bis-GMA: Bisphenol A diglycidyl ether dimethacrylate; HEMA: 2- hydroxyethyl methacrylate; Bis-EMA, bisphenol-A-ethoxylate glycidyl methacrylate; UDMA, urethane dimethacrylate; TEGDMA, triethylene glycol dimethacrylate.

Table 3. Means of µTBS in MPa (SD) on early and 6-months of storage obtained from regular (V0) and modified (V1) adhesives.

Adhesive / Viscosity	**24 h**	**6 m**
V0	26.55 (8.56)Bb	23.76 (7.70)bB
V1	35.14 (9.48)Aa	32.27 (10.19)aA

Mean values with different capital letter (in columns) are significantly different ($p<0.05$). Values with the same small letter (in lines) are not significantly different.

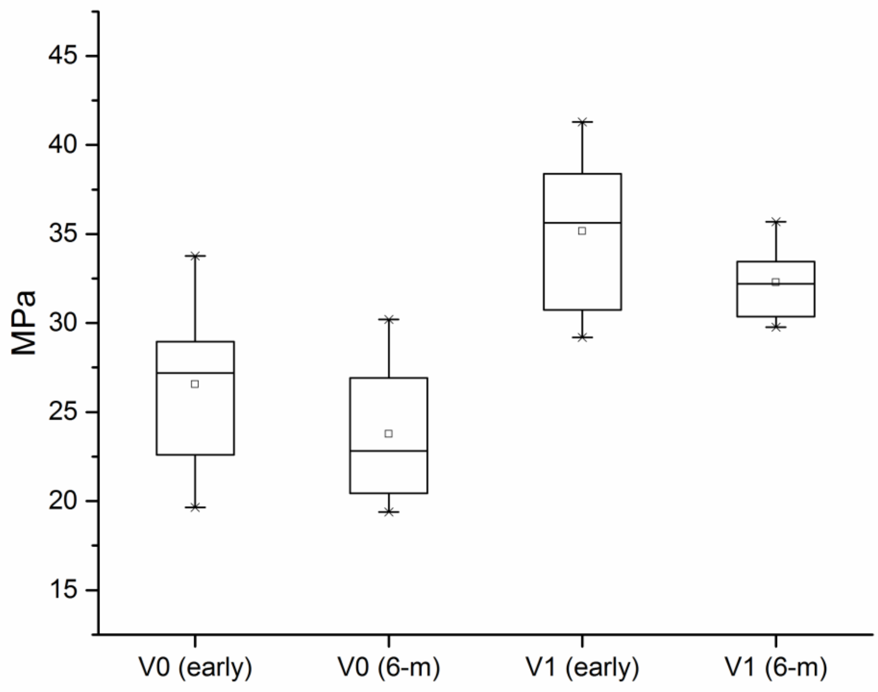

Figure 2. Box-plot and whiskers of means of μTBS of regular (V_0) and modified (V_1) adhesive.

Table 4. UTS means (sd) from regular (V_0) and modified adhesive (V_1).

Group	**V_0**	**V_1**
UTS	**32.46 (8.73)A**	**44.52 (7.79)B**

Mean values with different capital letter are significantly different ($p<0.05$).

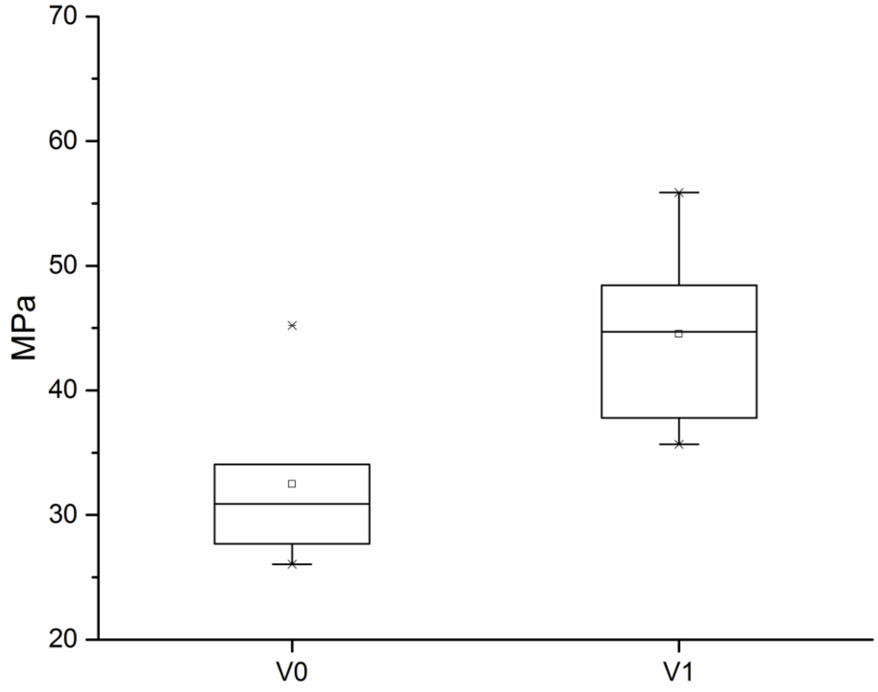

Figure 3. Box-plot and whiskers from means of UTS of regular (V_0) and modified (V_1) adhesive.

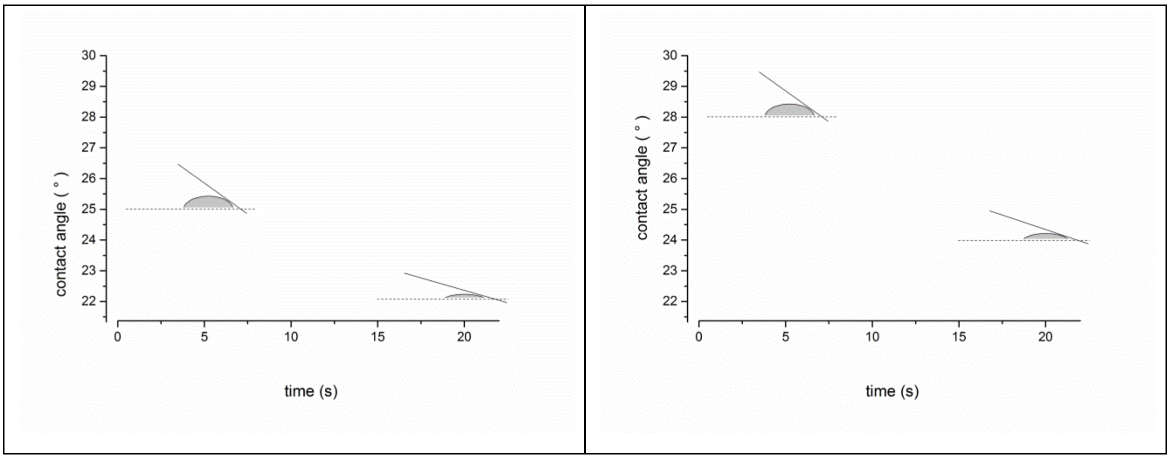

Figure 4. A and B. Graphic representation of contact angle (V_0 = A and V_1 =B) 5s and 20s after application.

2.2 ARTIGO 2 – EVALUATION OF DIFFERENT APPLICATION PROTOCOLS OF A UNIVERSAL ADHESIVE SYSTEM MODIFIED WITH ACRYLAMIDE IN THE DENTIN BOND STRENGTH

Este artigo será submetido ao periódico *Operative Dentistry*, *Elsevier*, ISSN: 1559-2863, Fator de impacto = 2.819; Qualis A1. As normas para publicação estão descritas no Anexo B

Evaluation of different application protocols of a universal adhesive system modified with acrylamide in the dentin bond strength

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Developed experimental design and methodology, performed statistical analysis and wrote manuscript

Key words: acrylamide, hybrid layer, microtensile bond strength, hydrolytic degradation

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ARTIGO 2:
EVALUATION OF DIFFERENT APPLICATION PROTOCOLS OF A UNIVERSAL ADHESIVE SYSTEM MODIFIED WITH ACRYLAMIDE IN THE DENTIN BOND STRENGTH

SUMMARY

Purpose: The aim of this in vitro study were: 1) to evaluate the influence of different protocols of adhesive application on the dentin bond strength; 2) to evaluate if the incorporation of acrylamide would increase the dentin bond strength of a universal adhesive system under multiple application protocols and 3) to determine the influence of acrylamide in the ultimate tensile strength of the modified adhesive.

Materials and Methods: Two Single Bond Universal (SBU) were used in this study. One with no modification on its composition (SBUV0) and one modified by adding of 0.05 µL of polyacrylamide per mL. (SBUV1). Ultimate tensile strength was performed to verify the intrinsic strength of the both versions. Then, six protocols were performed for each adhesive to evaluate the dentin bond strength. The protocols were: 1) Control – following the manufacturer´s instructions, 2) Warm Air – Using a warm air to evaporate the solvent, 3) Chlorexidine – Use of the chlorhexidine digluconate as dentin pre-treatment of the SBU, 4) Photoactivation for 40s –Adhesive photo-cured for 40 seconds instead of 10s, 5) Double application – Application of an extra layer of the adhesive system and 6) All-in-One – All the protocols combined. The samples were tested by microtensile test (µTBs) on early and long term. The failures were analyzed at 30× magnification and classified as adhesive/mixed, cohesive in resin, or cohesive in dentin.

Results: In the Ultimate Tensile Strength test, the SBUV1 (31.78Mpa) was statistically higher ($p<0.05$) than the SBUV0 (19.93Mpa). In the µTBs, three-way-ANOVA showed thar the groups with the SBUV1 showed higher results than the SBUV0 groups. Among the protocols, the "all-in-one" group showed the best results with a statistically difference compared with the control group and the 40s group who showed the worse results but the 40s groups wasn´t different statistically compared with the Warm air, chlorhexidine and douple application group.

Conclusion: The use of polyacrylamide improves the dentin bond strength of a universal adhesive in *in vitro* tests as well as some modifications of the protocol´s application but without a synergism between these two variables.

Key-words: Dentin. Dentin-bonding agents. Tensile Strength

INTRODUCTION

In vitro studies are important to the prediction of the adhesive performance of newly developed adhesives, when applied according to different protocols. Dentin adhesion have been improved but it is still a challenge to the restorative dentistry (1). The complex micromorphology is a difficult factor to reach an efficient hybridization (2). The universal adhesives simplified the application since they can be used for both self-etching and etch-and rinse (3).

For enamel, it has been shown that selective acid conditioning increases the bond strength when no etch-and-rinse technique is applied (4, 5). On the other hand, the etch-and-rinse strategy did not produce any improvement in the bond strength comparing to the self-etching strategy, but it may be used as a means of eliminating the critical steps of etching, washing, and drying the dentin (6, 7).

Studies have been conducted to minimize the degradation of the hybrid layer and extend the longevity of restorations by modifying the substrate and adjusting the protocol of the adhesive application (8). Some of these modifications, showed to be effective such as: the application of an extra layer of adhesive, which improves penetration of the resin monomers into the collagen fibrils and decreases the continuous exposure of collagen fibrils (9), increasing the adhesive curing time, which increases the degree of conversion and the solvent evaporation and decreases the continuous (10), applying of a warm stream of air, which increases the degree of conversion and solvent evaporation (11), and first applying a 2% chlorhexidine digluconate solution which inhibits MMPs activity and protects non-protected collagen fibrils (12).

To the authors knowledge, however, there is no mention in the literature of a comparison of all the cited modifications, while there has been no investigation of a universal adhesive system, since the available literature merely compares these modifications with the standard protocol. Therefore, we believe that the present study is the first to compare the proposed protocol’s alterations added by a chemical alteration of adhesive system.

Beyond the protocols, this study evaluated the effect on the dentin bond strength of a universal adhesive system added with acrylamide. The objective to add acrylamide into a universal adhesive system is to improve the hybrid layer, since acrylamides produce a more stable hybrid layer compared to dimethacrylates.

The present study aimed to evaluate the effect of different protocols use of a universal adhesive system, as well as, the addition of acrylamide, on the bond strength in dentin, over time.

The null hypotheses of the present study were: (a) there is no difference between the two adhesives tested (no addition of acrylamide and the one added of acrylamide) concerning microtensile bond strength; (b) there would be no difference among the protocols tested and; (c) The aging process for 6 months does not affect the dentin bond strength of the tested groups.

Materials and Methods

This study was submitted to the Institutional Ethics Committee of Federal University of Santa Maria and received their approval (n. 040959).

The Single Universal Bond adhesive system and Filtek Z250 composite resin, shade A2 (3M ESPE, St Paul, MN, USA) was used in the present study. Table 1 lists the material composition, manufacturer, and batch number.

Table 1.

Experimental Design

To check the "n" of the groups, the sample size was calculated for a power of 0.8 and an alpha of 0.05, using OriginPro software (OriginLab Co, Northampton, MA, USA).

To perform the microtensile bond strength test, 144 non carious human third molars were selected from the Institutional Bank of Teeth of Federal University of Santa Maria. After disinfection using 0.5% chloramine solution, they were stored in distilled water until use. The cusps were removed from the occlusal third to access the dentin on the pulpal wall. To standardize the smear layer and obtain a flat and smooth surface, they were polished for 45 s with wet 600-grit silicon-carbide paper. Then, the

teeth were randomly assigned into 12 groups (n = 6), according to the number of adhesive system versions (two) and adhesive protocols (six). Half of the samples were tested immediately (after 24 h of being stored in water) while the other half were tested after 6 months of (Table 2).
For the ultimate bond strength tests, only adhesives (versions) were used.

Adhesive Preparation (versions)

The Single Bond Universal adhesive was prepared as $SBUV_0$ and $SBUV_1$ to perform the chemical alteration tests, where V_0 corresponded to the original adhesive and V_1 corresponded to the chemically altered adhesive with 0.05 mL/ml of polyacrylamide. To prepare V_1, 0.05 mL of polyacrylamide was vigorously mixed into 1.0 ml of adhesive in a dark room, using a grail and pesitle for 1 min.

Table 2.

Tests

1. Microtensile Bond Strength Test

Adhesive Technique - composite resin restoration and bond strength test

The adhesive system type and adhesive protocols used to perform the bond restorations in each group are listed in Table 2.

Composite resin restorations (Filtek Z250 shade A2E – 3M ESPE, St Paul, MN, USA), 5 mm in height, were built over the adhered wall. The restored teeth were stored in distilled water for 24 h before being cut longitudinally into samples with a 0.8-mm^2 cross-section, using a double-face diamond saw. The samples were bonded to test devices using cyanoacrylate to determine the µTBS in an EMIC DL 1000 (Instron Brazil Ltd, S.J. Pinhais, Brazil). A 1-KN cell was used at a velocity of 1 mm/m, until failure.

The failures were analyzed at 30× magnification and classified as adhesive/mixed, cohesive in resin, or cohesive in dentin.

2. Ultimate Tensile Strength (UTS)

To assess the tensile strength, twelve specimens were prepared for each type of adhesive. A starch matrix, 10 mm in length with an orifice diameter of 1 mm, was employed to build up the specimens. The adhesive was collected from the bottle with a metallic cannula into a plastic syringe and then injected into the hole to complete the filling.

The adhesive was light-cured for 60 s with a monitored-irradiance 800-mW/cm^2 Olsen LED (Olsen Ind e Com S.A. Palhoça, Brazil). To promote water-absorption and facilitate the starch-matrix dissolution, half the specimens were stored in distilled water at room temperature to be tested after 24 h..

After the complete removal of the starch residues, the specimens were attached to testing devices using cyanoacrylate and subjected to a tensile force in an Emic DL 100 (Instron Brazil Ltd, S.J. Pinhais, Brazil). A 1-KN cell was used, at a velocity of 1 mm/m, until failure.

Statistical Analysis

The μTBS data were analyzed by three-way ANOVA and Tukey's post hoc test. Meanwhile, the UTS data were analyzed by two-way ANOVA and Tukey's post hoc test at $\alpha = 0.05$.

To preserve the power of the dataset, the interactions between variables adhesive x protocol, adhesive x aging, protocol x aging and adhesive x protocol x aging were investigated by Levene´s Test of Equality of Error Variances (SPSS 20.0) to evaluate the linear relationship between variables.

The interactions between variables μTBS x UTS were analyzed in pairs by two-way ANOVA and Tukey's post hoc test at $\alpha = 0.05$.

RESULTS

The means and standard deviation of the results of the microtensile bond strength tests of the studied groups are described in Table 3. There was a difference between the adhesives, the SBUV1 showed better results than the SBUV0 ($P = 0.02$) and among the protocols, the group All-in-one showed the best results (A), following the War Air, Chlorexidine and Double application groups (AB), then the 40s group (BC) and the control group (C) ($P < 0.001$). There was no interaction between the adhesive vs. aging ($P = 0.77$) or protocol vs. aging ($P = 0.78$), showing that the aging process did not decrease the dentin bond strength of all the groups that were studied. In fact, after 6 months of water storage, the dentin bond strength was found to increase, in all the groups studied ($P > 0.001$).

In general, the modified adhesive system, described as Single Bond Universal V1 (SBUV1), had the highest bond strength relative to SBUV0.

Among the protocols, group control produced the worst results. Statistically, the results were similar to the 40s group, but lower than with the other groups.

Although there was difference between the adhesives tested and the protocols tested, there was no interact between these two variables ($P = 0.71$).

The failure modes of all the groups and ages are shown in Figs. 1, 2, 3, and 4. Adhesive/mixed failures predominated (over 60% in all groups). However, cohesive failures in the dentin and resin were also observed in all the tested groups.

Between the intrinsic forces of the adhesives themselves, the ultimate bond strength test revealed a difference between SBUV0 and SBUV1. The values are listed in Table 3.

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Table 3.

DISCUSSION

Universal adhesives are the latest generation of adhesive systems used to hybridize dental hard tissues (3). Besides the adhesive strategy chosen by the dentist their main advantage is that they eliminate the steps of etching, washing, and drying and for this reason they are gaining popularity (13). However, popularity does not necessarily conform to efficiency. In some cases, a universal adhesive does not add any improvement in the dentin bond strength compared with other adhesive systems, even though they promote aceptable results (14, 15). To improve the efficacy of the Single Bond Universal adhesive, changes in the application protocol may be introduced. In the present study, modifications to the application protocol increased the dentin bond strength, relative to the control group. The null hypothesis 1, that there is no difference among the protocols had to be rejected. Similarly, the null hypothesis 2, which stated that there is no difference between the two adhesives systems (V0 and V1) had to be rejected.

To the best of our knowledge, this study was the first that promote chemically alteration of a universal adhesive with acrylamide. The modified adhesive, SBUV1, showed higher dentin bond strength than the unmodified adhesive, SBUV0.

Among the tested protocols, the control group exhibited the lowest dentin bond strength, regardless of the adhesive used. Statistically, only the 40 s group exhibited no difference relative to the control group. Since a longer exposure time produces a higher energy density and thus it causes the formation of free radicals, which initiate polymerization, this may result in a higher-molecular-weight and cross-linked polymer, thus improving the degree of conversion (8, 16, 17, 18). This could explain the statistical difference found in those studies which compared a standard protocol with a longer exposure polymerization time protocol in those adhesives that do not contain 10-MDP and which belong to an older generation of adhesives. Advances in the formulation and chemical composition of adhesives may have achieved a level of chemical engineering such that increasing the polymerization exposure time does not produce a major change in the bond strength.

The heat produced by the polymerization does not seem to have the same effect as a warm air stream. The benefits of a warm air stream, that is, the evaporation of the solvents, especially in ethanol/water-based adhesive systems, have already been focused by some previous studies (11, 19). The warm air stream increases the evaporation rate of solvent and decreases the viscosity of the adhesive, thus promoting a reduction in the amount of residual solvent and improving the wettability of the tooth surface (20). Although these studies did not test the Single Bond Universal. A high concentration of residual solvent could jeopardize resin-dentin bonds, but the use of a warm-air stream could minimize this risk by promoting the evaporation of the exceeding solvents (10).

The application of other protocols improved the dentin bond strength under different mechanisms. The application of an extra layer of adhesive was found to promote a higher penetration of the resin monomers into the collagen fibrils which could improve the dentin bond strength. This improvement was previously observed in other studies who tested self-etch adhesives such as iBond, Adper Prompt L-Pop, and Xeno III (9, 21) but their findings were not in agreement with those of Toledano et al. (22) who tested Futurabond (VOCO, Cuxhaven, Germany) in microtensile bond strength and those of Nakaoki et al. (23) who tested Xeno III and Adper Prompt L-Pop, in microshear bond strength test. The improvement caused by the addition of an extra layer of universal adhesive could be explained by the etching caused by the first layer, after which the additional layers of uncured acidic monomers could improve the etching ability of these adhesives by increasing the concentration of the acidic reagents in dentin with buffered hydroxyapatite (8, 9, 24).

The use of di-gluconate of 2% chlorhexidine led to an improvement in the dentin bond strength, relative to that of the control group. Although the consequences of inhibiting the MMPs arise only in the long term, the evaluation of the effects of using 2% chlorhexidine in the dentin before the application of the adhesive leads to an improvement in the baseline. However, it does not agree with the findings of Montagner et al. (25) in a systematic review and meta-analysis where they observed that the immediate bond strength results showed no difference between the 2% CHX and the control group. They found that the use of CHX promotes higher bond strength than the control group.

It was expected that the combined use of all these protocols would result in higher bond strength. The All-In-One group presented a higher bond strength than to the control and the 40s groups, but did not differentiate itself from the other groups, showing that a possible combination of the advantages of the different approaches did not necessarily result in a higher dentin bond strength.

The reason for this may be the same as that for the aging did not decrease the bond strength. The SBU is an adhesive that is a compound of Vitrebond co-polymers and 10-MDP which is able to produce more monomer-Ca salt in a stable and insoluble form, such that it could be used to form a water-stable adhesive interface (26). The advantages of this chemical bonding may require more than 24 h to become apparent and any decay could only be verified by aging over a period in excess of six months at undisturbed distilled water. This is one of the limitations of the study, since the aging of the samples may not have been long enough.

Despite the above, the use of polyacrylamide to endorse the SBU seems to have higher effect dentin bond strength. The SBUV1 exhibited better results than the SBUV0 ($P = 0.02$). All the procedures were applied with the vigorous rubbing application of the adhesive. This may explain why an even thicker layer of adhesive improves the dentin bond strength, which is in direct contradiction to usual practices related to adhesion. Wettability, flow, and contact angle all play important roles in ensuring effective adhesion. An adhesive that fails to flow across the entire surface, and which has a high contact angle and poor wettability, would offer only a poor degree of adhesion. This was not the case with the SBUV1. Its chemical formulation, along with the vigorous rubbing application may have overcome these possible shortfalls of a thicker adhesive layer.

A few studies have set out to test multimode adhesives such as SBU under different conditions to those recommended by the manufacturers. The present study was, to the knowledge of the authors, the first one comparing these different approaches. More in vitro studies comparing other adhesive systems, using different protocols and with the application of longer aging periods should be condicted.

CONCLUSION

The addition of acrylamide produces a more stable adhesive was found to improve the dentin bond strength of a multimode adhesive system. Some changes in the protocol appear to improve the dentin bond strength and preserve the hybrid layer for the first six months. Given the lack of research data, however, further studies addressing other adhesives systems, protocols, and longer aging periods are required.

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Table 1. Materials tested

Material	Composition	Manufacturer
Single Bond Universal (adhesive system) L:554836	MDP Phosphate monomer, Dimethacrylate resins, HEMA, Vitrebond^{TM} Copolymer, Filler, Ethanol, Water, Initiators, Silane	3M ESPE, Saint Paul, MN, USA
Flitek Z 350 (composite resin) L:5799667	Bis-GMA, UDMA, TEGDMA, Bis-EMA, Silcate, Zirconia	3M ESPE, Saint Paul, MN, USA

Abbreviations: MDP*, 10-methacryloyloxydecyl dihydrogenphosphate;* HEMA*, 2-hydroxyethyl methacrylate;* Bis-GMA*, bisphenol A diglycidyl methacrylate;* UDMA*, urethane dimethacrylate or 1,6-di(methacryloyloxyethylcarbamoyl)-3,30,5-trimethylhexaan;* TEGDMA*, triethylene glycol dimethacrylate;* Bis-HEMA*, bisphenol A diglycidyl methacrylate.*

Table 2. Studied groups according to adhesive type and adhesive protocol (adhesive technique/steps), storage time, and n

			Storage time and *n*	
GROUPS	**ADHESIVE SYSTEM versions**	**ADHESIVE TECHNIQUE / STEPS**	**Early**	**6 mo**
SBU_C (Control)	$SBUV_0$	1. Adhesive applied by agitation with a microbrush for 20 s 2. Gentle air-jet for 5 s 3. Photo-curing for 10 s	6	6
SBU_{WA} (Warm air)	$SBUV_0$	1. Adhesive applied by agitation with a microbrush for 20 s 2. Warm air-jet applied at 10 cm distance for 10 s 3. Photo-curing for 10 s	6	6
SBU_{CL} (Chlorexidine)	$SBUV_0$	1. 2% chlorhexidine digluconate solution applied for 60 s 2. Adhesive applied by agitation with a microbrush for 20 s 3. Gentle air-jet for 5 s 4. Photo-curing for 10 s	6	6
SBU_{PHO} (Photoativation for 40s)	$SBUV_0$	1. Adhesive applied by agitation with a microbrush for 20 s 2. Applying gentle air-jet for 5 s 3. Photo-curing for 40 s	6	6
SBU_{2AP} (Double aplication)	$SBUV_0$	1. Adhesive applied by agitation with a microbrush for 20 s 2. 2nd adhesive application by agitation with a microbrush for 20 s 2. Gentle air-jet for 5 s 3. Photo-curing for 10 s	6	6
SBU_{ALL} (All-in-one)	$SBUV_0$	1. 2% chlorhexidine digluconate solution applied for 60 s 2. Adhesive applied by agitation with a microbrush for 20 s 3. 2nd adhesive application by agitation with a microbrush for 20 s 4. Warm air-jet applied at 10 cm distance for 10 s 5. Photo-curing for 40 s	6	6
$SBUV_C$ (Control	$SBUV_1$	1. Adhesive applied by agitation with a microbrush for 20 s 2. Gentle air-jet for 5 s 3. Photo-curing for 10 s	6	6
$SBUV_{WA}$ (Warm air)	$SBUV_1$	1. Adhesive applied by agitation with a microbrush for 20 s 2. Warm air-jet WITH HAIR DRYER applied at 10 cm distance for 10 s 3. Photo-curing for 10 s	6	6
$SBUV_{CL}$ (clorexidine)	$SBUV_1$	1. 2% chlorhexidine digluconate solution applied for 60 s 2. Adhesive applied by agitation with a microbrush for 20 s 3. Gentle air-jet for 5 s 4. Photo-curing for 10 s	6	6
$SBUV_{PHO}$ (Photo for 40s)	$SBUV_1$	1. Adhesive applied by agitation with a microbrush for 20 s 2. Applying gentle air-jet for 5 s	6	6

		3. Photo-curing for 40 s		
$SBUV_{2AP}$ (Double application)	$SBUV_1$	1. Adhesive applied by agitation with a microbrush for 20 s 2. 2nd adhesive application by agitation with a microbrush for 20 s 2. Gentle air-jet for 5 s 3. Photo-curing for 10 s	6	6
$SBUV_{ALL}$ (All-in-one)TALVEZ MUDAR O NOME	$SBUV_1$	1. 2% chlorhexidine digluconate solution applied for 60 s 2. Adhesive applied by agitation with a microbrush for 20 s 3. 2nd adhesive application by agitation with a microbrush for 20 s 4. Warm air-jet applied at 10 cm distance for 10 s 5. Photo-curing for 40 s	6	6

$SBUV_0$ = Single Bond Universal with original viscosity; $SBUV_1$ = Single Bond Universal with 0.05 μL of polyacrylamide.

Table 2. Microtensile bond strength of two universal adhesives bonded to dentin using 6 application modes, with and without water storage (6 months). μTBs ± SD (no. of specimens tested/pre-tested failure).

Adhesive	Protocol	Aging	
		Baseline	6 months
SBUV0	Control	34.40 ± 5.4 (81/7) ^{C}	38.88 ± 11.7 (37/7) ^{C}
	40s	47.05 ± 13.4 (58/9) ^{BC}	45.91 ± 6.9 (70/3) ^{BC}
	Double Application	42.65 ± 8.2 (93/8) ^{AB}	56.24 ± 8.8 (67/7) ^{AB}
	Warm air stream	50.26 ± 7.9 (53/7) ^{AB}	49.19 ± 15.40 (63/9) ^{AB}
	Chlorexidine	40.20 ± 6.3 (57/7) ^{AB}	56.04 ± 9.2 (57/0) ^{AB}
	All-in-one	47.63 ± 15.97 (50/4) ^{A}	56.76 ± 11 (53/3) ^{A}
SBUV1	Controle	40.21 ± 7.27 (67/8) ^{C}	45.31 ± 12.13 (61/2) ^{C}
	40s	40.92 ± 10.03 (74/7) ^{BC}	50.42 ± 6.7 (71/11) ^{BC}
	Double Application	50.50 ± 12.13 (84/9) ^{AB}	56.67 ± 10.59 (59/2) ^{AB}
	Warm Air Stream	47.93 ± 9.7 (71/9) ^{AB}	54.44 ± 8.13 (61/0) ^{AB}
	Chlorexidine	55.38 ± 6.18 (39/5) ^{AB}	55.63 ± 8.70 (57/2) ^{AB}
	All-in-One	55.72 ± 7.76 (54/5) ^{A}	61.28 ± 8.45 (66/3) ^{A}

Those values marked with the same superscript letter are not statistically different ($p > 0.05$).

Figure 1. Fracture pattern distribution (%) for each protocol in the SBUV0 baseline group (SBUV0 – Baseline).

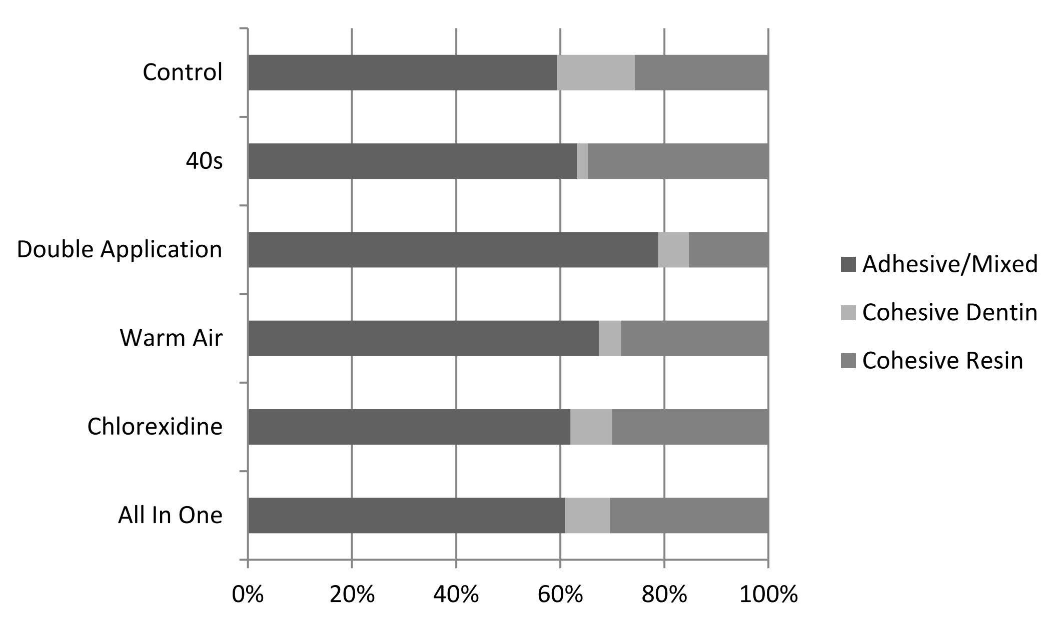

Figure 2. Fracture pattern distribution (%) for each protocol in the SBUV1 baseline group (SBUV1 – Baseline)

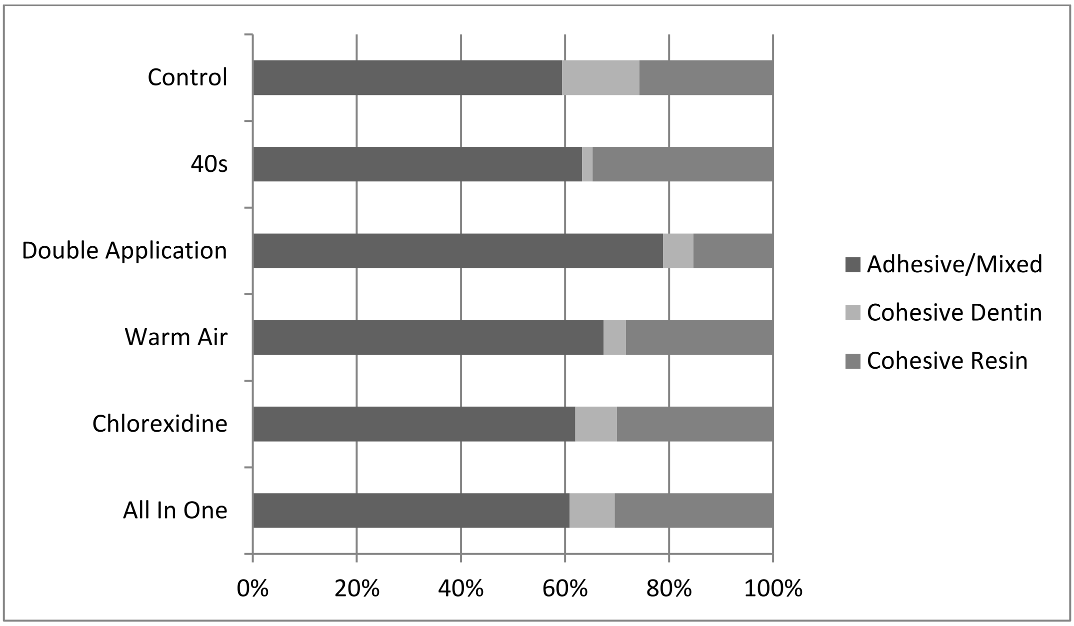

Figure 3. Fracture pattern distribution (%) for each protocol in the SBUV0 aged group (SBUV0 – Aged)

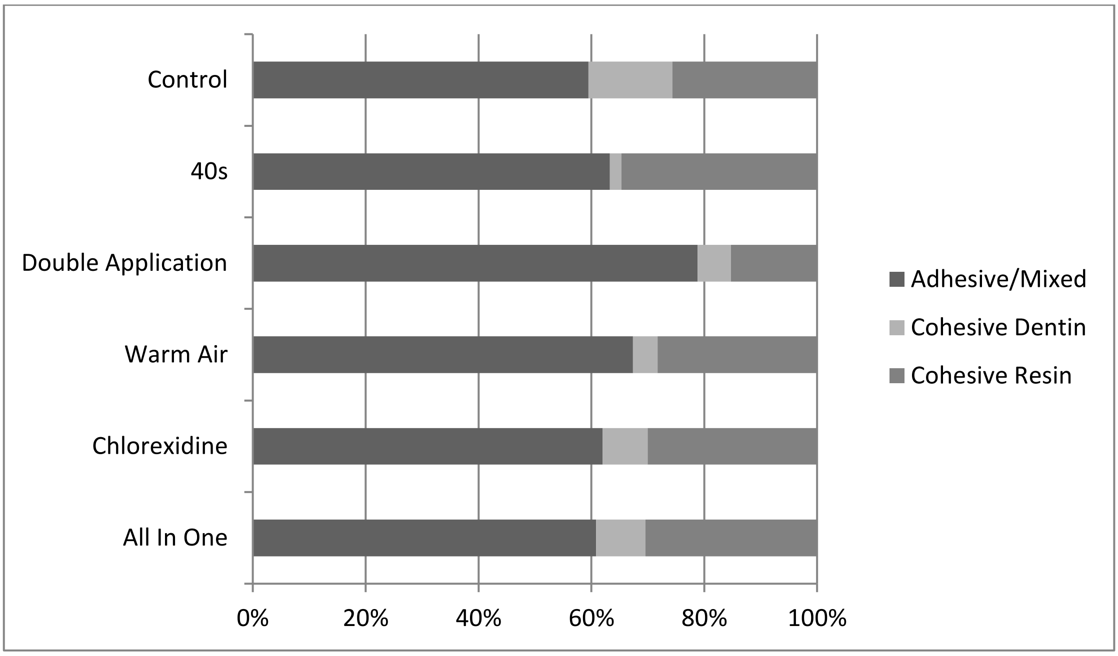

Figure 4. Fracture pattern distribution (%) for each protocol in the SBUV1 Aged group (SBUV1 – Aged).

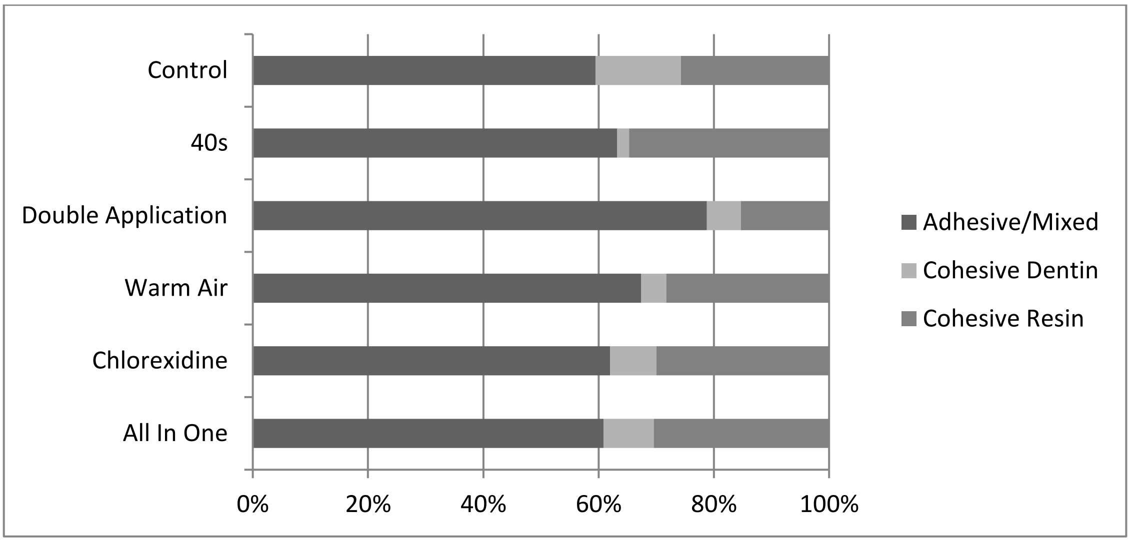

Table 3. Ultimate bond strength. Data for 6 specimens

Adhesive / Sample	SBUV0	SBUV1
Stick 1	20.7	27.4
Stick 2	23.2	23.4
Stick 3	17.6	33.5
Stick 4	19.3	35.8
Stick 5	23	37
Stick 6	19.2	33.6
Overall Media ± SD	**19.83^{B} ± 2.24**	**31.78^{A} ± 5.27**

3 DISCUSSÃO

A adição de uma acrilamida no Single Bond Universal resultou em melhora na resistência de união à dentina quando os espécimes foram submetidos ao ensaio de microtração. Isso foi observado nos dois artigos, onde no segundo também foi constatada uma melhora na resistência de união após os 6 meses de armazenamento. O aumento da resistência intrínseca do adesivo, medido pelo ensaio de ultimate, também foi observado em ambos os artigos.

A incorporação de um espessante como a acrilamida tornaria o adesivo mais viscoso, diminuindo sua molhabilidade e aumentando o ângulo de contato. O aumento do ângulo de contato foi verificado no goniômetro, onde o adesivo modificado com acrilamida apresentou maior ângulo de contato nos dois tempos testados (5s e 20s). Porém, esse evidente aumento no ângulo de contato não foi suficiente para prejudicar a resistência de união. As razões discutidas nos artigos para isso ter ocorrido focam na maneira como o adesivo é aplicado na dentina (aplicação ativa) e nas possíveis vantagens que um adesivo mais espesso poderia trazer na estabilidade da camada híbrida, em especial ao longo do tempo.

Entre as vantagens estão o fato das acrilamidas serem mais resistentes à degradação hidrolitica comparado aos grupos acrilatos. Os grupos amida e carbóxilo interagem com os aminoácidos das fibrilas de colágeno presentes na dentina formando ligações de hidrogênio mais estáveis do que os monômeros acrilatos com colágeno (MOSZNER, SALZ, ZIMMERMANN . 2005; NISHIYAMA, et al. 2010). Outra vantagem da adição da acrilamida é o aumento da resistência intrínseca do adesivo modificado polimerizado. Essa maior resistência intrínseca foi observada nos dois artigos através de ensaios de ultimate. A acrilamida traz, portanto, benefícios químicos e mecânicos ao adesivo dentinário.

A adição de outras substâncias para melhorar as propriedades do adesivo não é novidade na literatura. A adição de nanopartículas de cobre (GUTIÉRREZ, et al. 2017.; ESSA, KHALLAF. 2016) e prata (MORONES. et al. 2005.; CORRÊA. et al. 2015) melhoram as propriedades do adesivo e tem efeito antimicrobiano, dificultando a progressão de novas lesões de cárie na interface dente-restauração. A incorporação de princípios ativos de plantas e sementes como as Proantocianidinas, deixando os adesivos “bioativos” e proporcionando vantagens como inibição da produção de ácido por streptococcus mutans, aumento da estabilidade mecânica e redução da degradação do colágeno também são exemplos de produtos adicionados à composição do adesivo para melhorar seu desempenho (SILVA, et al. 2015.; SANTOS , KAROL, RUSSO, et al. 2011).

Porém, todas essas abordagens necessitam da aquisição e manuseio de outro produto a ser aplicado junto com o adesivo dentinário. Existem maneiras de melhorar a performance do adesivo sem que haja a necessidade de obter novos produtos. A simples mudança de protocolo de aplicação dos sistemas adesivos pode promover uma melhora considerável. Aplicar duas vezes o adesivo, fotopolimerizar pelo dobro de tempo, usar ar quente para evaporar o solvente do adesivo, usar digluconato de clorexidina antes da aplicação do adesivo são alguns exemplos. Algumas dessas mudanças estão ao alcance do clínico e podem facilmente ser empregadas na prática clínica. Cada modificação de protocolo melhora o desempenho do adesivo através de diferentes mecanismos de ação. A aplicação de uma camada extra de adesivo melhora a penetração dos monômeros resinosos nas fibrilas colágenas e diminui a exposição das mesmas. Prolongar o tempo de fotopolimerização aumenta o grau de conversão do adesivo, aumenta a evaporação de solvente e diminui a exposição das fibras colágenas. Aplicar um jato de ar quente além de melhorar a evaporação do solvente, aumenta o grau de conversão. Já o uso da clorexidina antes do adesivo inibe a atividade das Metaloproteinases (MMPs), protegendo as fibrilas colágenas que, eventualmente, não são inpregnadas pelo adesivo, no procedimento adesivo.

A soma desses benefícios, aliado com a melhora nas propriedades mecânicas do adesivo promovidas pela poliacrilamida poderiam ter aumentado ainda mais o desempenho do adesivo quanto à resistência de união à dentina. No segundo artigo, foi verificado que a adição da acrilamida, assim como as mudanças no protocolo de aplicação do adesivo, aumentou a resistência de união. Porém, não houve interação entre os diferentes mecanismos de ação. A adição de acrilamida não potencializou os benefícios das modificações de protocolo e vice-versa. Como esses foram os primeiros estudos a avaliar o desempenho de um sistema adesivo com incorporação da acrilamida, testando-o em diferentes protocolos de aplicação, é necessário que outros estudos investiguem os possíveis efeitos benéficos dessa sinergia e corroborem ou refutem os achados dessa pesquisa.

Por se tratar de um estudo inédito, longo, com muitas variáveis (adesivo – e suas versões -, envelhecimento e protocolo de aplicação) opta-se primeiro por realizar um estudo in vitro. Outros estudos laboratoriais são necessários para avaliar diferentes parâmetros (nanoinfiltração, nanodureza, efeito antimicrobiano e grau de conversão), utilizando outros sistemas adesivos que não apenas o Single Bond Universal, além de ser adotado um maior tempo de envelhecimento, bem como diferentes métodos, para averiguar a degradação da camada híbrida.

Estudos clínicos e laboratoriais, no entanto, também são necessários para averiguar possíveis efeitos da acrilamida à polpa e se o aumento na resistência intrínseca do adesivo e o aumento da resistencia de união observadas nesse estudo são confirmados, *in vivo*.

4 CONCLUSÃO

A incorporação de baixa concentração de acrilamida em um sistema adesivo universal aumenta a resistência de união à dentina e a resistência intrínsica do adesivo. Algumas mudanças de protocolo também melhoram a resistência de união à dentina, contudo não parecem ter sido, necessariamente, sinergiscos com a adição da acrilamida. Mais estudos laboratorias e clínicos são necessários para confirmar as reais vantagens das alterações realizadas no intuito de melhorar a adesão e a resistência à degradação da camada híbrida.

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ANEXO A – NORMAS JOURNAL OF ADHESIVE DENTISTRY

The Journal of Adhesive Dentistry: GUIDELINES FOR AUTHORS

The Journal of Adhesive Dentistry is a bi-monthly journal that publishes scientifically sound articles of interest to practitioners and researchers in the field of adhesion to hard and soft dental tissues. The Journal publishes several types of peer-reviewed original articles:

1. Clinical and basic science research reports – based on original research in adhesive dentistry and related topics.
2. Reviews topics – on topics related to adhesive dentistry
3. Short communications – of original research in adhesive dentistry and related topics. Max. 4 printed pages, including figures and references (max. characters 18,000). High priority will be given to the review of these papers to speed publication.

4a. Invited focus articles – presenting a position or hypothesis on a basic science or clinical subject of relevant related topics. These articles are not intended for the presentation of original results, and the authors of the articles are selected by the Editorial Board.

4b. Invited commentaries – critiquing a focus article by addressing the strong and weak points of the focus article. These are selected by the Editorial Board in consultation with the focus article author, and the focus article and the commentaries on it are published in sequence in the same issue of the Journal.

5. Invited guest editorials – may periodically be solicited by the Editorial Board.
6. Proceedings of symposia, workshops, or conferences – covering topics of relevance to adhesive dentistry and related topics.
7. Letters to the Editor – may be submitted to the editorin-chief; these should normally be no more than 500 words in length.

SUBMISSION INSTRUCTIONS Submission of manuscripts in order of preference:

1. Submission via online submission service (www.manuscriptmanager.com/jadd). Manuscript texts should be uploaded as PC-word files with tables and figures preferably embedded within the PC-word document. A broad range of file formats are acceptable. No paper version required but high resolution photographs or illustrations should be

sent to the editorial office (see below). Online submissions are automatically uploaded into the editorial office's reviewer assignment schedule and are therefore processed immediately upon upload.

2. Submission via e-mail as a PC-word document (wintonowycz@quintessenz.de). Illustrations can be attached in any format that can be opened using Adobe Photoshop, (TIF, GIF, JPG, PSD, EPS etc.) or as Microsoft PowerPoint Documents (ppt). No paper version required but high resolution photographs or illustrations should be sent to the editorial office.
3. One paper copy of the manuscript plus a floppy diskette or CD-ROM (mandatory) containing a PC-word file of the manuscript text, tables and legends. Figures should be included on the disk if possible in any format that can to be opened using Adobe Photoshop, (TIf, GIf, JPG, PSD, EPS etc.) or as a Microsoft PowerPoint Document (ppt).

Mailing address:
Quintessenz Verlags-GmbH, Karin Wintonowycz
The Journal of Adhesive Dentistry,
Ifenpfad 2-4, D–12107 Berlin, Germany
Illustrations that cannot be sent electronically will be scanned at the editorial office so that they can be sent to reviewers via e-mail along with the manuscript to expedite the evaluation process. Resubmitted manuscripts should also be submitted in the above manner. Please note that supplying electronic
versions of your tables and illustrations upon resubmission will assure a faster publication time if the manuscript is accepted. Review/editing of manuscripts. Manuscripts will be reviewed by the editor-in-chief and at least two reviewers with expertise within the scope of the article. The publisher reserves the right to edit accepted manuscripts to fit the space available and to ensure conciseness, clarity, and stylistic consistency, subject to the author's final approval. Adherence to guidelines. Manuscripts that are not pre pared in accordance with these guidelines will be returned to the author before review.

MANUSCRIPT PREPARATION

• The Journal will follow as much as possible the recommendations of the International Committee of Medical Journal Editors (Vancouver Group) in regard to preparation of manuscripts and authorship (Uniform requirements for manuscripts submitted to biomedical journals. Ann Intern Med 1997;126: 36-47).

• Title page. The first page should include the title of the article (descriptive but as concise as possible) and the name, degrees, job title, professional affiliation, contribution to the paper (e.g., idea, hypothesis, experimental design, performed the experiments in partial fulfillment of requirements for a degree, wrote the manuscript, proofread the manuscript, performed a certain test, consulted on and performed statistical evaluation, contributed substantially to discussion, etc.) and full address of all authors. Phone, fax, and e-mail address must also be provided for the corresponding author, who will be assumed to be the first listed author unless otherwise noted. If the paper was presented before an organized group, the name of the organization, location, and date should be included.

• 3-8 keywords.

• Structured abstract. Include a maximum 250-word structured abstract (with headings Purpose, Materials and Methods, Results, Conclusion).

• Introduction. Summarize the rationale and purpose of the study, giving only pertinent references. Clearly state the working hypothesis.

• Materials and Methods. Present materials and methods in sufficient detail to allow confirmation of the observations. Published methods should be referenced and discussed only briefly, unless modifications have been made. Indicate the statistical methods used, if applicable.

• Results. Present results in a logical sequence in the text, tables, and illustrations. Do not repeat in the text all the data in the tables or illustrations; emphasize only important observations.

• Discussion. Emphasize the new and important aspects of the study and the conclusions that follow from them. Do not repeat in detail data or other material given in the Introduction or Results section. Relate observations to other relevant studies and point out the implications of the findings and their limitations.

• Acknowledgments. Acknowledge persons who have made substantive contributions to the study. Specify grant or other financial support, citing the name of the supporting organization and grant number.

• Abbreviations. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

• Trade names. Generic terms are to be used when ever possible, but trade names and manufacturer should be included parenthetically at first mention.

• Clinical Relevance. Please include a very brief (2 sentences or 3 lines) clinical relevance statement.

REFERENCES

• All references must be cited in the text, according to the alphabetical and numerical reference list.

• The reference list should appear at the end of the article, in alphabetical and numerical sequence.

• Do not include unpublished data or personal communications in the reference list. Cite such references parenthetically in the text and include a date.

• Avoid using abstracts as references.

• Provide complete information for each reference, including names of all authors. If the reference is part of a book, also include title of the chapter and names of the book‘s editor(s).

Journal reference style:

1. Turp JC, Kowalski CJ, Stohler CS. Treatment- seeking patters of facial pain patients: Many possibilities, limited satisfaction. J Orofacial Pain 1998;12:61-66.

Book reference style:

1. Hannam AG, Langenbach GEJ, Peck CC. Computer simulations of jaw biomechanics. In: McNeill C (ed). Science and Practice of Occlusion. Chicago: Quintessence, 1997:187-194.

ILLUSTRATIONS

• All illustrations must be numbered and cited in the text in order of appearance.

• Submitted figures should meet the following minimum requirements: – High-resolution images should have a width of 83 mm and 300 dpi (for column size). – Graphics (bar diagrams, schematic representations, drawings) wherever possible should be produced in Adobe Illustrator and saved as AI or EPS files. – All figures and graphics should be separate files – not embedded in Word or Power Point documents.

Upon article acceptance, high-resolution digital image files must be sent via one of the following ways:

1. As an e-mail attachment, if the files are not excessively large (not more than 10 MB), to our production department: Steinbrueck@quintessenz.de

2. Online File Exchange Tool: Please send your figures with our Online File Exchange Tool. This web tool allows you to upload large files (< 350.0 MB) to our server. Please archive your figures with a maximum size of 350 MB first. Then upload these archives with the following link: http://files.qvnet.de/JAD/, password: IAAD. Please name the archive with your name and article number so we can identify the figures. Line drawings–Figures, charts, and graphs should be professionally drawn and lettered large enough to be read after reduction. Good-quality computer-generated laser prints are acceptable (no photocopies); also provide electronic files (eps, ai) if possible. Lines within graphs should be of a single weight unless special emphasis is needed. Legends–Figure legends should be grouped on a separate sheet and typed double-spaced.

TABLES

• Each table should be logically organized, on a separate sheet, and numbered consecutively.

• The title and footnotes should be typed on the same sheet as the table.

MANDATORY SUBMISSION FORM

The Mandatory Submission Form, signed by all authors, must accompany all submitted manuscripts before they can be reviewed for publication. Electronic submission: scan the signed form and submit as JPG or TIF file.

PERMISSIONS & WAIVERS

• Permission of author and publisher must be obtained for the direct use of material (text, photos, drawings) under copyright that does not belong to the author.

• Waivers must be obtained for photographs showing persons. When such waivers are not supplied, faces will be masked to prevent identification. For clinical studies the approval of the ethics committee must be presented.

PAGE CHARGE

The first 8 printed pages in an article are free of charge. For excess pages, the charge is €140 per printed page. The approximate number of characters on a printed page is approximately 6,800. Please also consider the number and size of illustrations.

ANEXO B - NORMAS DA OPERATIVE DENTISTRY

Normas para publicação: Operative Dentistry

INSTRUCTIONS TO AUTHORS

New Instructions as of 20 September 2008

Operative Dentistry requires electronic submission of all manuscripts. All submissions must be sent to Operative Dentistry using the Allen Track upload site. Your manuscript will only be considered officially submitted after it has been approved through our initial quality control check, and any problems have been fixed. You will have 6 days from when you start the process to submit and approve the manuscript. After the 6 day limit, if you have not finished the submission, your submission will be removed from the server. You are still able to submit the manuscript, but you must start from the beginning. Be prepared to submit the following manuscript files in your upload:

- A Laboratory or Clinical Research Manuscript file must include:
  - a title
  - a running (short) title
  - a clinical relevance statement
  - a concise summary (abstract)
  - introduction, methods & materials, results, discussion and conclusion
  - references (see Below)
  - The manuscript **MUST NOT** include any:
    - identifying information such as:
      - Authors
      - Acknowledgements
      - Correspondence information
    - Figures
    - Graphs
    - Tables
- An acknowledgement, disclaimer and/or recognition of support (if applicable) must in a separate file and uploaded as supplemental material.
- All figures, illustrations, graphs and tables must also be provided as individual files. These should be high resolution images, which are used by the editor in the actual typesetting of your manuscript. Please refer to the instructions below for acceptable formats.
- All other manuscript types use this template, with the appropriate changes as listed below.

Complete the online form which includes complete author information and select the files you would like to send to Operative Dentistry. Manuscripts that do not meet our formatting and data requirements listed below will be sent back to the corresponding author for correction.

GENERAL INFORMATION

- All materials submitted for publication must be submitted exclusively to Operative Dentistry.
- The editor reserves the right to make literary corrections.
- Currently, color will be provided at no cost to the author if the editor deems it essential to the manuscript. However, we reserve the right to convert to gray scale if color does not contribute significantly to the quality and/or information content of the paper.
- The author(s) retain(s) the right to formally withdraw the paper from consideration and/or publication if they disagree with editorial decisions.
- International authors whose native language is not English must have their work reviewed by a native English speaker prior to submission.
- Spelling must conform to the American Heritage Dictionary of the English Language, and SI units for scientific measurement are preferred.
- While we do not currently have limitations on the length of manuscripts, we expect papers to be concise; Authors are also encouraged to be selective in their use of figures and tables, using only those that contribute significantly to the understanding of the research.
- Acknowledgement of receipt is sent automatically. If you do not receive such an acknowledgement, please contact us at editor@jopdent.org rather than resending your paper.
- **IMPORTANT:** Please add our e-mail address to your address book on your server to prevent transmission problems from spam and other filters. Also make sure that your server will accept larger file sizes. This is particularly important since we send page-proofs for review and correction as .pdf files.

REQUIREMENTS

- **FOR ALL MANUSCRIPTS**

    1. **CORRESPONDING AUTHOR** must provide a WORKING / VALID e-mail address which will be used for all communication with the journal.
       NOTE: Corresponding authors MUST update their profile if their e-mail or postal address changes. If we cannot contact authors within seven days, their manuscript will be removed from our publication queue.

    2. **AUTHOR INFORMATION** must include:
        - full name of all authors
        - complete mailing address for each author
        - degrees (e.g. DDS, DMD, PhD)
        - affiliation (e.g. Department of Dental Materials, School of Dentistry, University of Michigan)

    3. **MENTION OF COMMERCIAL PRODUCTS/EQUIPMENT** must include:
        - full name of product
        - full name of manufacturer
        - city, state and/or country of manufacturer

4. **MANUSCRIPTS AND TABLES** must be provided as Word files. Please limit size of tables to no more than one US letter sized page. (8 ½ ” x 11”)

5. **ILLUSTRATIONS, GRAPHS AND FIGURES** must be provided as TIFF or JPEG files with the following parameters
   - line art (and tables that are submitted as a graphic) must be sized at approximately 5” x 7” and have a resolution of 1200 dpi.
   - gray scale/black & white figures must have a minimum size of 3.5” x 5”, and a maximum size of 5” x 7” and a minimum resolution of 300 dpi and a maximum of 400 dpi.
   - color figures must have a minimum size of 2.5” x 3.5”, and a maximum size of 3.5” x 5” and a minimum resolution of 300 dpi and a maximum of 400 dpi.
   - color photographs must be sized at approximately 3.5” x 5” and have a resolution of 300 dpi.

- **OTHER MANUSCRIPT TYPES**

1. **CLINICAL TECHNIQUE/CASE STUDY MANUSCRIPTS** must include:
   - a running (short) title
   - purpose
   - description of technique
   - list of materials used
   - potential problems
   - summary of advantages and disadvantages
   - references (see below)

2. **LITERATURE AND BOOK REVIEW MANUSCRIPTS** must include:
   - a running (short) title
   - a clinical relevance statement based on the conclusions of the review
   - conclusions based on the literature review…without this, the review is just an exercise
   - references (see below)

- **FOR REFERENCES**

REFERENCES must be numbered (superscripted numbers) consecutively as they appear in the text and, where applicable, they should appear after punctuation.

The reference list should be arranged in numeric sequence at the end of the manuscript and should include:

1. Author(s) last name(s) and initial (ALL AUTHORS must be listed) followed by the date of publication in parentheses.
2. Full article title.
3. Full journal name in italics (no abbreviations), volume and issue numbers and first and last page numbers complete (i.e. 163-168 NOT attenuated 163-68).
4. Abstracts should be avoided when possible but, if used, must include the above plus the abstract number and page number.
5. Book chapters must include chapter title, book title in italics, editors' names (if appropriate), name of publisher and publishing address.
6. Websites may be used as references, but must include the date (day, month and year) accessed for the information.
7. Papers in the course of publication should only be entered in the references if they have been accepted for publication by a journal and then given in the standard manner with "In press" following the journal name.
8. **DO NOT** include unpublished data or personal communications in the reference list. Cite such references parenthetically in the text and include a date.

EXAMPLES OF REFERENCE STYLE

- Journal article: two authors
  Evans DB & Neme AM (1999) Shear bond strength of composite resin and amalgam adhesive systems to dentin *American Journal of Dentistry* **12(1)** 19-25.

- Journal article: multiple authors
  Eick JD, Gwinnett AJ, Pashley DH & Robinson SJ (1997) Current concepts on adhesion to dentin *Critical Review of Oral and Biological Medicine* **8(3)** 306-335.

- Journal article: special issue/supplement
  Van Meerbeek B, Vargas M, Inoue S, Yoshida Y, Peumans M, Lambrechts P & Vanherle G (2001) Adhesives and cements to promote preservation dentistry *Operative Dentistry* **(Supplement 6)** 119-144.

- Abstract:
  Yoshida Y, Van Meerbeek B, Okazaki M, Shintani H & Suzuki K (2003) Comparative study on adhesive performance of functional monomers *Journal of Dental Research* **82(Special Issue B)** Abstract #0051 p B-19.

- Corporate publication:
  ISO-Standards (1997) ISO 4287 Geometrical Product Specifications Surface texture: Profile method – Terms, definitions and surface texture parameters *Geneve: International Organization for Standardization* **1st edition** 1-25.

- Book: single author
  Mount GJ (1990) *An Atlas of Glass-ionomer Cements* Martin Duntz Ltd, London.

- Book: two authors
  Nakabayashi N & Pashley DH (1998) *Hybridization of Dental Hard Tissues* Quintessence Publishing, Tokyo.

- Book: chapter
  Hilton TJ (1996) Direct posterior composite restorations In: Schwarts RS, Summitt JB, Robbins JW (eds) *Fundamentals of Operative Dentistry* Quintessence, Chicago 207-228.

- Website: single author
  Carlson L (2003) Web site evolution; Retrieved online July 23, 2003 from: http://www.d.umn.edu/~lcarlson/cms/evolution.html

- Website: corporate publication
  National Association of Social Workers (2000) NASW Practice research survey 2000. NASW Practice Research Network, 1. 3. Retrieved online September 8, 2003 from:http://www.socialworkers.org/naswprn/default

ANEXO C – PARECER EMITIDO PELO CEP

UNIVERSIDADE FEDERAL DE SANTA MARIA/ PRÓ-REITORIA DE PÓS-GRADUAÇÃO E

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação do desempenho de um sistema adesivo universal aplicado em diferentes protocolos e alteração na viscosidade.

Pesquisador: ALEXANDRE HENRIQUE SUSIN

Área Temática:

Versão: 1

CAAE: 48595915.6.0000.5346

Instituição Proponente: Universidade Federal de Santa Maria/ Pró-Reitoria de Pós-Graduação e

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.218.989

Apresentação do Projeto:

Pesquisa corresponde a uma projeto de tese de Doutorado vinculada ao Programa de Pós-Graduação em

UNIVERSIDADE FEDERAL DE SANTA MARIA/ PRÓ-REITORIA DE PÓS-GRADUAÇÃO E

Continuação do Parecer: 1.218.989

Outros	20150825112029666.pdf	26/08/2015 10:09:13	ALEXANDRE HENRIQUE SUSIN	Aceito
Cronograma	CRONOGRAMA.docx	26/08/2015 10:14:59	ALEXANDRE HENRIQUE SUSIN	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	JUSTIFICATIVA_DE_AUSENCIA.docx	26/08/2015 10:19:28	ALEXANDRE HENRIQUE SUSIN	Aceito
Folha de Rosto	folhaderostoMP.pdf	26/08/2015 10:00:36	ALEXANDRE HENRIQUE SUSIN	Aceito
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_578920.pdf	26/08/2015 10:20:13		Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

SANTA MARIA, 08 de Setembro de 2015

Assinado por:
CLAUDEMIR DE QUADROS
(Coordenador)