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EFEITO DA CLOREXEDINA ÁCIDA NA RESISTÊNCIA DE UNIÃO IMEDIATA E EM LONGO PRAZO DE SISTEMAS ADESIVOS ATUAIS

Santa Maria, RS
2016

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Dissertação de Mestrado apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Ciências Odontológicas, área de concentração em Odontologia, ênfase em Dentística Restauradora, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do título de **Mestre em Ciências Odontológicas.**

Orientador: Prof. Dr. Alexandre Henrique Susin

Santa Maria, RS

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Aprovado em 28 de julho de 2016:

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(Presidente/Orientador)

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Santa Maria, RS
2016

DEDICATÓRIA

Dedico este trabalho aos meus pais, **Cleci e Lázaro**. Agradeço a Deus por tê-los como pais! Muito obrigada por sempre acreditarem em mim, pelo cuidado em me instruir com valores e por serem pessoas maravilhosas, com este amor incondicional. Dedico este trabalho a vocês, que me permitiram crescer com tranquilidade, buscar meus sonhos e desfrutar das oportunidades. Dedico a vocês esta vitória!

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“Talvez não tenha conseguido fazer o melhor, mas lutei para que o melhor fosse feito. Não sou o que deveria ser, mas Graças a Deus, não sou o que era antes”.

Marthin Luther King

RESUMO

EFEITO DA CLOREXEDINA ÁCIDA NA RESISTÊNCIA DE UNIÃO IMEDIATA E EM LONGO PRAZO DE SISTEMAS ADESIVOS ATUAIS.

AUTORA: Clecila Müller
ORIENTADOR: Alexandre Henrique Susin

As metaloproteinases de matriz (MMPs) têm sido extensivamente estudadas, e inibidores de MMP têm sido utilizados como agentes de pré-tratamento dental previamente procedimentos adesivos em dentina porque reduzem a degradação do colagénio e melhoraram a resistência de união, sendo o inibidor mais utilizado a clorexidina (CHX). O objetivo deste estudo foi analisar o efeito de diferentes valores de pH da CHX na resistência de união de um adesivo universal e um adesivo autocondicionante, imediatamente e após 12 meses de envelhecimento. Métodos: superfícies planas de dentina de setenta e dois molares humanos devidamente preparados para o estudo foram divididos aleatoriamente em 12 grupos de acordo com superfícies pré-tratados com CHX com diferentes valores de pH (CHX 5.8 ou CHX 2,9) aplicadas durante 60 s, antes do antes da aplicação doadesivo e abordagem de união (Scotchbond Universal: nas estratégias autocondicionante e condicionamento ácido total) ou um adesivo autocondicionante (Clearfil SE bond). Restaurações de resina composta foram construídas incrementalmente. Os grupos foram examinados no imediatamente e após 12 meses de armazenamento de água, palitos com área de secção transversal de 0,8 mm² foram testados para resistência de união (µTBS). Dois palitos de cada dente foram selecionados a fim de avaliação em MEV. As médias de µTBS foram analisadas separadamente para cada sistema adesivo usando one-way ANOVA e teste de Tukey (α = 0,05). Resultados: Os dados mostraram que CHX 5,8 e 2,9 CHX não têm diferenças estatísticas cedo, enquanto CHX 2.9 na longo prazo, preserva a resistência de união. O substrato tratado com CHX 2,9 preservada a resistência de ligação em todos os sistemas adesivos em contraste com os grupos de auto-adesivas etch tratados com CHX 5,8 a longo prazo. Conclusões: O tratamento com clorexidina baixo pH preservar a resistência de união de restaurações adesivas no longo prazo em todos adesivo significado strategies.Clinical: O estudo pode ajudar a prever o desempenho clínico de sistemas adesivos de forma mais eficaz no longo prazo reduzindo o pH do pré-tratamento solução de CHX.

Palavras-chave: Inibição enzimática. Adesivos multimodo. Camada Híbrida. Mataloproteinases de matriz. Microtração. pH.

ABSTRACT

EFFECT OF ACIDIC CHLORHEXIDINE ON EARLY AND ON LONG TERM DENTIN BOND STRENGTH OF CURRENT ADHESIVE SYSTEMS

AUTHOR: Clecila Müller
ADVISOR: Alexandre Henrique Susin

Matrix metalloproteinases (MMPs) have been studied extensively, and MMP inhibitors have been used as dental pretreatment agents prior to dentin bonding because they reduce collagen degradation and improve bonding strength, the most used inhibitor is chlorhexidine (CHX). The aim of this study was to analyze the effect of different pH values of CHX solution on the microtensile bond strength of a universal and a self-etch adhesives, early and after 12 months of aging. Methods: Flat dentin surfaces from seventy two human molars properly prepared for the study, and were randomly assigned to 12 groups according surfaces pretreated with CHX with different pH values (CHX 5.8 or CHX 2.9) applied during 60 s, prior to adhesive application, and bonding approach (Scotchbond Universal: self-etching and etch-and-rinse strategies) or a self-etch adhesive (Clearfil SE Bond). Composite buildups were constructed incrementally. The groups were examined early and after 12 months of water storage, bonded sticks with cross-sectional areas of 0.8 mm² were tested for microtensile bond strength (μTBS). Two sticks from each tooth were selected in order to evaluate with SEM. The μTBS means were analyzed separately for each adhesive system using one-way ANOVA and Tukey's tests ($\alpha = 0.05$). Results: The data showed that CHX 5.8 and CHX 2.9 did not have statistic differences early, while CHX 2.9 on long term, preserves the bond strength. The substrate treated with CHX 2.9 preserved the bond strength in all adhesive systems in contrast to the self-etch adhesive groups treated with CHX 5.8 in the long term. Conclusions: Treatment with low-pH chlorhexidine preserve the bond strength of adhesive restorations in the long-term in all adhesive strategies.Clinical significance:The study could help to predict the clinical performance of adhesive systems more effectively on long term reducing the pH of pretreatment solution of CHX.

Keywords: Enzyme inhibition, Multimode Adhesive, Hybrid layer, Matrix metalloproteinase, Microtensile, pH.

LISTA DE ABREVIATURAS E SIGLAS

°C	Graus Celsius
MEV	Microscóspio Eletrônico de Varredura
MPa	Megapascal
mm	Milímetros
mm/min	Milímetros por minuto
mm²	Milímetros quadrado
mW/cm²	Mili-Watt por centímetro ao quadrado
s	Segundos
µm	Micrometro
µTBS	Teste de Microtração
x	Número de aumento
n	Número de amostras
N	Newton
pH	Potencial hidrogeniônico
p	Nível de significância
rpm	Rotações por minuto
%	Porcentagem
=	Igual a
ANOVA	Análise de Variância
USA	Estados Unidos da América
F	Força
SBU	Scocthbond Universal
CSEB	Clearfil SE Bond
TE	Total-etching
SE	Self-etching
RS	Rio Grande do Sul
UFSM	Universidade Federal de Santa Maria
LED	Light emitter diode) diodo emissor de luz
et al.	E outros (abreviatura de *et alli*)
mL	Mililitro

SUMÁRIO

1 INTRODUÇÃO

A odontologia adesiva foi fundada com o condicionamento do esmalte, idealizado por Buonocore em 1955, que propiciou a união entre substrato dentário e restauração; A descrição do mecanismo de formação da camada híbrida, ocorrida pela ação dos adesivos sobre substrato dentinário, realizada por Nakabayashi em 1982, o que proporcionou a compreensão dos fundamentos de interação entre tecidos dentários e sistemas adesivos, na formação da interface adesiva. Toda esta evolução trouxe benefícios no quesito preservação de estrutura dental sadia e estética, porém ainda são encontradas limitações no que diz respeito ao selamento do preparo. A maioria dos sistemas adesivos tem um bom desempenho em curto prazo, mas a degradação que ocorre na intereface dente-restauração é ainda um desafio a ser enfrentado para proporcionar maior longevidade às restaurações.

Com a evolução tecnológica dos materiais adesivos, a fim de simplificar a realização do procedimento e serem obtidos melhores resultados de resistência de união para a dentina, foram introduzidos no mercado os sistemas adesivos universais, que podem ser utilizados na técnica do condicionamento ácido total ou autocondicionante (PERDIGÃO et al., 2014), como por exemplo, o sistema adesivo Single Bond Universal. De acordo com o fabricante, esse sistema apresenta como proposta maior adesão ao esmalte e estabilidade hidrolítica, independente da técnica de aplicação e do grau de umidade dentinária. Assim, ele pode ser utilizado pela técnica do condicionamento total, seletivo ou como autocondicionante, para a adesão de restaurações em dentina úmida ou seca.

O mecanismo de adesão ao substrato dentinário é bastante investigado, visto que, diferente do que ocorre no esmalte, o procedimento adesivo em dentina é desafiador por conta da heterogeneidade desse tecido (PERDIGÃO, 2010; SALVIO et al., 2013). A dentina é composta 70% de material inorgânico, seguido de 20% de material orgânico e 10% de água, notoriamente diferente dos 96% de conteúdo mineral presente no esmalte. Trata-se de um complexo biológico hidratado e poroso, composto por fluídos e arquitetura tubular que se encontra cercada por uma zona peritubular altamente mineralizada, inseridos em uma matriz intertubular, que além de cristais de apatita apresenta-se predominantemente constituída por colágeno tipo I, proteínas não colagenosas, glicoproteínas e outras biomoléculas, as quais são produzidas durante a síntese da matriz extracelular e se mantém confinadas no tecido dentinário após o fim do processo de mineralização (MARSHALL et al., 1997; SPENCER et al., 2012).

Sendo assim, conceitualmente a hibridização representa o principal mecanismo de retenção dos sistemas adesivos. A camada híbrida, resultante da impregnação dos monômeros dos sistemas adesivos nos espaços interfibrilares produzidos pela remoção dos minerais do substrato, é considerada a principal responsável pela retenção micromecânica das restaurações de resina e também responde, em grande parte, pelo selamento da dentina. A estabilidade da interface de união depende da formação de uma compacta e homogênea camada híbrida (NAKABAYASHI et al., 1982). Logo, esta estrutura é a zona mais vulnerável da interface adesiva, onde ocorre a maioria das falhas e a concentração de tensões (REIS et al., 2013).

Dentre os diferentes fenômenos que ocorrem na interface de união resina-dentina, alguns são considerados fundamentais na degradação da camada híbrida como: incompleta infiltração na dentina desmineralizada pelos monômeros resinosos, a alta permeabilidade da interface de união, a sub-polimerização dos monômeros e a ativação da atividade colagenolítica de enzimas endógenas, que possuem afinidade por tecidos a base de colágeno. Fibras colágenas uma vez que se mantiverem desprotegidas pelos monômeros adesivos após o condicionamento acido tornam-se susceptíveis à hidrólise e degradação enzimática mediada por proteases, como as MMPs da matriz dentinária e cisteínas catepsinas (PASHLEY et al., 2004; TEZVERGIL-MUTLUAY et al., 2013).

As MMPs que apresentam capacidade de degradação da matriz extracelular são representadas por 23 endopeptidases, que tem metabolismo dependentes de zinco e cálcio, sendo que cinco destas estão presentes na dentina, sendo as MMP – 2, - 8 e – 9 as mais frequentes. Entretanto, a atividade colagenolítica destas enzimas pode ser suprimida por inibidores de proteases a médio e longo prazo, dentre eles esta o digluconato de clorexidina (CARRILHO et al., 2007; BRESCHI et al., 2009; BRESCHI et al., 2010b).

Proporcionando melhora da integridade da camada híbrida e estabilidade na resistência de união resina-dentina, esta solução possui vários estudos acerca da concentração ideal da mesma (BRESCHI et al., 2009, 2010b), tempo de aplicação (LOGUERCIO et al., 2009) e substrato a qual é aplicada (REIS et al., 2013) , porém não é de conhecimento dos autores que haja na literatura estudos sobre o efeito da clorexidina acidificada para preservação da camada híbrida e resistência de união, nem imediatamente e nem a longo prazo. Assim, este estudo teve como objetivo, verificar o comportamento de duas soluções de clorexedine com diferentes pHs (2,9 e 5,8), na resistência de união à dentina de, um adesivo universal e um autocondicionante.

2 ARTIGO – "EFFECT OF ACIDIC CHLORHEXIDINE ON EARLY AND ON LONG TERM DENTIN BOND STRENGTH OF CURRENT ADHESIVE SYSTEMS".

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EFFECT OF ACIDIC CHLORHEXIDINE ON EARLY AND ON LONG TERM DENTIN BOND STRENGTH OF CURRENT ADHESIVE SYSTEMS

Short tittle: Effects of different pH values of chlorhexidine on dentin bond strength.

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Keywords: Enzyme inhibition, Multimode Adhesive, Hybrid layer, Matrix metalloproteinase, Microtensile, pH.

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ABSTRACT

Matrix metalloproteinases (MMPs) have been studied extensively, and MMP inhibitors have been used as dental pretreatment agents prior to dentin bonding because they reduce collagen degradation and improve bonding strength, the most used inhibitor is chlorhexidine (CHX). The aim of this study was to analyze the effect of different pH values of CHX solution on the microtensile bond strength of a universal and a self-etch adhesives, early and after 12 months of aging. Methods: Flat dentin surfaces from seventy two human molars properly prepared for the study, and were randomly assigned to 12 groups according surfaces pretreated with CHX with different pH values (CHX 5.8 or CHX 2.9) applied during 60 s, prior to adhesive application, and bonding approach (Scotchbond Universal: self-etching and etch-and-rinse strategies) or a self-etch adhesive (Clearfil SE Bond). Composite buildups were constructed incrementally. The groups were examined early and after 12 months of water storage, bonded sticks with cross-sectional areas of 0.8 mm^2 were tested for microtensile bond strength (μTBS). Two sticks from each tooth were selected in order to evaluate with SEM. The μTBS means were analyzed separately for each adhesive system using one-way ANOVA and Tukey's tests ($\alpha = 0.05$). Results: The data showed that CHX 5.8 and CHX 2.9 did not have statistic differences early, while CHX 2.9 on long term, preserves the bond strength. The substrate treated with CHX 2.9 preserved the bond strength in all adhesive systems in contrast to the self-etch adhesive groups treated with CHX 5.8 in the long term. Conclusions: Treatment with low-pH chlorhexidine preserve the bond strength of adhesive restorations in the long-term in all adhesive strategies.Clinical significance:The study could help to predict the clinical performance of adhesive systems more effectively on long term reducing the pH of pretreatment solution of CHX.

INTRODUCTION

Restorative dentistry is highly dependent on the adhesive procedures. After introducing by Buonocore, 1955, the acid etching was widely accepted as a way to provide retention to acrylic resin restorations. The description of the concepts and mechanism of hybridization and the importance of monomers impregnation involving the collagen fibrils in the deep demineralized dentin were revealed by Nakabayshi, 1982, and it is basically accepted until today.

Currently, the adhesive systems are able to permeate conditioned enamel and dentin in the etch-and-rinse approaching, or dissolve / modify the smear layer and simultaneously etch and impregnate hard tissues with monomers, on the self-etching [1].

Recently, a new generation of adhesives called "multimode" or "universal" was introduced as a way to be used safer, versatile, and conveniently to the dentists [2-4]. Stability and longevity of adhesive interface are non-solved requirements on bonded restorations [5-7]. Complex and inefficient interactions between dentin and monomeric contents reveal the dentin a critic tissue on terms of sealing and stability of hybrid layer due the intricate micromorphology besides the moisture, organic phase and variable permeability and physiological changes in distinct regions and over time [6,8,9]. Then, especially on long term, deboning may occur from remained unprotected and degraded collagen fibrils on the base of hybrid layer compromising the longevity of resin composite restorations [10]. According the above, it is clear that hydrolytic degradation of resin/dentin interface is attributed to the combined deterioration of unprotected collagen fibrils and non polymerizable adhesive monomers [7,10,11].The deterioration by hydrolysis is mediated by MMPs from dentin matrix via enzymatic degradation of collagen fibrils [12-14]. The MMPs are enzymes that regulate pathological and physiological activities of tissues containing collagen. They exist in latent form in dentin substrates and they are activated by caries lesions and acid defy as acid conditioning prior the adhesive' applying. Removed salts from dentin by acid conditioners leave unprotected collagen fibrils which may be degraded by action of the MMPs.

In vitro and *in vivo* studies have shown efficient on inhibiting the effects of MMPs in the hybrid layer degradation. Dentin pre-treatments with solutions such as carbodimide, digluconate of chlorhexidine, galardin, benzalkonium chloride, and tetracyclines may be used as inhibitors to reduce collagen fibril degradation with advantage of do not negatively influence the immediate bond strength [15-17]. Chlorhexidine is the most studied solution in the prevention or retardation of deleterious enzymatic effects by inactivation of MMP-2, MMP-8, and MMP-9 resulting in more stable adhesive interface on the long-term [18,19].

Since the positive effect of chlorhexedine on regular pH on the preserving of integrity and stability of hybrid layer was widely reported on long term of storage [18] could be speculated that on obtaining a 2% aqueous solution of chlorhexidine with lower pH (2.9) the effect could be enlarged to twelve and more months due the subsuperficial deposition of chlorexhedine closely to the eventual unprotected collagen fibrils. Since the pH of acidic chlorhexidine is not more acid than current self-etching adhesives it is expected that the effects of previous acidic chlorexedine do not interfere on the conditioning promoted by acidic monomers from adhesive system.

The aim of this study was to analyze the effect of different pH values of CHX solution on the microtensile bond strength of a universal and a self-etch adhesives, early and after 12 months of aging.

The null hypothesis was that lower chlorhexidine pH does not affect the early and long-term bond strengths of self-etch and a universal adhesive system.

MATERIALS AND METHODS

This study was approved by a local Ethic's Committee. The required "n" of teeth per group was six (n=6), calculated thorough sample size function with 80% of power using Origin Pro 2015 software, based on the parameters obtained from current literature. Seventy-two caries-free human third molars were obtained from the Teeth Bank. The teeth were cleaned and then disinfected for 1 week with 0.5% chloramine solution and stored in distilled water at room temperature until the use in this study.

Preparation of acidic chlorhexidine solution

Acidification of chlorhexidine digluconate solution was conducted in a laboratory of clinical analyses. A pHmeter was calibrated with buffer solutions supplied by manufacturers. The electrode was immersed in a test tube that contained 2 mL of 2% chlorhexidine digluconate to obtain the original pH value. In order to acidification to pH 2.9 (pH referring to the half of commercially available solution), phosphoric acid was added until the pH be reached This process was performed weekly and on demand since it was found that acidified chlorhexidine solution solution remained no stable stable after this period.

Specimen preparation

All procedures were carried out by a single trained operator, in a laboratoratory with controlled temperature and humidity. A flat coronal dentin surface was obtained by sectioning off the oclusal surface in the medium third with a double-face diamond cutting disc (KG Sorensen, Ind. & Com. Ltd., Cotia, Brazil) coupled in a hand straight piece. The exposed dentin surfaces were standardized by polished with 600-grit silicon carbide paper under running water for 60 s to create a standard smear layer. Subsequently, the prepared surfaces were observed under a 30 x of magnification in a stereomicroscope (Discovery V20, Zeiss, Oberkochen, Germany) to confirm the absence of enamel “islands” and / or any communication with pulp chamber. Then, the teeth were stored in distilled water at 37 °C until use.

The seventy-two teeth were randomized into two groups according pH of chlorhexidine solution (5.8 or 2.9). Both groups were again divided in three others according adhesive system used (Scotchbond Universal, 3M ESPE, St Paul MN, USA) to self-etch and total-etch technique or Clearfil SE Bond (Kuraray Corp, Osaka, Japan), as described by Table 2. After this step, six teeth per groups were tested immediately and 6 were storage in distilled water at 37ºC during one year for proceed the bond strength test, resulting in 12 groups.

Resin Composite Restoration and Microtensile Bond Strength Test

Immediately after adhesive’s photo-curing, resin composite restorations were built (Filtek Z350 XT; shade A2, 3M ESPE, St. Paul, MN, USA) in two increments of 2.0 mm each individually light-cured with an LED unit with a checked 800 mW/cm^2 light intensity (LED Olsen; Olsen Ind. e Com. S/A, Palhoça, SC, Brazil) for 20 s. The restored teeth were stored in distilled water for 24 h at 37 °C, and then they were longitudinally sectioned in mesio-distal and buccal-lingual directions, in order to produce sticks of 0.8 mm^2 of cross-section on bonded interface, with a low-speed diamond disc in a cutting machine (Labcut 1010; Extec Co. Enfield, MA, USA). Half of the groups were tested by microtensile bond strength in 24 hr (early) and the other half was stored in distilled water (37 °C) for 12 months before testing. The sticks were bonded with cyanoacrylate in a specific device and tested on microtensile forces in a testing machine EMIC DL 100 (Instron Instruments Brazil, SJ Pinhais, PR, Brazil) using a load cel of 0.5 N at 0.5 mm / s of crosshead speed, . The μTBS values were calculated by dividing the load at failure by the cross-sectional bonding area. After the test, all fractured specimens were observed in 30 x of magnification and classified according the failure mode:

adhesive/mixed (failure at resin/dentin interface or mixed when also presenting cohesive failure of the dentin or resin) or, cohesive (failure exclusively in dentin or resin composite).

Scanning electron microscopy preparation

Four sticks from each group were aleatory selected for analysis. The test specimens were sequentially polished using silicon-carbide paper (800, 1200, and 2000-grit) and cleaned in an ultrasonic basket for 10 min. Subsequently, the samples were dehydrated and fixed with glutaraldehyde in 2.5% solution 0.1 M sodium cacodylate buffer, for 2 h. The samples were then dehydrated in ascending grades of ethanol: 50% and 75% for 5 min, and 100% for 30 min. The samples were mounted on aluminum metallized bases in a Desk II Sputter Coater (Denton Vacuum, Moorestown, NJ, USA). Resin–dentin interfaces were analyzed under a scanning electron microscope (JEOL A 110; Jeol Ltd., Tokyo, Japan) operated at 12 kV. Imaging at magnifications of 800× and 1000 times× of each sample was obtained in the adhesive interface..

Statistical analysis

A two-factor analysis of variance at 5% significance level was applied to evaluate separately the effect of chlorhexidine solution pH in the interaction with different adhesives and the rate of sample storage. After observing the normality of the data distribution (Levene's test) and the equality of the variances, data obtained from μTBS (MPa) were analyzed separately for each adhesive system using one-way ANOVA; pretreatment, adhesive system, adhesive strategy, and storage time were used as independent variables and compared with couples considering each adhesive system and pH of chlorhexidine solution. Tukey's post-hoc multiple comparison test was performed for multiple comparisons at 5% significance level.

RESULTS

One-way ANOVA (5% of significance level) was separately applied to evaluate the interactions between chlorhexidine pHs and adhesive systems and storage time of the samples. It was not found statistical significant difference when analyzing results of SBU TE + chlorhexidine 5.8 and to SBU TE + chlorhexidine 2.9, on early and on long term (p=0.83 an p=0.54, respectively).

To SBU SE + chlorhexidine 5.8 it was found significant difference (p=0.024) between early and on long term (40,04 and 31,12 MPa respectively) while analysis of SBU SE+ chlorhexidine 2.9 the bond strength was preserved since it was not found significant differences (p=0.83). From CSEB + chlorhexidine 5.8 it was found significant differences (p=0.015) while CSEB + chlorhexidine 2.9 it was not found statistical differences (p=0.75) Table 3 and figure 1.

DISCUSSION

MMP inhibitor agents are used to prolong the durability of resin–dentin bond. In 2004, Pashley et al. [8] showed satisfying evidence of the effectiveness of inhibiting dentin collagenolytic enzyme with the application of inhibitor agents prior to the adhesive system to preserve the interface [14, 20-22]. Since then, several studies have been conducted to develop adequate chlorexidine solution to preserve the hybrid layer. Studies lay emphasis on parameters such as concentration and application time, than on pH concentration [19,23].

This study focused on introducing of an altered pH chlorhexidine solution as alternative to preserve bond strength on long term. Despite several studies had been reporting the effects of regular pH chlorhexidine solutions on dentin bonding preservation [24-28], the altered pH may promote a sub-superficial chlorhexidine deposition in order to enlarge its effect as MMPs inhibitor.

This topic is less likely explored considering that the hybrid layer degrading enzymes are stimulated by acids pH. However, this study showed that the results between the groups that received CHX with manufacture pH and the test group (CHX 2.9) in the bond strength category were similar, independent of the adhesive used on early. Nevertheless, this was not found in the long term, where the groups receiving CHX 2.9 showed results higher than control groups, using the self-etch strategy, therefore the null hypothesis was partial accepted.

Self-etching and Universal adhesive systems generally have mild pH, such as 1.8 from Clearfil SE Bond and 2.7, from SBU, that is efficient on dentin conditioning. Regular pH of available 2% chlorhexidine aqueous solution is 5.8 and the altered pH solution was established in 2.9 to do not interfere on the effect of the adhesives as dentin conditioners. To obtain stable bonding, effective impregnation surrounding collagen fibrils is required [13]. The pre-treatment with CHX 2.9 presented superficial exposition of collagen networks combined with the opening of dentinal tubules (figure 2), assisting adhesives to penetrate into the collagen network, enhancing bonding between the dentin and composite resin.

The quality of the hybrid layer and the bonding strength was significantly improved as well, was similar between TE groups and SE groups pre- treated with CHX 2.9, suggesting CHX 2.9 improves the micromechanical interaction and prevent MMP activity over collagen fibrils in the base of hybrid layer .

Contradicting several studies which found that chlorhexidine used prior the bonding technique preserves bond strength on long term [18,20,21,26,27], this study found discrepant results comparing early with 12 months to SBU on self-etching approaching and CSEB, both pre-treated with CHX 5.8, respectively: 40.05 to 31.12 and 43.62 to 35.82. The *in vitro* tests performed on 12 months could be more predictable of the effect of preserving of hybrid layer than on 6-months [13, 14]. Typically, bond strength decreases after 3 or 6 months of aging, after water storage for a long time [27]. In this instance the use of chlorhexidine in pH 2.9 was able to preserve bond strength on 12 months for both adhesives on self-etch mode to SBU and to CSEB. This might be explained by prior incorporation of the smear layer in the presence of CHX with pH 2.9 in the subsurface dentin, leaving a dentin surface energy suitable for diffusion of adhesive monomer.

Results obtained in SBU TE groups pre-treated with chlorhexidine in the pHs of 5.8 and 2.9 on early have no statistical differences, in accordance with current literature [10,22,28]. Thus, it clearly shows that CHX in acid pH fulfills the purpose of preserve bond strength when the collagen fibrils are not extremely exposed by a previous acid conditioning as it is performed on a total-etch approach. It is clear that bond degradation occurs over time at the bottom of the hybrid layer in the different adhesive systems [29,30]. The durability of this interface is affected by the degradation of the resin components occurring via hydrolysis of collagen fibrils and degradation by MMPs and cysteine cathepsins [9,22].

The adhesive monomers ability to diffuse into the demineralized collagen matrix is invariably impaired. The main reason for CHX2.9 related bond strength improvement with self-etch strategies might be attributed to the interaction between CHX and the dentin substrate/adhesive. The chemical and structural characteristics of chlorhexidine determine the ability to interact with and modify the dentin matrix and the consequent impact on the stability of the vulnerable collagen at the base layer, acting mainly on the inhibition of cysteine cathepsins, collagenases (MMP-8), and gelatinases (MMP-2,-9). Current evidence suggests that these host enzymes are responsible for the degradation of the dentin matrix after demineralization. At the concentrations used, the CHX inhibitory mechanism is thought to occur via calcium chelation. The sequestration of calcium and zinc ions hampers the activation of the catalytic domains within MMPs [7,12].

Previous studies [32] showed that applying 2% chlorexidine after acid etch to minimize the degradation of resin–dentin bonds does not negatively influence the immediate bond strength. Similar results were observed in this study, corroborating the findings of Ricci et al. (2010) [25]. Furthermore, this study found similar results in groups that followed the total etch strategy, regardless of the pH of chlorhexidine solution and the time where the results remained without statistically significant difference. Changing the pH of chlorhexidine solution can ensure space for their action, without competing directly with chelators and ions of calcium, adhesive solvents, and uncured monomers.

Such acidity brings some concern when applying it after acid etching, once some additional demineralization may occur leading to over-etching and consequent unfilled and unprotected collagen. However, no negative effects on bond strength were observed when CHX2.9 was applied. This finding can be justified by the interaction with dentin and consequent reduction of acidity. Also, this acid solution has pH value superior to self-etch adhesives and phosphoric acid, so no detrimental effects to dentin. Therefore mentioned phenomenon was proven by Zhou et al. in 2011[33], evaluating the collagenolytic activity that occurred when the primer and adhesive solutions from CSEB were used at different times and different concentrations of CHX as pre-treatment. The longer time the primer remained on the substrate, the greater the degradation in the groups receiving low concentrations of solution concluding that only concentrations of 2% of CHX showed some efficacy in the inhibition of MMPs, despite the concentration of 0,2% should be also efficient inhibitory effect, but there is still controversy about the optimal inhibitory concentration, as it is reported by Collares et al., 2013[34] and Montagner et al., 2014 [27]. This finding suggests that CHX competes with MMPs by collagen binding, which could explain the high concentration of inhibitor solution needed for its efficient action. When applied in higher concentrations, CHX may oversaturate the enzyme binding sites, and remain bound to collagen fibrils for later release. But it is questionable how long the CHX is able to preserve the hybrid layer [27]. A very positive point is its property CHX has a high substantivity, one cannot rule out that the CHX applied during the adhesive procedure may be easily diluted. The present study suggest, if concentrations of CHX with low pH can secure their place in a subsurface, which will play its inhibiton function of MMPs longer, as can be seen in the test groups, where the bond strength was maintained long-term stable.

Self-etch approaching to SBU and CSEB showed better results in bond strength using 2.9 chlorhexidine in 12 months of storage. Probably the MMPs were not inhibited by 5.8 chlorhexidine when using self-etching adhesives and could be speculated that acidic chlorhexidine (2.9) works as mild acid conditioner able to allow the molecules of chlorhexidine act over collagen fibrils in the surface and also in the subsurface of dentin, before being highly unprotected as it happens in the aggressive demineralization as it occur using total-etching adhesives.

After one year of storage, a significant decrease in bond effectiveness to dentin substrate was observed for both adhesives tested. Over time, storing the sticks in water may have accelerated the degradation. The presence of water also may have caused swelling and reduction in the frictional forces between the polymer chains as well as hydrolysis of the filler matrix interfaces, leading to a decrease in the mechanical properties of the resin. Thus, after the long term storage, mainly an increase of cohesive failure in composite resin and mixed failure of composite resin, adhesive, and dentin were observed for both adhesives [35-37].

Despite the advantages of using 2% chlorhexidine with low pH, the process requires one more step in the restorative procedure, which contrasts with the simplified clinical technique proposed, but the evidence of stability of the hybrid layer for a long time justify this additional step in the restorative procedure. Others studies should be developed, as this is a pioneering study in modifying the pH of CHX to find the ideal pH solution, as well as application time to preserve bond strength on ageing.

CONCLUSION

Within the limitation of this study, it is possible to conclude that, the pH values of CHX influence significantly the microtensile bond strength of the tested adhesive systems. The substrate treated with CHX 2.9 preserved the bond strength in all adhesive systems in contrast to the self-etch adhesive groups treated with CHX 5.8 when aged for 12 months.

Acknowledgments

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TABLES

Table 1. Composition and materials used.

Materials	Composition
Adhesive Scotchbond Universal (3M ESPE – St. Paul, MN, USA)	MDP, phosphate monomer, dimethacrylate resins, HEMA, modified methacrylate, polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane
Adhesive Clearfil SE Bond (Kuraray –Medical Inc., Kurashiki, Japan)	Primer: MDP, HEMA, camphorquinone, hydrophilic dimethacrylate, water. Bond: MDP, Bis-GMA, HEMA, camphorquinone, hydrophobic dimethacrylate, N,N-diethanol ptoluidine bond, colloidal silica
Resin Composite Z350XT (3M ESPE – St. Paul, MN, USA)	Bis-GMA, uretane dimethacrylate (UDMA), TEGDMA, bisfenol-A, Bis-EMA, silicate, zircon
2% Chlorhexidine (Villevie – Joinville, SC, Brazil)	2% Chlorhexidine digluconate
2% Chlorhexidine (2.9) (FARMATOX UFSM)	2% Chlorhexidine digluconate, phosphoric acid
37% Phosphoric acid (FGM – Joinville, SC, Brazil)	37% Phosphoric acid, aqueous gel base

Abbreviations: Bis-GMA, bisphenol-A diglycidyl ether dimethacrylate; HEMA, hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate

Table 2. Description of groups characteristics and application mode

Groups characteristics	Groups	Application mode
SBU TE	**CHX 5,8** **CHX 2,9**	• Etch the dentin with phosphoric acid for 15s; • Followed by rinsing at the same time, with jet air/water • Remove the excess water with absorbent paper; • Application without rubbing 60 s the correspondent solution of chlorhexidine; • Removal of excess with absorbent paper; • Apply adhesive with a microbrush and rub it in for 20 s; • Gently air blow-dry for 5 s until no visible movement of the material is observed; • Light cure for 10 s.
SBU SE	**CHX 5,8** **CHX 2,9**	• Application without rubbing for 60 s the correspondent solution of chlorhexidine; • Removal of excess with absorbent paper; • Apply adhesive with a microbrush and rub it in for 20 s; • Gently air blow-dry for 5 s until no visible movement of the material is observed; • Light cure for 10 s.
CSEB	**CHX 5,8** **CHX 2,9**	• Application without rubbing for 60 s of chlorhexidine with pH 5,8; • Removal of excess with absorbent paper; • Primer to tooth surface and leave for 20 s; • Blow dry; • Apply bond to the tooth surface and then create a uniform film using a gentle air flow; • Light cure for 10 s.

Table 3. Mean, ± standard deviation (SD) for dentin bond strength of each combination of adhesive, pH value and time.

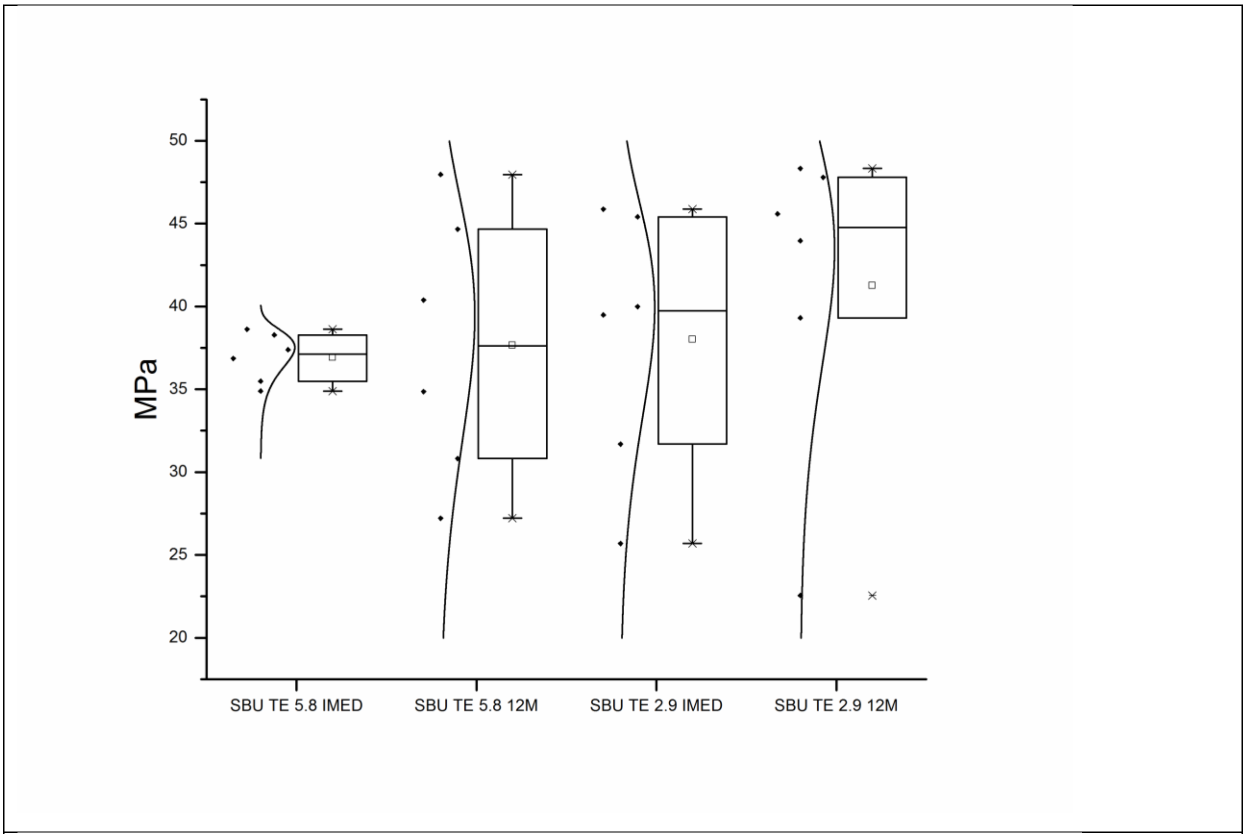
Adhesive system	SBU TE 5.8	SBU TE 2.9	SBU SE 5.8	SBU SE 2.9	CSEB 5.8	CSEB 2.9
Early	36.92 (7.49) A,a	38.02 (7.93) A,a	40.05 (7.10) A,a	42.34 (3.16) A,a	43.62 (12.55) A,a	38.86 (18.40) A,a
Long term	37.65 (8.08) A,a	41.26 (9.72) A,a	31.12 (4.30) B,b,	43.40 (12.10) A,a	24.47 (9.97) B,b	35.82 (14.06) A,a

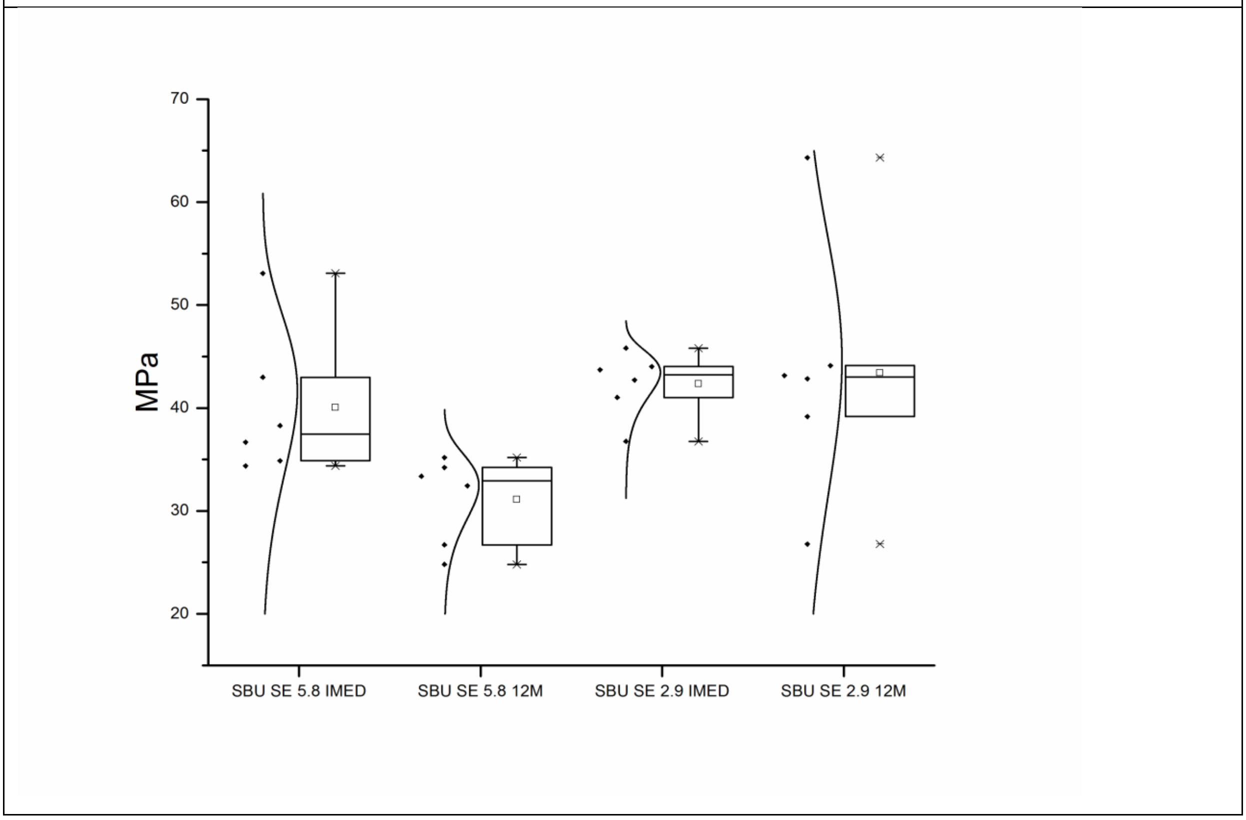
Abbreviation:
Data are presented as the mean (standard deviation) in megapascals (MPa; n = 6 teeth). Identical capital letters in a column indicate the absence of any statistically significant difference. Identical lowercased letters in a row within the same pH solution between 24 h and 12 months water storage indicate the absence of any statistically significant difference. Comparisons within storage period between adhesive and different pH are statistically significant.

Table 4. Distribution of failure types (%) on early and on long term of storage.

		Adhesive	Cohesive in Dentin	Cohesive in Resin
Early	SBU TE 5.8	58	3	39
	SBU TE 2.9	87	-	13
	SBU SE 5.8	62	1	37
	SBU SE 2.9	53	9	38
	CSEB 5.8	78	3	19
	CSEB 2.9	77	2	21
Long Term	SBU TE 5.8	92	-	8
	SBU TE 2.9	97	-	3
	SBU SE 5.8	85	-	15
	SBU SE 2.9	72	-	28
	CSEB 5.8	70	-	30
	CSEB 2.9	77	-	23

FIGURES


MPa
50
45
40
35
30
25
20
SBU TE 5.8 IMED
SBU TE 5.8 12M
SBU TE 2.9 IMED
SBU TE 2.9 12M


MPa
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SBU SE 5.8 IMED
SBU SE 5.8 12M
SBU SE 2.9 IMED
SBU SE 2.9 12M

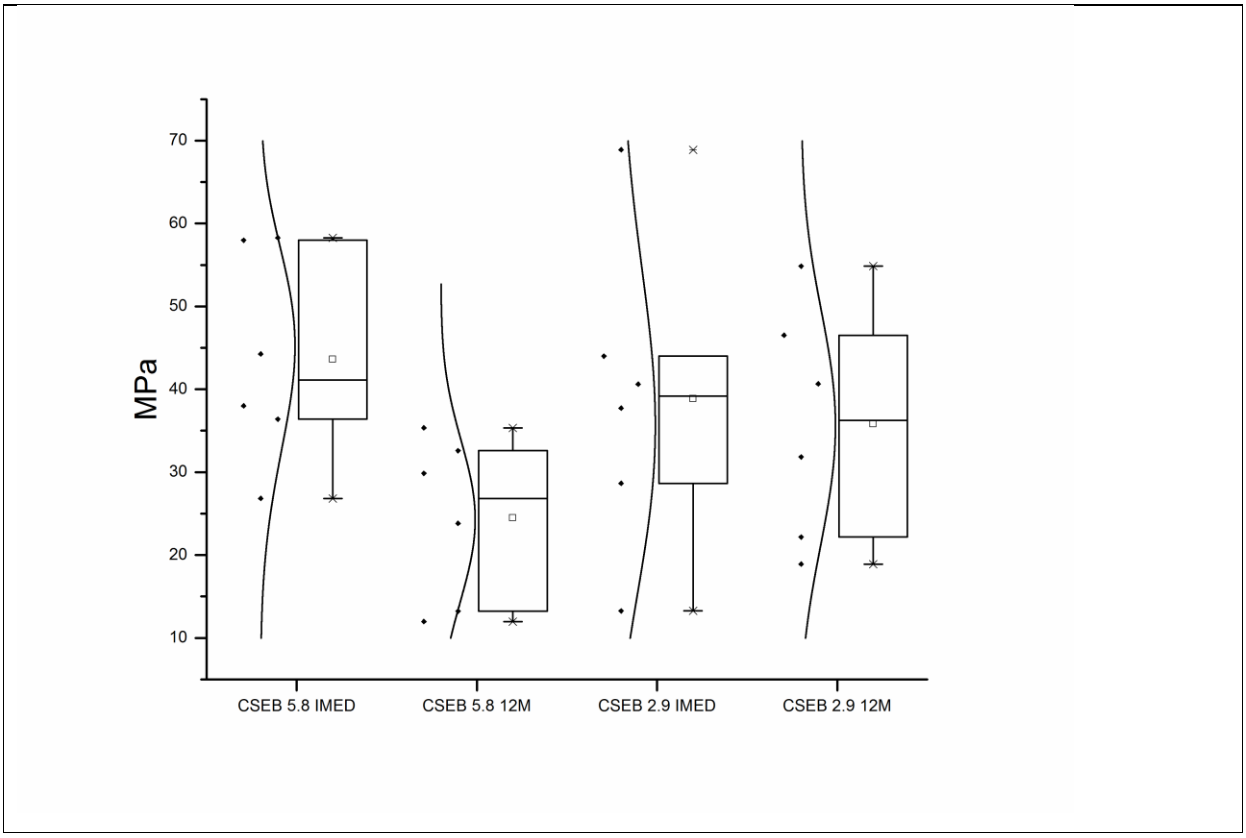

Figure 1: Box plot of dentin bond strength values for the different pH values, adhesives and time. SBU= Single Bond Universal, TE= Total-etching, SE= Self-etching, CSEB= Clearfil SE Bond, 5.8 = pH value, 2.9= pH value.

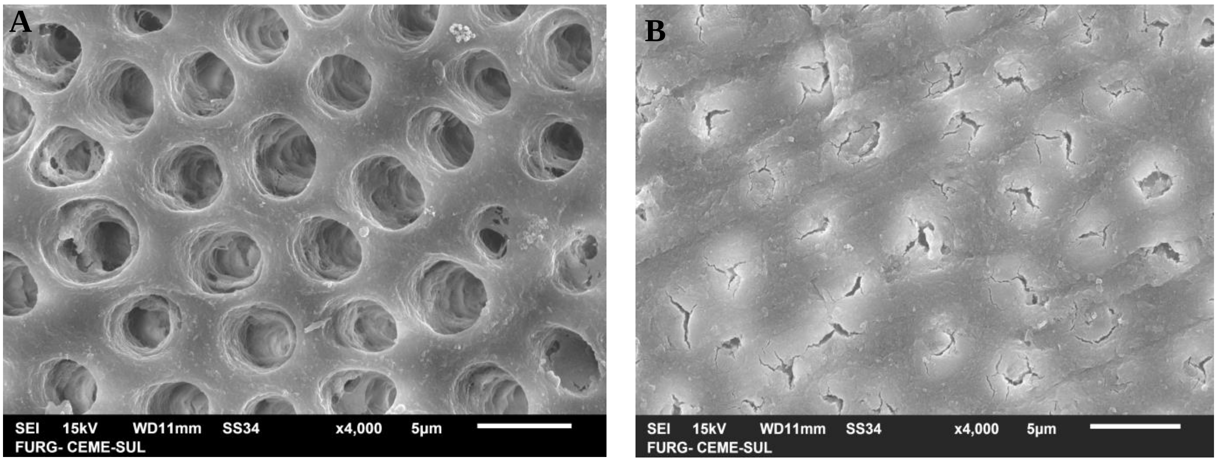

Figure 2: SEM images of surfaces of specimens. A) Surface trated with CHX 2.9; B) Surface trated with CHX 5.8.

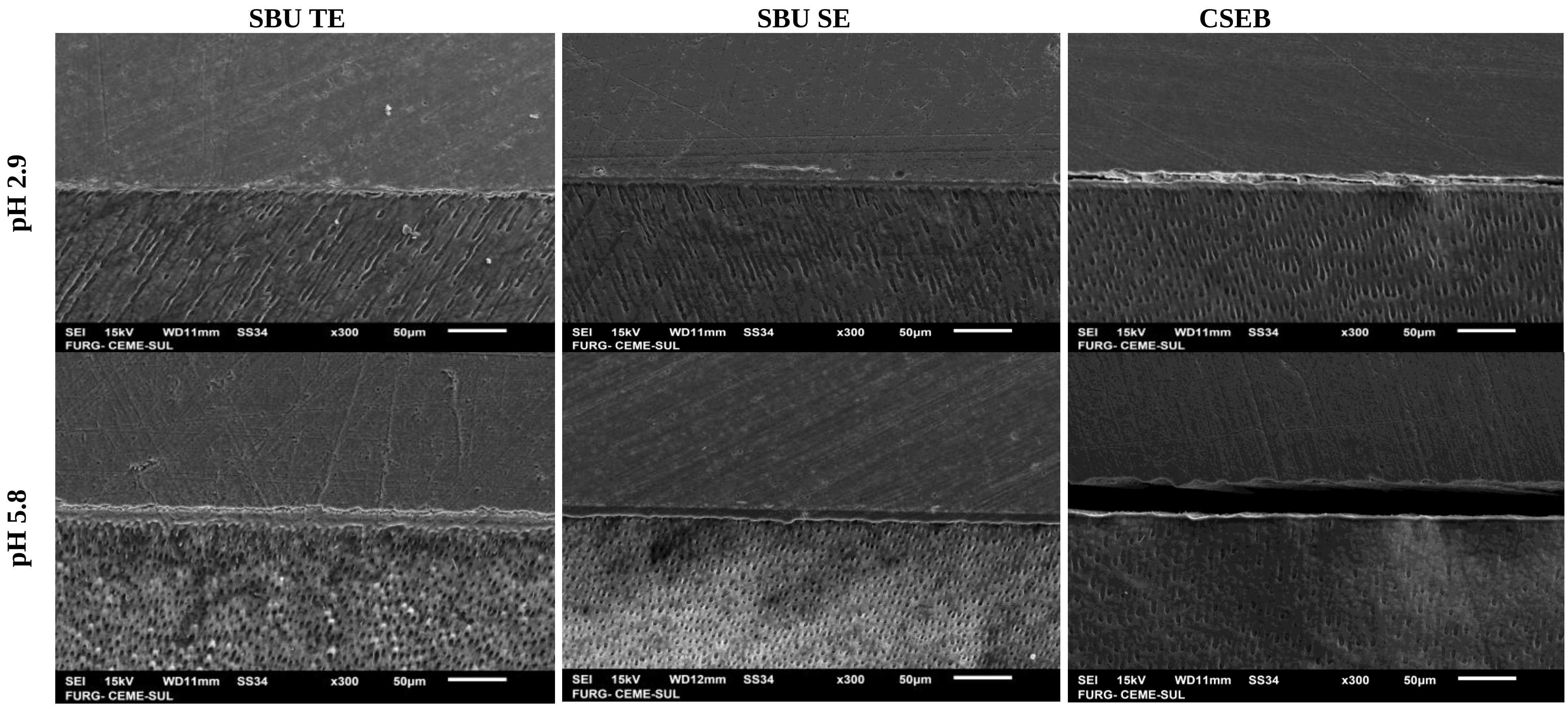

Figure 3: SEM images of interface of all groups

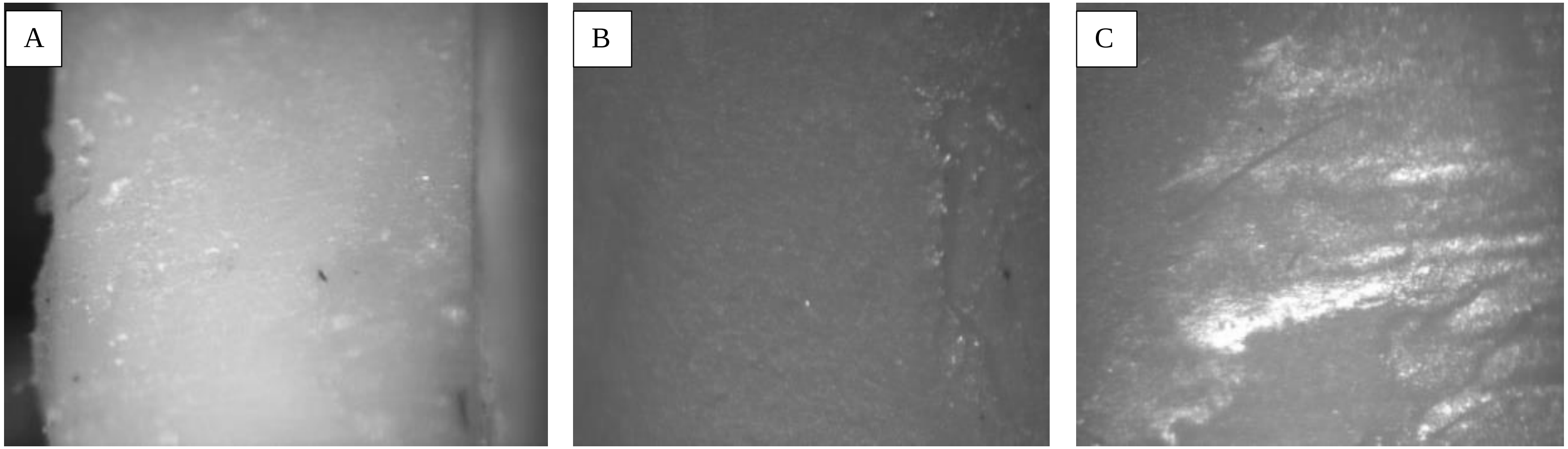

Figure 4: Images failures mode (x300 increase). Legends: A) Adhesive; B) Coesive in dentin and C) Coesive in resin.

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Reference to a journal publication:
[1] J. van der Geer, J.A.J. Hanraads, R.A. Lupton, The art of writing a scientific article, J. Sci. Commun. 163 (2010) 51–59.
Reference to a book:
[2] W. Strunk Jr., E.B. White, The Elements of Style, fourth ed., Longman, New York, 2000.
Reference to a chapter in an edited book:
[3] G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z. Smith (Eds.), Introduction to the Electronic Age, E-Publishing Inc., New York, 2009, pp. 281–304.
Reference to a website:
[4] Cancer Research UK, Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/, 2003 (accessed 13.03.03).

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The following list will be useful during the final checking of an article prior to sending it to the journal for review. Please consult this Guide for Authors for further details of any item.
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• E-mail address
• Full postal address
All necessary files have been uploaded, and contain:
• Keywords
• All figure captions
• All tables (including title, description, footnotes)
Further considerations
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ANEXO B – Aprovação Comitê de Ética:

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

Continuação do Parecer: 1.154.495

disponiveis, evite pendencias e agilize a tramitacao do seu projeto.

Conclusões ou Pendências e Lista de Inadequações:

O projeto não apresenta mais pendências.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

SANTA MARIA, 20 de Julho de 2015