UNIVERSIDADE FEDERAL DO CEARÁ
FACULDADE DE FARMÁCIA, ODONTOLOGIA E ENFERMAGEM.
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
DOUTORADO EM ODONTOLOGIA

FRANCISCO CLÁUDIO FERNANDES ALVES E SILVA

AVALIAÇÃO DO USO DO ETANOL COMO PRÉ-TRATAMENTO DA DENTINA NA RESISTÊNCIA DE UNIÃO DE UM ADESIVO SIMPLIFICADO DE CONDICIONAMENTO TOTAL

FORTALEZA
2014

FRANCISCO CLÁUDIO FERNANDES ALVES E SILVA

AVALIAÇÃO DO USO DO ETANOL COMO PRÉ-TRATAMENTO DA DENTINA NA RESISTÊNCIA DE UNIÃO DE UM ADESIVO SIMPLIFICADO DE CONDICIONAMENTO TOTAL

Tese apresentada ao Programa de Pós-Graduação em Odontologia da Faculdade de Farmácia, Odontologia e Enfermagem da Universidade Federal do Ceará, como requisito parcial para obtenção do Título de Doutor em Odontologia.

Área de Concentração: Clínica Odontológica

Orientador: Prof. Dr. Vicente de Paulo Aragão Saboia

FORTALEZA

2014

Dados Internacionais de Catalogação na Publicação
Universidade Federal do Ceará
Biblioteca de Ciências da Saúde

S58a Silva, Francisco Cláudio Fernandes Alves e.
Avaliação do uso do etanol como pré-tratamento da dentina na resistência de união de um adesivo simplificado de condicionamento total / Francisco Cláudio Fernandes Alves e Silva. – 2014.
69 f. : il.

Tese (Doutorado) - Universidade Federal do Ceará. Faculdade de Farmácia, Odontologia e Enfermagem. Programa de Pós-Graduação em Odontologia, Fortaleza, 2014.
Área de concentração: Clínica Odontológica.
Orientação: Prof. Dr. Vicente de Paulo Aragão Saboia.

1. Etanol. 2. Adesivos Dentinários. 3. Corantes. I. Título.

CDD 617.695

FRANCISCO CLÁUDIO FERNANDES ALVES E SILVA

AVALIAÇÃO DO USO DO ETANOL COMO PRÉ-TRATAMENTO DA DENTINA NA RESISTÊNCIA DE UNIÃO DE UM ADESIVO SIMPLIFICADO DE CONDICIONAMENTO TOTAL

Tese apresentada ao Programa de Pós-graduação em Odontologia da Faculdade de Farmácia, Odontologia e Enfermagem da Universidade Federal do Ceará, como requisito parcial para a obtenção do título de Doutor em Odontologia.

Área de concentração: Clínica Odontológica

Aprovada em 27/02/14

BANCA EXAMINADORA

Prof. Dr. Vicente de Paulo Aragão Saboia
Universidade Federal do Ceará - UFC

Prof. Dr. Flávio Augusto Pereira Gomes
Universidade de Fortaleza - UNIFOR

Profa. Dra. Márcia Rosa Sobreira Cavalcante
Universidade de Fortaleza - UNIFOR

Profa. Dra. Celiane Mary Carneiro Tapety
Universidade Federal do Ceará - UFC

Prof. Dr. Mário Áureo Gomes Moreira
Universidade Federal do Ceará - UFC

Dedico essa tese:

Ao meu pai (LUIS SILVA), que com sabedoria soube educar os filhos através de ações e atitudes, me deixando como herança o maior bem que um filho pode desejar: a educação e o nome.

À minha mãe (ADAISA), guerreira, amável e zelosa pelos filhos, sempre ao meu lado, mesmo à distância.

À minha querida e amada esposa (ANDRÉA), presente que Deus em um de seus dias mais inspirados, reservou-a para mim.

Aos meus filhos (ETHEL e SÁVIO), meu tesouro.

AGRADECIMENTOS

À DEUS, antes de tudo.

Ao professor Dr. VICENTE DE PAULO ARAGÃO SABOIA, porque além de ser meu orientador, amigo e colega de turma, é acima de tudo meu irmão. Obrigado Primo! Saiba que você é um dos grandes responsáveis por essa minha caminhada na pós-graduação.

Ao Dr. VICTOR PEREIRA FEITOSA, pela imensa e valiosa contribuição durante a escrita dos capítulos dessa tese.

Ao ex-coordenador do Programa de Pós-Graduação da Faculdade de Farmácia, Odontologia e Enfermagem (FFOE/UFC), Prof. Dr. SÉRGIO LIMA SANTIAGO e à atual coordenadora Profa. Dra. LIDIANY KARLA AZEVEDO RODRIGUES pelo apoio e abnegação à pesquisa odontológica.

Aos Profs. Drs. LIDIANY KARLA AZEVEDO RODRIGUES, MÔNICA YAMAUTI e VICTOR PEREIRA FEITOSA pela participação na banca de minha pré-defesa e pelas valiosas contribuição e sugestões ao meu trabalho.

A todos os professores do PPGO, pela convivência e ensinamentos durante o curso de Doutorado.

Aos meus colegas da turma de doutorado BRUNO CARVALHO DE VASCONCELOS, JULIANA PAIVA MARQUES LIMA ROLIM, MARY ANNE SAMPAIO DE MELO, PAULA GOES PINHEIRO, REBECA BASTOS ROCHA ARAÚJO e VANARA FLORÊNCIO PASSOS, pela boa convivência e troca de experiências.

Ao amigo JIOVANNE RABELO NERI, pela inestimável contribuição na estatística dos trabalhos realizados, além da agradável convivência no laboratório durante as pesquisas.

Ao Prof. e amigo EMMANUEL ARRAES, por ser responsável por recarregar meus níveis de alegria com seus papos agradáveis e alegres, sempre que me encontrava atarefado com os trabalhos laboratoriais.

Aos voluntários do estudo *in vivo*, meu respeito, minha admiração e gratidão.

Às funcionárias da Pós-Graduação em Odontologia da UFC, LÚCIA RIBEIRO MARQUES LUSTOSA e JANAINE MARQUES LEAL, pelo auxílio e disponibilidade.

Ao laboratório LAFICA - Laboratório de Farmacologia da Inflamação e do Câncer (Departamento de Morfologia - UFC), na pessoa da Profa. Dra. RENATA FERREIRA DE CARVALHO LEITÃO, pela inestimável ajuda na microscopia ótica.

À FUNCAP - Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico, pelo seu apoio financeiro através de bolsa de estudo, ajuda que foi fundamental na execução das pesquisas.

Aos meus familiares e amigos pela compreensão às muitas horas em que estive ausente para a dedicação ao estudo.

A todos que de maneira direta ou indireta ajudaram na concretização de mais uma etapa em minha vida.

Meus sinceros agradecimentos!

RESUMO

O objetivo desse estudo foi avaliar o efeito do pré-tratamento da dentina com etanol na resistência de união de um sistema adesivo simplificado de condicionamento total. Para isso, foram realizados estudos *in vitro* e *in vivo*. O primeiro estudo "Different concentrations of ethanol as dentin pre-treatment to bonding of an etch-and-rinse adhesive", avaliou a resistência de união à dentina com a aplicação de etanol anterior à aplicação de um adesivo simplificado de condicionamento total (Single Bond 2) e analisou as características morfológicas da interface resina-dentina. Materiais e Métodos: Vinte e quatro terceiros molares foram distribuídos aleatoriamente em quatro grupos (n=5) de acordo com a concentração de etanol: controle (sem etanol, técnica convencional), 50%, 70% e 100%. Nos grupos experimentais, o etanol foi aplicado durante 20 segundos. Em seguida o adesivo (Adper Single Bond 2) foi aplicado e platôs de resina composta foram construídos de forma incremental. Os dentes foram seccionados e submetidos ao teste de microtração (1 mm/min). ANOVA one-way e teste *post hoc* Tukey foram aplicados para analisar os dados estatísticos (α=0,05). Microscopia ótica (MO) utilizando coloração Tricrômio de Masson foi usado para observar as características das interfaces resina-dentina. Resultados: o grupo 50% não apresentou diferença do grupo controle ($p>0,05$). No entanto, os grupos 70% e 100% apresentaram um aumento na resistência de união ($p<0,001$ e $p=0,003$ respectivamente), sem diferença entre estes dois grupos. MO mostrou uma diminuição discreta de fibras colágenas expostas na interface adesiva dos espécimes dos grupos tratados com etanol 70% e 100%. Conclusão: O pré-tratamento da dentina utilizando etanol aumenta a resistência de união da interface resina-dentina quando aplicado em concentrações de 70% ou 100%. No segundo estudo "The use of ethanol as pre-treatment of dentin on bond strength of a two-step etch-and-rinse adhesive - an *in vivo* study", vinte terceiros molares sadios, em função oclusal e indicados para exodontia, foram divididos aleatoriamente em 4 grupos e tiveram cavidades Cl I preparadas e restauradas aplicando-se ou não etanol 70% por 20 s. Os dentes foram extraídos imediatamente (24 h) ou após 18 meses. Quatro dentes de cada grupo (n=4) foram submetidos ao teste de micro tração e um foi seccionado para análise em MO, utilizando a técnica de coloração Tricrômio de Masson. Análise estatística (ANOVA two way e Holm Sidak *post hoc*, α=0,05) mostrou que os grupos tratados sem etanol (24 h e 18 meses) e o grupo imediato tratado com etanol (24 h) apresentaram resistência de união semelhantes ($p>0,05$) e estatisticamente superiores ao grupo que teve tratamento com etanol e extraídos com 18 meses ($p<0,05$). A análise em MO mostrou áreas pigmentadas em vermelho,

indicando presença de fibras de colágeno expostas nos grupos imediatos (24 h) e a ausência dessas áreas nos grupos envelhecidos (18 meses), Já nos grupos tratados com etanol e extraídos após 18 meses foram observadas áreas sem pigmentação vermelha indicando a degradação do colágeno exposto. Conclusão: o uso de etanol 70% na dentina previamente a aplicação de um sistema adesivo de condicionamento total simplificado diminui a resistência de união.
Palavras-chave: Etanol. Adesivos Dentinários. Corantes.

ABSTRACT

The aim of this study was to evaluate the effect of pretreatment of dentin with ethanol on bond strenght (BS) of a two-step etch-and-rinse adhesive system and morphologically observe the hybrid layer (HL). And for that, two studies have been realized: one *in vitro* and other *in vivo*. In the first study, twenty four third molars were collected and randomly assigned into four groups (n=5) according to the ethanol concentration: control (traditional water-wet technique), 50vol.%, 70vol.% and 100vol.%. In experimental groups, ethanol was applied for 20 s. Bonding procedures (Adper Single Bond 2) were performed and resin composite was built up incrementally. Bonded teeth were sectioned into 0.8mm² sticks. The specimens were submitted to microtensile bond strenght test (μTBS). One-way ANOVA and *post hoc* Tukey's test were applied to analyze statistical the data (α=0.05). Light microscopy (LM) assisted by Masson's trichrome staining was used to observe the features of resin-dentin interfaces. Results: Control BS was not different from ethanol 50vol.% ($p>0.05$). However, the pre-treatment using ethanol 70vol.% and 100vol.% showed increase on BS ($p<0.001$ and $p=0.003$ respectively) with no difference between these two groups. LM showed a discrete decrease in denuded collagen fibrils to teeth treated with 70vol.% and 100vol.%. Conclusion: The pre-treatment of dentin using 70vol.% or 100vol.% ethanol may increase the initial BS of resin/dentin interface. In the second study, twenty healthy third molars, in function, and suitable for extraction were divided into 4 groups, Cl I cavities were prepared and restored applying or not 70% ethanol for 20 s. Teeth were collected immediately (24 h) or after 18 months. Four teeth of each group (n=4) were subjected to microtensile bond strenght (μTBS) and one tooth was observed under light microscopy (LM), using Masson's trichrome staining. Statistical analysis (ANOVA Two Way and Holm Sidak *post hoc*, $\alpha = 0.05$) showed that the groups treated without ethanol (24 h and 18 m) and the group treated with ethanol (24 h) showed similar BS ($p>0.05$) and statistically superior to the group treated with ethanol and extracted after 18 months ($p<0.05$). The LM analysis revealed a small amount of denuded collagen fibrils in immediate groups (24 h) and absence of these fibrils on aged groups (18 mos), while in the group treated with ethanol and extracted after 18 m, presented areas without pigmentation on HL. Conclusion: the use of 70vol.% ethanol on dentin prior to the adhesive system decreases the BS and increases the degradation of resin/dentin interface over time.

Keywords: Ethanol. Dentin-Bonding Agents. Coloring Agents.

SUMÁRIO

1 INTRODUÇÃO GERAL

O mecanismo de adesão amplamente utilizado na odontologia atual foi difundido pelo uso de materiais resinosos empregados com a técnica de condicionamento ácido (Buonocore, 1955). Desde então, vários estudos têm sido desenvolvidos com a finalidade de aperfeiçoar essa adesão do material restaurador ao substrato dentário (HASHIMOTO *et al*., 2000; Van MEERBEEK *et al*., 2003; DeMUNCK *et al.*, 2003; TAY; PASHLEY, 2003; PASHLEY *et al*., 2007). Embora a adesão ao esmalte apresente alto índice de sucesso clínico, na dentina ainda é considerada deficiente, principalmente a longo prazo, devido à heterogeneidade e à presença de água nesse tecido (PASHLEY *et al*., 2003).

A adesão ao dente é obtida através da infiltração de monômeros resinosos do sistema adesivo no tecido (esmalte/dentina) parcialmente desmineralizado pelo condicionamento ácido. Após a polimerização *in situ* da resina, essa camada de interdifusão entre material polimérico e tecido dental é denominada camada híbrida (CH) (NAKABAYASHI; KOJIMA; MASHUARA, 1982). A camada híbrida propicia resistência de união (RU) entre o material restaurador e o dente (TAY *et al*., 2008) que promove a adesão e durabilidade das restaurações. A água é responsável por manter as fibras colágenas (expostas após a desmineralização) expandidas para a correta infiltração dos monômeros e formação da camada híbrida. Entretanto, a água em excesso também impede a infiltração de monômeros mais hidrófobos e prejudica a polimerização da resina, facilitando a degradação da união ao longo do tempo (CARVALHO *et al*., 2003; De MUNCK *et al.*, 2003). Além disso, uma interface adesiva deficiente possui fibras colágenas não adequadamente envolvidas por resina, estando mais susceptíveis à degradação hidrolítica e enzimática (MAZZONI *et al*., 2006; GARCIA-GODOY *et al*., 2007). Portanto, na formação da camada híbrida, durante a aplicação de adesivos convencionais (com passo de condicionamento e lavagem separados), a remoção do excesso de água remanescente é considerada essencial para o sucesso do procedimento adesivo (TAY; PASHLEY, 2003).

Para auxiliar na remoção do excesso de água, facilitar a infiltração dos monômeros hidrofóbicos, melhorar a polimerização da resina e permitir a formação de uma camada híbrida mais homogênea, tem sido proposta a aplicação de etanol na dentina condicionada previamente à aplicação do adesivo, em uma técnica conhecida como “ethanol wet bonding” (PASHLEY *et al*., 2007). Essa técnica consiste na aplicação de concentrações crescentes de etanol, começando com a concentração de 50%, seguida de 70%, 80%, 95% e 100% (SADEK

et al., 2010; OSÓRIO *et al*., 2010). O aumento gradual na concentração de etanol é um passo necessário para prevenir o colapso das fibras colágenas e permitir a total troca da água por etanol, dentro da dentina desmineralizada (LIU *et al*., 2011; GUIMARÃES *et al*., 2012).

Os monômeros hidrofóbicos presentes nos adesivos são solúveis em etanol e os monômeros hidrofílicos são mais solúveis em água (SAURO *et al*., 2010). Dessa forma, é possível que esses adesivos possam apresentar melhor penetração em dentina desmineralizada e saturada com etanol devido à sua melhor solubilidade e consequente afinidade com o substrato saturado com etanol. Assim, a aplicação do etanol apresentaria dois mecanismos de ação para o aumento da infiltração dos monômeros na dentina desmineralizada: a remoção da água e o aumento da solubilidade destes resultando em maior penetração na dentina (PASHLEY *et al.*, 2007).

Os passos clínicos adicionais da técnica "ethanol-wet bonding" seriam justificados pela obtenção de um substrato dentinário com menos conteúdo de água e desta forma mais adequado à infiltração dos monômeros hidrofóbicos do adesivo, podendo resultar em valores de resistência de união superiores aos da técnica convencional e uma interface mais durável e resistente à degradação (HOSAKA *et al*., 2009; SADEK *et al*., 2010). Para essa técnica, diferentes tempos de saturação da dentina com etanol são descritas na literatura, variando de 20 s a 3,5 min (CARVALHO *et al*., 2003; PASHLEY *et al*., 2007; HOSAKA *et al*. 2009; SADEK *et al*., 2010; LIU *et al*., 2011). No entanto, essa técnica é considerada clinicamente inviável uma vez que acrescenta mais etapas, demanda mais tempo e torna a aplicação do adesivo mais sensível. Com a finalidade de deixa-la clinicamente viável, tem-se sugerido uma técnica simplificada, onde apenas uma aplicação de etanol a 100% por 60 segundos aplicado por 7 vezes demonstrou resultados promissores (LI *et al*., 2012; SADEK *et al*. 2010). No entanto, nosso trabalho sugere a aplicação de etanol a 70% por 20s, em virtude de resultados prévios terem demonstrado aumento de resistência de união utilizando essa técnica (vide Capítulo 1). Somando-se a isso a evaporação rápida do etanol a 100% pode não remover totalmente o excesso de água.

Devido à carência de estudos clínicos na literatura utilizando a técnica de saturação da dentina com etanol e considerando que o principal objetivo desta é permutar a água dentro da dentina desmineralizada pelos monômeros hidrofóbicos do adesivo, o presente estudo, baseado em resultados de testes laboratoriais prévios e com o objetivo de tornar a técnica clinicamente viável, ajustou esse passo clínico utilizando solução de etanol em uma concentração de 70% aplicado em passo único por 20 s.

2 PROPOSIÇÃO

Essa tese de doutorado será apresentada em capítulos, tendo como objetivos:

Capítulo 1 - Avaliar, *in vitro*, o efeito do pré-tratamento da dentina com etanol em diferentes concentrações na resistência de união de um adesivo simplificado de condicionamento total.

Capítulo 2 - Avaliar, *in vivo*, o efeito do pré-tratamento da dentina com etanol 70%, na resistência de união de um sistema adesivo simplificado de condicionamento total.

3 CAPÍTULOS

Esta tese está baseada no Artigo 46 do Regimento Interno do Programa de Pós-graduação em Odontologia da Universidade Federal do Ceará que regulamenta o formato alternativo para dissertações de Mestrado e teses de Doutorado e permite a inserção de artigos científicos de autoria ou coautoria do candidato. Por se tratar de pesquisas envolvendo seres humanos, ou partes deles, os projetos de pesquisa deste trabalho foram submetidos à apreciação do Comitê de Ética em Pesquisa da Universidade Federal do Ceará, tendo sido aprovados (Processo #107/10; Processo #108/10) anexos A e B. Assim sendo, esta tese é composta de dois capítulos contendo artigos que serão submetidos para publicação em periódicos científicos.

Capítulo 1:

"Avaliation of Different Concentrations of Ethanol as Dentin Pre-treatment on Bonding of an Etch-and-rinse Adhesive"

Francisco C.F.A. e Silva, Victor P. Feitosa, Vicente P.A. Saboia.

Esse artigo será submetido ao periódico "Journal of Esthetic and Restorative Dentistry".

Capítulo 2

"Ethanol as dentin pre-treatment on the bonding performance of a two-step etch-and-rinse adhesive - an *in vivo* study"

Francisco C.F.A. e Silva, Jiovanne R. Neri, Victor P. Feitosa, Vicente P.A. Saboia.

Esse artigo será submetido ao periódico "The Journal of Adhesive Dentistry".

3.1 Capítulo 1

Avaliation of Different Concentrations of Ethanol as Dentin Pre-treatment to Bonding of an Etch-and-rinse Adhesive

Francisco C. F. A. e Silva[1],

Victor P. Feitosa[1],

Vicente P. A. Saboia[1]

[1] Post graduate Program in Dentistry, Faculty of Pharmacy, Dentistry and Nursing, Federal University of Ceará, Brazil, w/n Cap Francisco Pedro Street - Rodolfo Teófilo, Zip Code: 60430-170, Phone- #558533668410, Fax- #558533668232

Correspondence: Francisco Cláudio Fernandes Alves e Silva (Email: claudiofernandes@me.com), Post-graduate Program in Dentistry, Faculty of Pharmacy, Dentistry and Nursing, Federal University of Ceará, Fortaleza, Ceará, Brazil. Rua Cap. Francisco Pedro - Zip Code: 60430-170, Phone- #558533668401/Fax- #558533668232

ABSTRACT

Objective: To evaluate the bond strenght (BS) to dentin pre-treated with ethanol in single application for 20 s after etching and before application of a two-step etch-and-rinse adhesive and to analyze the morphological features at the resin-dentin interface.

Materials and Methods: Twenty four third molars were collected and randomly assigned into four groups (n=5) according to the ethanol concentration: control (traditional water-wet technique), 50vol.%, 70vol.% and 100vol.%. In experimental groups, ethanol was applied for 20 s and gently air-dried for 5 s. Bonding procedures (Adper Single Bond 2) were performed and resin composite was built up incrementally. Bonded teeth were sectioned into 0.8mm² sticks. These specimens were submitted to microtensile bond strenght test (μTBS). One-way ANOVA and Tukey's *post hoc* test were applied to statistically analyze the data (α=0.05). Light microscopy (LM) assisted by Masson's trichrome staining was used to observe the features of resin-dentin interfaces. Results: Control BS was not different from ethanol 50vol.% ($p>0.05$). However, the pre-treatment using ethanol 70vol.% and 100vol.% showed increase on BS ($p<0.001$ and $p=0.003$ respectively) with no difference between these two groups. LM Showed a discrete decrease in denuded collagen fibrils to teeth treated with 70vol.% and 100vol.%. Conclusion: The dentin pre-treatment with ethanol may increase the initial BS of resin/dentin interface when using 70vol.% or 100vol.% ethanol. Ethanol in 70vol.% also is able to decrease the thickness of the resin-sparse collagen fibrils.

Keywords: dentin bonding; ethanol-saturated dentin, microtensile bond strenght.

INTRODUCTION

Resin-dentin bonds are less durable than resin-enamel bonds[1-3]. In fact, this is due to dentin bonding relies on organic components which are not remarkable in enamel bonding[4-8]. Even though the moisture after etching is essential for successful bonding using etch-and-rinse adhesives, it also affects the long-term bonding stability[9,10]. Although immediate bond strengths of contemporary adhesives have been acceptable and in some cases relatively high[6,7,11-13], substantial decreases occurred after several sorts of aging[2,8,14,15].

Resin-dentin bonding uses a partially demineralized dentin collagen matrix as the scaffold for resin infiltration, to produce a hybrid layer that couples the adhesives, the resin composite and the underlying mineralized dentin[8]. However, the permeation of resin monomers within exposed collagen fibrils is not complete[8,16] and one of the reasons to this occur is the presence of residual water from the etch-and-rinse technique[17]. Water is responsible for maintaining the collagen fibrils (exposed after demineralization) optimally expanded for the infiltration of the comonomer blend and formation of the hybrid layer[18]. Nevertheless, excessive water also impairs the infiltration of hydrophobic monomers and precludes the polymerization of the resin facilitating the degradation of the bond over time[5,9,19]. Furthermore, the resin-sparse collagen fibrils are more susceptible to hydrolytic and enzymatic degradation[20,21]. Therefore, the control of water content (dentin wetness) after etching is important to create a reliable resin-dentin bond[1,3,8,22,23].

In order to assist the removal of residual water and improve the infiltration of hydrophobic monomers into exposed collagen fibrils, as well as to allow the formation of a homogeneous hybrid layer, it has been proposed to saturate the etched dentin with ethanol prior to application of the two-step etch-and-rinse adhesives, the so-called "ethanol-wet bonding"[24]. This technique involves the application of a gradual and increasing concentration

of ethanol, starting with 50vol.%, 70vol.%, 80vol.%, 95vol.% and finally 100vol.%[24-28]. Such process is required to allow complete exchange of water by ethanol within the partially demineralized dentin[23,29]. These additional clinical steps are justified to obtain a more adequate infiltration of hydrophobic monomers of the adhesive[28] as well as less water content which could result in higher bond strength and more durable resin-dentin interfaces[25-27,30]. However, this technique is considered clinically unattainable as it is laborious, time consuming and technique sensitive. In an attempt to make the ethanol pre-treatment clinically feasible, an alternative and simplified technique has been suggested, in which seven applications of 100vol.% ethanol are conducted, showing promising results[25-27,31,32]. Although this simplified technique presented high bond strength, it is still time consuming and unattractive to be used clinically. It would be interesting to observe the effects of ethanol pre-treatment in a single application during a shorter period prior to the adhesive application, in order to make the technique clinically acceptable.

Therefore, the aim of this study was to evaluate the bond strength and morphological features of resin-dentin bonds using ethanol pre-treatment after the etching procedures by means of a single ethanol application for 20 s. The null hypothesis tested is that ethanol-saturated dentin does not interfere on BS.

MATERIALS AND METHODS

Tooth preparation

Twenty four recently extracted, caries-free human third molars were used after protocol was approved by the Research Ethics Committee Institution (Process #107/10). The teeth were stored in 0.01% thymol solution at 4°C and used within 2 months after extraction. After being copiously rinsed in running water, cleaned, and pumiced, tooth crowns were cut flat using a low-speed diamond saw under water irrigation (Isomet, Buehler; Lake Bluff,

USA), and a standardized smear layer was produced on the exposed coronal dentin using 600-grit wet silicon carbide paper mounted on a polishing machine (Aropol 2V – Arotec; São Paulo, SP, Brazil). The teeth were randomly assigned into four groups (n=5) according to the ethanol concentration: control (water), 50vol.%, 70vol.% and 100vol.%. Each tooth was etched with 35% phosphoric acid gel (Scotchbond Etchant, 3M ESPE; St Paul, MN, USA) for 15 s and rinsed thoroughly with distilled water for 15 s. The excess of water was removed from the surface with absorbent paper (Mellita; São Paulo, SP, Brazil). In experimental groups, the different concentrations of ethanol were applied for 20 s in a single application. Thereafter, the adhesive (Adper Single Bond 2 – M/ESPE) and resin composite (Z350 XT - 3M/ESPE St. Paul, MN, USA) applications were performed according to the manufacturer's instructions. Each resin composite increment was light cured for 20 s with 600 mW/cm^2, using a light-curing unit (XL 3000, 3M-ESPE). The details of all materials used are described in Table 1.

Microtensile bond strenght (μTBS)

Following storage in distilled water at 37 ºC for 24 h, each restored tooth was longitudinally sectioned in both "x" and "y" directions, across the bonded interface using a diamond blade (Isomet 1000, Buehler Ltd., Lake Bluff, USA) to obtain sticks with cross-sectional areas of approximately 0.8 mm^2. Each stick was measured with a digital caliper (Absolute Digimatic, Mitutoyo, Tokyo, Japan) and fixed to a test apparatus (Bencor Multi-T Device - Danville Engineering, San Ramon, CA, USA) using cyanoacrylate glue (Zapit, Dental Ventures of America, Corona, CA, USA) and stressed to failure with tensile force in a universal testing machine (Instron 4411, Canton, MA, USA) at a cross-head speed of 1 mm/min and data were collected in MPa. The bond strengths from same bonded tooth were averaged and the mean was used as a statistical unit. The fractured beams were analyzed

using a stereomicroscope (Stemi 2000-C, Carl Zeiss Jena GmbH, Germany) at 50x magnification and the failure mode classified as cohesive in dentin (CD), cohesive in resin (CR), adhesive (A) or mixed (M).

Statistical analysis

SPSS 17.0 (SPSS, Chicago, IL, USA) software was used to perform the statistical analysis. One-way ANOVA was applied to analyze the μTBS data (MPa) complemented by Tukey's multiple comparison *post hoc* test with significance level set at α=5%. Premature failures were noted but not included in the statistical analysis.

Light microscopy – Masson's trichrome

One tooth from each group was sectioned serial 1 mm thick resin-dentin slabs, which were fixed on a glass holder with cyanoacrylate glue (Super Bonder flex gel – Henkel Ltd., Düsseldorf, Germany) and polished with SiC papers on increasing grits size (800, 1000, 1200 and 2500) under running water (Buehler, Lake Bluff, IL, USA), reducing the slabs to about 150 μm in thickness. After polishing, the specimens were treated with Masson's trichrome staining technique, as previously described[33]. This staining technique has high affinity for cationic elements normally found in mineralized type I collagen, resulting in blue color. The acid etching of dentin causes the removal of these cationic elements and exposes collagen fibers showing a red pigmentation. These exposed collagen fibrils, showed by LM images, represented by a thin red colored layer at the HL, is called "red zone". Using this microscopic technique, lower incidence of red zones in the interface indicates less denuded collagen fibrils[5,11,12]. The composite resin usually stains in beige color. After all staining procedures, the specimens were covered with a glass coverslip and analyzed under light microscopy (LM)

at 400x magnification (Olympus BH-2, Tokyo, Japan). The evaluation of Masson's Trichrome was performed qualitatively.

RESULTS

Microtensile bond strenght - μTBS

One-way ANOVA test showed a statistically significant difference on BS between groups ($p<0.001$). Tukey multiple comparison *post hoc* test showed no difference between control and ethanol 50vol.% ($p>0.05$). Dentin pre-treatment with ethanol 70vol.% and 100vol.% showed statistically higher BS ($p<0.001$ and $p=0.003$, respectively), with no difference between these two treatments ($p>0.05$). There was no difference between dentin pre-treatment with ethanol 50vol.% and with water (control). Mixed failures (M) were the most common fracture pattern observed in all groups. Mean bond strength and failure mode distribution are summarized in Table 2.

Light microscopy

Representative light microscopy (LM) images of the adhesive interface showed presence of denuded collagen fibrils in all groups, demonstrated by red zones representing non-encapsulated/exposed collagen, able to react with the stain (Fig. 1 - arrows). A thicker red zone (resin-sparse collagen) can be seen along the whole adhesive interface of control and 50vol.% groups (Fig. 1A and 1B) suggesting less ability of these techniques in replacing the residual water by polymers. When dentin was treated with 70vol.% and 100vol.% ethanol, images showed less red zones indicating better infiltration of monomers during the hybrid layer formation (Fig. 1C and 1D).

DISCUSSION

In the present study, it could be observed that dentin pre-treatment using 50vol.% ethanol applied for 20 s showed no statistical significant difference on BS ($p>0.05$) in comparison with the traditional water-wet technique (control group). Additionaly, based on morphological features of the resin-dentin interfaces imaged by LM (Fig. 1A and 1B), we could suggest that the effectiveness in replacing water was the same for these groups. Contrariwise, 70vol.% and 100vol.% ethanol presented a significant increase on BS (Table 2) compared with control group ($p<0.05$). The null hypothesis was rejected once 70vol.% and 100vol.% ethanol increased BS despite 50vol.% presented similar BS to control (water). The microscopical survey showed that dentin pre-treatment using both 70vol.% and 100vol.% ethanol achieved higher resin infiltration than using 50vol.% ethanol or water-wet bonding, as demonstrated by the less amount of red zones (Fig. 1C and 1D respectively). These findings are not in agreement with those of Sadek *et al*. (2010), who afirm that 100vol.% ethanol applied for 30 s induced low bond strength. On the other hand, Hosaka *et al*. (2009) showed acceptable performance of 100vol.% ethanol appllied for 1 min.

Authors have reported that the interfibrillar spaces between collagen fibrils contain a hydrogel composed of proteoglycans[34,35]. It has been discussed that the presence of this hydrogel may interfere with comonomer infiltration during bonding[5,11,12]. However, the ethanol removes the water from these spaces, causing the hydrogel to collapse, and shrinks the dehydrated collagen fibrils in a greater extent than the overall shrinkage of the collagen mesh[16], thereby enlarging the interfibrillar spaces and allowing more resin infiltration resulting in a resin-dentin interface with less denuded collagen fibrils[30]. In the LM analysis, the dentin pre-treatment with 70vol.% and 100vol.% ethanol (Fig. 1C and 1D, respectively) presented less incidence of red zones than the control and 50vol.% ethanol (Fig. 1A and 1B, respectively). It may be suggested that the former groups achieved improved resin infiltration

and reduced resin-sparse collagen which could likely decrease the degradation of the resin-dentin interface.

Although ethanol-saturated dentin might be a better substrate for adhesive infiltration, it may be taking into account that ethanol evaporates faster than water, due to its higher vapor pressure[24]. Thus, the high volatility of 100vol.% ethanol and its rapid evaporation may lead collagen fibrils to collapse unless a continuous and time-consuming aplication is performed[24-27] which sometimes reduces the clinical feasibility of the technique. Although 70vol.% ethanol also has a relatively high volatility, the presence of water in its composition decreases its evaporation thereby facilitating the exchange of water and maintenance of the interfibrillar spaces. In fact, this procedure allows better infiltration of the resin comonomer blend[31]. However, in order to accomplish the optimal resin infiltration, the operator needs to avoid over-wet and over-dry situations which is easier to be controled using 70vol.% ethanol[24-27,31]. Furthermore, the water present in 70vol.% ethanol facilitates the passage of ethanol through the bacteria cell wall which is one of the well-known antibacterial properties of ethanol[36].

The hydrophobic monomers are more soluble in ethanol than in water[37], and most of the dental adhesives are currently ethanol/water solvated mixtures of different sorts of monomers. Nevertheless, the degradation of the resin-dentin interface is remarkably reduced using more hydrophobic monomers which have higher affinity to ethanol-saturated dentin[38]. Therefore, it is possible that most of current adhesives may exhibit enhanced penetration in ethanol-wet partially demineralized dentin matrix[38].

In an etched and rinsed dentin, the surface remains water-saturated preventing the collagen shrinkage, and after the experimental treatments the ethanol concentration would certainly diminish. In other words, the 50vol.% ethanol when in contact with this water-saturated dentin would be diluted (<50vol.% ethanol), leaving the partially demineralized dentin predominantly water-wet. The same dilution could occur with 70vol.% and 100vol.%

ethanol creating <70vol.% and <100vol.% ethanol concentrations in the substrate respectively. However, even after the dilution, these concentrations (70vol.% and 100vol.%) would likely saturate the partially demineralized dentin into a predominantly ethanol-wet surface due to the higher amount of ethanol. As previosly demonstrated[24], the overall solubility parameter of dental adhesive resins is higher in ethanol than in water. Therefore, the solubility and infiltration of the comonomer blend in a predominantly ethanol-wet substrate woud be improved. This may explain the outcomes observed in the present investigation for 70vol.% and 100vol.% ethanol pre-treatment (Table 2 and Fig. 1). Indeed, in 50vol.% ethanol pre-treated dentin, this solvent was not able to significantly improve the BS and resin infiltration due to the increasing in the overall water content.

Despite the limitations of this *in vitro* study, the simplified application of 70vol.% and 100vol.% ethanol prior to the application of a two-step etch-and-rinse adhesive showed to be effective in terms of initial dentin bonding performance. However, further investigations should be conducted to confirm the effectiveness of such dentin pre-treatments.

CONCLUSION

It may be concluded that dentin pre-treatment with ethanol increased the bond strength of the resin-dentin interface when 70vol.% or 100vol.% concentrations were used. Yet, both concentrations of ethanol decreased the incidence of resin-sparse collagen.

ACKNOWLEDGMENTS

This study was supported by FUNCAP (BMD-0008-00071.01.07/09).

REFERENCES

1) Tay FR, Pashley DH. Have dentin adhesives become too hydrophilic? J Can Dent Assoc 2003 Dec;69(11):726-731.
2) Vaidyanathan TK, Vaidyanathan J. Recent Advances in the theory and mechanism of adhesive resin bonding to dentin: a critical review. J Biomed Mater Res B Appl Biomater 2009 Feb;88(2):558-78.
3) Van Meerbeek B, De Munck J, Yoshida Y, *et al*.Buonocore Memorial Lecture. Adhesion to enamel and dentin: current status and future challenges. Oper Dent 2003 May-Jun;28(3):215-35.
4) Hashimoto M, Ohno H, Kaga M, *et al. In vivo* Degradation of resin-dentin bonds in humans over 1 to 3 Years. J Dent Res 2000 Jun;79(6):1385-91.
5) Carvalho RM, Mendonça JS, Santiago SL, *et al*.Effects of HEMA/solvent combinations on bond strength to dentin. J Dent Res 2003 Aug;82(8):597-601.
6) Loguercio AD, Reis A, Ballester RY. Polymerization Shrinkage: effects of constraint and filling technique in composite restorations. Dent Mater 2004 Mar;20(3):236-43.
7) Loguercio AD, Moura SK, Pellizaro A, *et al.* Durability of enamel bonding using two-step self-etch systems on ground and unground enamel. Oper Dent 2008 Jan-Feb;33(1):79-88.
8) Breschi L, Mazzoni A, Ruggeri A, *et al.* Dental adhesion review: aging and stability of the bonded interface. Dent Mater 2008 Jan;24(1):90-101.
9) De Munck J, Van Meerbeek B, Yoshida Y, *et al.* Four-year water degradation of total-etch adhesives bonded to dentin. J Dent Res 2003 Feb;82(2):136-40.
10) Garcia-Godoy F, Tay FR, Pashley DH, *et al.* Degradation of resin-bonded human dentin after 3 years of storage. Am J Dent 2007 Apr;20(2):109-13.

11) Nishitani Y, Yoshiyama M, Donnelly AM, *et al*. Effects of resin hydrophilicity on dentin bond strength. J Dent Res 2006a;114:160-66..

12) Nishitani Y, Yoshiyama M, Donnelly AM, *et al*. Effects of resin hydrophilicity on dentin bond strength. J Dent Res 2006b;85:1016-21.

13) Saboia VP, Silva FC, Nato F, *et al.* Analysis of differential artificial ageing of the adhesive produced by a two-step etch-and-rinse adhesive. Eur J Oral Sci 2009 Oct;117(5):618-24.

14) Tay FR, Pashley DH, Suh BI, *et al.* Single-step adhesives are permeable membranes. J Dent 2002 Sep-Nov;30(7-8):371-82.

15) Tay FR, Pashley DH. Water treeing - A potential mechanism for degradation of dentin adhesives. Am J Dent 2003 Feb;16(1):6-12.

16) Tay FR, Pashley DH, Kapura RR, *et al.* Bonding BisGMA to dentin - A proof of concept for hydrophobic dentin bonding. J Dent Res 2007 Nov;86(11):1034-39.

17) Toledano M, Osorio R, Osorio E, *et al.* Durability of resin-dentin bonds: effects of direct/indirect exposure and storage media. Dent Mater 2007 Jul;23(7):885–92.

18) Nakabayashi N, Nakamura M and Yasuda N. Hybrid layer as a dentin-bonding mechanism. J. Esthetic Dent 1991 Jul-Ago;3(4):133-38.

19) Sano H, Takatsu T, Ciucchi B, *et al.* Tensile properties of resin-infiltrated demineralized human dentin. J Dent Res 1995 Apr;74(4):1093-102.

20) Mazzoni A, Pashley DH, Nishitani Y, *et al.* Reactivation of inactivated endogenous proteolytic activities in phosphoric acid-etched dentin by etch-and-rinse adhesives. Biomaterials 2006 Sep;27(25):4470-76.

21) Garcia-Godoy F, Tay FR, Pashley DH, *et al.* Degradation of resin-bonded human dentin after 3 years of storage. Am J Dent 2007 Apr;20(2):109-113.

22) van Dijken JW, Sunnergardh-Grönberg K, Lindberg A. Clinical long-term retention of etch-and-rinse and self-etch adhesive systems in non-carious cervical lesions. A 13 years evaluation. Dent Mater 2007 Sep;23(9):1101-07.

23) Liu Y, Tjäderhane L, Breschi L, *et al.* Limitations in bonding to dentin and experimental strategies to prevent bond degradation. J Dent Res 2011 Aug;90(8):953-68.

24) Pashley DH, Tay FR, Carvalho RM, *et al.* From dry bonding to water-wet bonding to ethanol-wet bonding. A review of the interactions between dentin matrix and solvated resins using a macromodel of the hybrid layer. Am J Dent 2007 Feb;20(1):7-20.

25) Sadek FT, Pashley DH, Nishitani Y, *et al.* Application of the hydrophobic resin adhesive to acid-etched dentine with an alternative wet bonding technique. J Biomed Mater Res A. 2008 Jan;84(1):19-29.

26) Sadek FT, Castellan CS, Braga RR, *et al.* One-year stability of resin-dentin bonds created with a hydrophobic ethanol-wet bonding technique. Dent Mater 2010 Apr;26(4):380-86.

27) Sadek FT, Mazzoni A, Breschi L, *et al.* Six-month evaluation of adhesives interface created by a hydrophobic adhesive to acid-etched ethanol-wet bonded dentine with simplified dehydration protocols. J Dent 2010 Apr;38(4):276-83.

28) Osorio E, Toledano M, Aguilera FS, *et al.* Ethanol wet-bonding technique sensitivity assessed by AFM. J Dent Res 2010 Nov;89(11):1264-69

29) Guimarães LA, Almeida JCF, Wang L, *et al.* Effectiveness of immediate bonding of etch-and-rinse adhesives to simplified ethanol-saturated dentin. Braz Oral Res 2012 Mar-Apr;26(2):177-82.

30) Hosaka K, Nishitani Y. Tagami J, *et al.* Durability of resin-dentin bonds to water- vs. ethanol-saturated dentin. J Dent Res 2009 Feb;88(2):146-51.

31) Li F, Liub XY, Zhang L, *et al.* Ethanol-wet bonding technique may enhance the bonding performance of contemporary etch-and-rinse dental adhesives. J Adhes Dent 2012 Apr;14(2):113-20.

32) Huang X, Li L, Huang C, Du X. Effect of Ethanol-wet bonding with hydrophobic adhesive on caries-affected dentine. Eur J Oral Sci 2011 Aug;119(4):310-15.

33) Garcia Del Moral R. Coloraciones para fibras colagenas y elasticas del tejido conjuntivo, Coloraciones para sustancia amiloidea, Cap 10. p.175–8. In: Garcıa del Moral R, editor. Laboratorio de Anatomıa Patologica. Madrid: McGraw-Hill/Interamericana de España; 1993.

34) Embery G, Hall R, Waddington R, *et al.* Proteoglycans in dentinogenesis. Crit Rev Oral Biol Med 2001;12(4):331-349.

35) Pereira PN, Bedran-de-Castro AK, Durante WR, Yamauti M. Removal of noncollagenous components affects dentin bonding. J Biomed Mater Res B: Appl Biomater 2007 Jan;80(1):86-91.

36) Chambers ST, Peddie B, Pithie A. Ethanol disinfection of plastic-adherent micro-organisms. J Hosp Infect. 2006 Jun;63(2):193-6.

37) Cadenaro M, Breschi L, Rueggeberg FA, *et al.* Effect of adhesive hydrophilicity and curing time on the permeability of resins bonded to water vs. ethanol- saturated acid-etched dentin. Dent Mater 2009 Jan;25(1):39-47.

38) Sauro S, Toledano M, Aguilera FS, *et al.* Resin-dentin bonds to EDTA-treated vs. acid- etched dentin using ethanol wet-bonding. Dent Mater 2010 Apr;26(4):368-79.

Table 1. Materials used in the study

Comercial brand	Main components	Mode of application	Manufacturer
Adper Single Bond 2	Ethanol, bis-GMA, HEMA, GDMA, polycarboxylic acid copolymer, UDMA, water ter, CQ, EDMAB, DPIHFP	Apply two layers of the adhesive resin, gently air-stream for 5 s and light-cure for 10 s	3M/ESPE
Scotchbond Etchant	35% phosphoric acid, water, silica	Apply a layer for 15 s and wash for 15 s.	3M/ESPE
Filtek Z350 XT	-Bis-GMA, UDMA, Bis-EMA, TEGDMA resins, zirconium, silica	Apply increments of 2 mm and light-cure for 20 s.	3M/ESPE

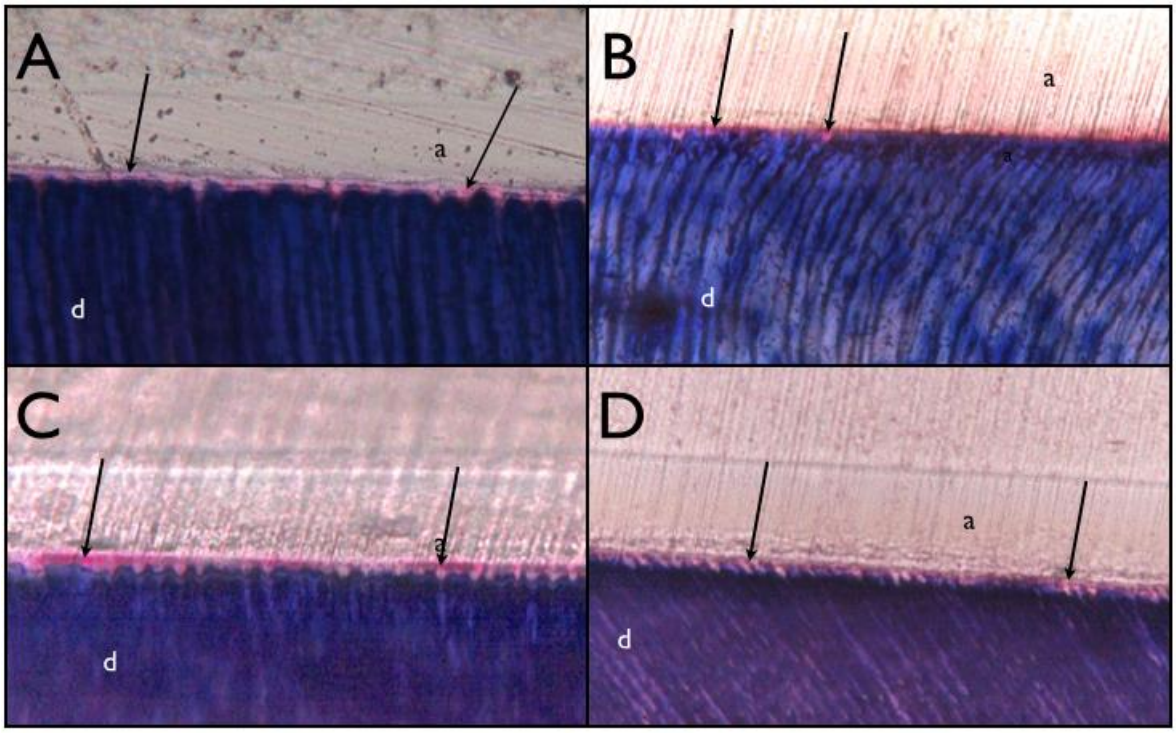
Table 2. Bond strenght and Distribution of Fracture Modes

Dentin Pre-Treatment	**μTBS***	**Failure mode - %****			
		CD	CR	M	A
Water (control)	*34,8 ± 9,8 (45)* ^{b}	9	17	65	9
50vol.%	*36,1 ± 9,4 (59)* ^{b}	12	15	62	11
70vol.%	*41,5 ± 8,8 (53)* ^{a}	10	12	71	7
100vol.%	*40,9 ± 8,4 (57)* ^{a}	13	11	69	7

***** Bond Strength values are means ± Standard Deviations (beams/group). Different superscripts indicate statistical diferences ($p<0.05$).

******Failure modes pattern: CD - cohesive in dentin; CR - cohesive in resin; M - mixed; A - adhesive.

Fig. 1 - Light micrographs of Masson's Trichrome of resin-dentin interface



A) Control - water; B) 50vol.% ethanol; C) 70vol.% ethanol; D) 100vol.% ethanol. a - adhesive; d - dentin. Arrow - red zone layer indicating denuded and unprotected collagen fibrils.

3.2 Capítulo 2

Ethanol as dentin pre-treatment on the bonding performance of a two-step etch-and-rinse adhesive - an *in vivo* study

Francisco C. F. A. e Silva[1],

Jiovanne R. Neri[1]

Victor P. Feitosa[1],

Vicente P. A. Saboia[2]

[1] PhD Student, Faculty of Pharmacy, Dentistry and Nursing, Federal University of Ceará, Fortaleza, Ceará, Brazil.

[2] Associate Professor, Department of Restorative Dentistry, Faculty of Pharmacy, Dentistry and Nursing, Federal University of Ceará, Fortaleza, Ceará, Brazil. Idea, experimental design, co-wrote manuscript.

Correspondence: Francisco Cláudio Fernandes Alves e Silva (Email: claudiofernandes@me.com), Post-graduate Program in Dentistry, Faculty of Pharmacy, Dentistry and Nursing, Federal University of Ceará, Fortaleza, Ceará, Brazil. Rua Cap. Francisco Pedro - Zip Code: 60430-170, Phone- #558533668401/Fax- #558533668232

ABSTRACT

Purpose: To evaluate the use of 70vol.% ethanol as a dentin pre-treatment on the bond strenght (BS) of a two-step etch-and-rinse adhesive.

Materials and Methods: Resin composite Class I restorations were clinically bonded to acid-etched dentin of human sound third molars using Adper Single Bond 2 (SB, 3M-ESPE) and randomly divided into two major groups: dentin saturated with water (control) or 70vol.% ethanol (ethanol). The teeth were divided into two subgroups: immediately extracted and tested after 24h, and extraction after 18 months. Bonded-teeth (Adper Single bond 2) were cut into resin-dentin sticks which were tested by microtensile bond strength. The failure mode was thereafter evaluated. Data were statistically analyzed using two-way ANOVA and Holm-Sidak *post hoc* test ($\alpha = 0.05$). Additional bonded resin-dentin slabs from each group were examined under light microscopy (LM) using Masson's trichrome staining technique.

Results: The lowest BS was obtained by ethanol pre-treated dentin after aging whilst other groups presented similar BS. The LM analysis showed presence of resin-sparse collagen fibrils in groups examined immediately (24 h) and the presence of several gaps due to collagen degradation at the interfaces of ethanol pre-treated aged specimens (18 mos).

Conclusion: The use of 70vol.% ethanol in dentin prior to the application of a two-step etch-and-rinse adhesive should be avoided once it resulted in a significant drop on the bond strength.

Keywords: bond strength, ethanol wet-bonding, hybrid layer.

INTRODUCTION

The current concept of adhesion to dentin relies on the infiltration of monomers from the adhesive resin within the etched dentin tissue (collagen mesh), in order to allow the formation of the so-called hybrid layer.[29] The hybrid layer is considered the foremost factor to achieve high bond strength (BS) between the restorative composite and the dentin.[5,43] During this process, water is responsible for maintaining the collagen fibrils expanded which allows resin penetration and formation of hybrid layer after the subsequent polymerization of the resin.[12,29] Although water has been important in this bonding mechanism, especially using etch-and-rinse adhesives, it is also associated with the degradation of the resin-dentin bonds.[5,9,12,19] In order to simplify the application of dentin adhesives, the two-step etch-and-rinse and one-step self-etch adhesives were developed. However, they are intrinsically more hydrophilic than the multi-step versions. In fact, such hydrophilic polymers result in significant water sorption [23,27], which also cause the decrease of mechanical properties. Additionally, acid-etching exposes and activates matrix metalloproteinases (MMPs) in the dentin which are able to accelerate the collagen degradation promoted by water.[25,30,43] The residual water entrapped surrounding the collagen fibrils may impair the diffusion of more hydrophobic monomers [36,42,44] potentially contributing to a rapid resin-dentin interfacial degradation.[7,48] Thus, the residual water must be removed as much as possible to attain an optimal resin infiltration.[47] Solvents such as acetone and ethanol are added to the adhesive resin blend to decrease its viscosity as well as to facilitate the evaporation of water from the interfibrilar spaces, thereby contributing to the formation of the hybrid layer.[12] Unfortunately, the complete removal of this water is unattainable in user-friendly protocols.

In a clinical attempt to dehydrate the exposed collagen fibrils, a simplified dehydration protocol applying 100vol.% ethanol three times for 1 min prior to the adhesive application

was tested.[24] Although this technique seems promising, it is still time-consuming and ethanol applied at this concentration may not be totally effective due to its fast evaporation.[40]

The use of 70vol.% ethanol as a dentin pre-treatment could be useful to reduce the overall residual water content to improve the more hydrophobic monomers infiltration (Li *et al.*, 2012) and potentially to attain antimicrobial properties (Chambers *et al.*, 2006).

Therefore, the aim of this study was to evaluate clinically the effect of such dentin pre-treatment adjunctively used with a two-step etch-and-rinse adhesive on the dentin bond strength and interfacial morphology. The null hypothesis to be tested was that 70vol.% ethanol dentin pre-treatment does not interfere on the bonding performance of a two-step etch-and-rinse adhesive.

MATERIALS AND METHODS

This research protocol was approved by the appropriate institutional Research Ethics Committee (Process #108/10). Eight volunteers of both genders, with ages from 18 - 30 years, who presented all four sound third molars, erupted, in function and orthodontically scheduled for extraction were selected. After all patients had signed an informed consent form, the clinical procedures were carried out.

Bonding procedures

From the same patient, four teeth were randomly assigned to receive a restorative adhesive procedure. After acid etching, 70vol.% ethanol was applied an two teeth

(experimental group) and in the control group (two teeth) ethanol was not applied. From each treatment, one tooth was extracted immediately (24 h) and another tooth extracted at 18 months. The same clinical procedures were performed for all teeth: local anesthesia using anesthetics without vasoconstrictor (MEPISV 3% - DFL, Rio de Janeiro, Brazil); rubber dam isolation; and Class I cavity preparation for composite resin, with continuous enamel cavosurface margins using diamond burs #3131 (KG Sorensen, São Paulo, Brazil) under abundant water cooling. In order to obtain standardization, the depth of the cavities was controlled using the half-length of the diamond burs as reference (4 mm) in the central sulcus following the long axis of the teeth, and the width was controlled by the inter-cuspal distance. The teeth were radiographically examined prior cavities preparation to avoid the use of teeth with morphological defects or caries. All restorative procedures were performed by a single trained operator. Cavities were etched with 35% phosphoric acid (Scotchbond Etchant; 3M-ESPE, St. Paul, USA) for 15 s and thoroughly rinsed with water for 15 s. The excess of water was removed with absorbent papers (Mellita; São Paulo, Brazil) according to the etch-and-rinse technique. Afterwards, the experimental groups were saturated with 70vol.% ethanol for 20 s. In all groups, the two-step etch-and-rinse adhesive Adper Single Bond 2 (3M/ ESPE, St. Paul, USA) was applied to the cavity walls and light-activated with an halogen lamp for 10 s (Optilux 500; Kerr, Danbury, USA) at 600 mW/cm^2, as periodically controlled by a radiometer (Demetron; Kerr). The build-ups were constructed with a resin composite shade A2 (Filtek Z350 XT; 3M/ESPE, St. Paul, USA) in 1mm-thick increments light-activated for 20 s each. The commercial brand, components, mode of application, and manufacturers of the materials used in the study are presented in Table 1.

The volunteers were informed about oral hygiene and teeth were periodically followed up clinically and radiographically.

Microtensile bond strenght (μTBS)

Teeth were collected, using minimal traumatic technique of extraction in order to avoid damages to dental structures. Bonded-teeth were cut into beam-shaped specimens with a cross-sectional area of approximately 0.8 mm^2 using a slow-speed water-cooled diamond saw (Isomet 1000, Buehler. Lake Bluff, USA). Each beam was measured with a digital caliper (Absolute Digimatic, Mitutoyo, Tokyo, Japan), fixed to a test apparatus (Bencor Multi-T Device - Danville Engineering, San Ramon, USA) using cyanoacrylate glue (Zapit, Dental Ventures of America, Corona, USA), submitted to microtensile bond strength test in a universal testing machine (Instron 4411, Canton, USA), stressed until failure with a tensile force at a speed of 1 mm/min, data were collected in MPa. The data (MPa) attained from the beams of the same resin-bonded tooth were averaged and the mean bond strength was used as one unit for statistical analysis. Immediately after testing, the debonded beams were dried and stored at room temperature until analysis of the fracture pattern using a stereomicroscope (Stemi 2000-C, Carl Zeiss Jena GmbH, Germany) at 50X magnification. Failure mode was classified as cohesive failure in dentin (CD), cohesive in resin (CR), adhesive (A) or mixed failure (M).

Statistical Analysis

SPSS 17.0 (SPSS, Chicago, IL, USA) software was used to perform the statistical analysis. A two-way ANOVA was applied to the μTBS data to analyze the factors “dentin pre-treatment” (water *vs.* ethanol) and “aging” (24 h *vs.* 18 mos), complemented by Holm-Sidak multiple comparison *post hoc* test ($p<0.05$). The significance level was set at $\alpha=0.05$

and statistical unit was tooth (n=4). Premature failures were noted, but not included in the data analysis.

Light Microscopy – Masson's trichrome

One tooth from each group was sectioned in only one direction to obtain 1 mm thick dentin-resin slabs, which were fixed on a glass holder with cyanoacrylate glue (Super Bonder flex gel – Henkel Ltd., Düsseldorf, Germany) and polished with SiC papers on increasing fine grits (800, 1000, 1200 and 2500) under running water (Buehler, Lake Bluff, IL, USA) reducing the slabs to about 150 µm in thickness. After polishing, the specimens were treated with Masson's trichrome staining technique as previously described.[15] This staining technique has high affinity for cationic elements normally found in mineralized type I collagen, resulting in blue color. The acid etching of dentin causes the removal of these cationic elements and exposes collagen fibers showing a red pigmentation. These exposed collagen fibrils, showed in the LM images, represented by a thin red colored layer at the HL, is called "red zone". Using this microscopic technique, lower incidence of red zones at the interface indicates less denuded collagen fibrils.[9,30] The composite resin usually stains in beige color. After all staining procedures, the specimens were covered with a glass coverslip and analyzed under light microscopy (LM) at 400x magnification (Olympus BH-2, Tokyo, Japan). The evaluation of Masson's Trichrome was performed qualitatively.

RESULTS

Microtensile bond strenght (μTBS)

Two-way ANOVA test showed there is a statistically significant interaction between treatment and aging ($p=0.008$). Holm-Sidak multiple comparison *post hoc* test showed that the bond strenght was not affected by ethanol pre-treatment 24 h after restoration ($p=0.430$). After 18 months, no bond strenght reduction was observed in water-saturated dentin ($p=0.096$), but a significant drop in bond strength was attained using ethanol pre-treatment ($p<0.001$), with a statistically significant difference between the two dentin treatments after aging ($p<0.001$). Mixed failures (M) were the most common fracture pattern observed in all groups. In the ethanol groups, adhesive failures (A) were three times more frequent after 18 months than in 24 h. In the control groups, this failure pattern remained predominant. Mean bond strengths, standard deviations and failure mode distribution are summarized in Table 2.

Light Microscopy – Masson's trichrome

LM showed resin-sparse collagen fibrils within the resin-dentin interfaces in immediate groups, demonstrated by the red zones (Fig. 1A and 1C), with greater intensity in the group treated with ethanol. For the aged groups (18 months), the group treated with water showed some isolated, discrete and less colored red zones at the bonded interface and white zone suggesting the absence of either polymers or collagen fibrils which were likely degraded (Fig. 1B), while the group treated with ethanol showed no red zone but white zone (Fig. 1D).

DISCUSSION

Based on the outcomes aforementioned, the null hypothesis was rejected once ethanol pre-treated dentin showed significant decrease on the bond strength after 18 months in clinical function.

The residual water in the dentin matrix and the hydrophilic domains of contemporary dental adhesives make the hybrid layer behave as a semi-permeable membrane, which permits water permeation through the bonded interface even after polymerization.[20,43] By dentin acid-etching, the etchant dissolves interfibrilar apatite crystallites and exposes the collagen, creating spaces between and inside the collagen fibrils.[48] Several authors have reported the presence of a hydrogel composed of proteoglycans in these spaces.[13,17,32,35,46] Studies affirm that the presence of this hydrogel may interfere with monomer infiltration during bonding and the removal of water from these spaces result in hydrogel collapse.[4,6,23] The solvated resins of contemporary two-step etch-and-rinse adhesives do not remove all the residual water from interfibrilar spaces[5,18,26] leaving small amounts of water into partially demineralized dentin which may hinder the hydrophobic comonomer blend to optimally coat the exposed collagen.[16,18] Based on the solubility parameters theory of Hansen,[1,3,28] ethanol is miscible with both hydrophobic monomers and water, which makes this substance an appropriate alternative to facilitate the penetration of hydrophobic monomers into a water-wet substrate. The so-called ethanol-wet bonding technique relies on filling spaces between the fibrils with ethanol,[34] thereby replacing all water in the partially demineralized dentin by ethanol. It may permit hydrophobic comonomer blend to infiltrate the spaces along the etched substrate properly, providing less resin-sparse collagen and consequently durable resin-dentin bonds.[31,34,40].

Experiments with different concentrations of ethanol have been applied in a simplified protocol, using also 70vol.% and 100vol.%.[24,31,34,41] However, when a simplified protocol is applied using ethanol at high concentration (*i.e* 100vol.%), the replacement of water may not be totally efficient to promote a completely saturated substrate, resulting in relatively low infiltration of hydrophobic monomers.[21,30,33] A suitable explanation for this occurrence could be the high vapor pressure of 100vol.% ethanol which is almost three times higher than that of water.[34] In spite of 70vol.% ethanol has a relatively high volatility, the presence of water in its composition decreases its evaporation time and vapor pressure in comparison with 100vol.%, thereby facilitating the exchange of water and the maintenance of interfibrillar spaces.[24] Yet, water facilitates the passage of ethanol through the bacteria cell wall which attains its well-known antibacterial properties.[11] Moreover, 70vol.% ethanol dentin pre-treatment showed a significant increase on the initial bond strength of Adper Single Bond 2 (unpublished observations, 2013). Therefore, 70vol.% ethanol could be a feasible alternative as a dentin pre-treatment as used in this study.

In spite of using ethanol, our study did not apply the ethanol-wet-bonding technique but rather a simple protocol (70vol.% ethanol for 20 s) of dentin pre-treatment to advocate the well-known characteristics of ethanol. The immediate results were promising, showing ethanol with similar bond strength of the control group. Nevertheless, the amount of red zone (resin-sparse collagen) was lower in water-treated group (Fig. 1A). However, when the ethanol pre-treatment was used in such an adverse situation as clinical function ageing (18 months) the results was discouraging once the bond strength strikingly decreased and the resin-dentin interface was severely degraded (Fig. 1D). The negative outcomes of ethanol pre-treated aged group could be explained by the ineffectiveness of 70vol.% ethanol to remove all residual water from interfibrilar spaces. By the contrary, the amount of water in its composition may have increased the moisture of the partially demineralized substrate

impairing an efficient coating of exposed collagen fibrils by resin (represented by the increase in the red zones, Fig. 1C) and jeopardizing the solvent evaporation.

Our findings are in accordance with those of Huang *et al*.[22] and Barros *et al*.[2] who found a decrease on the bond strength and significant collagen degradation in specimens pre-treated with 100vol.% ethanol and submitted to thermocycling or immersion in NaOCl. Conversely, Carvalho *et al*.,[8] Hosaka *et al*.,[21] and Pashley *et al*.[34] showed a strong increase on bond strength using the ethanol-wet bonding technique. Although Pashley's *in vitro* study (2007)[34] had been performed using a macro-model of hybrid layer, differently from an *in vivo* study in which some variables are difficult to control such as thickness of the smear layer, cavity preparation, dentin moisture, intrapulpal pressure.[14,38] However, in a clinical situation, resin-dentin interfaces are only partially in contact with environmental fluids, since outer resin-bonded enamel has been shown to prevent water uptake.[8,14,37] In such circumstances, these resin-dentin bonds may come in contact with fluids *in vivo* only via pulpal pressure through dentinal tubules (Pashley, 1998)[33] or by residual water in etched and rinsed dentin.[8]

White spaces found in the resin-dentin interfacial microscopies after ageing where there was the red zone staining (Fig. 1B and 1D) suggest the breakdown of resin-infiltrated dentin[14] and/or collagenolytic degradation which may be governed by host-derived factors, such as the action of endogenous collagenolytic enzymes on partially exposed collagen fibrils.[7] Nevertheless, the ethanol pre-treatment resulted in a significant decrease on bond strength in the long-term which was not observed using water (control). Furthermore, the ethanol aged group (18 months) showed an increase in adhesive failures (Table 2) when compared with ethanol immediate (24 h) which is another indication of resin-dentin degradation. It may be suggested that the disappearance of resin-sparse collagen layer, as aforementioned and the appearance of gaps in the hybrid layer may have contributed to achieving this lower bond strength (Table 1). However, the correlation between the decrease on bond strength and these

gaps at the resin-dentin interface is complex and still unclear as discussed in previous publication.[5]

Finally, further research is required to explain the many possible reasons for the degradation promoted by 70vol.% ethanol dentin pre-treatment.

CONCLUSION

It can be concluded that the use of 70vol.% ethanol in dentin after phosphoric acid etching and water rinsing, prior to the application of a two-step etch-and-rinse adhesive decreases the bond strength and increases the resin-dentin interfacial degradation *in vivo*. The proposed dentin pre-treatment using ethanol was not clinically successful.

ACKNOWLEDGMENTS

This work was supported by FUNCAP (BMD-0008-00071.01.07/10).

REFERENCES

1. Asmussen E, Unu S. Solubility parameters, fractional polarities, and bond strengths of some intermediary resins used in dentin bonding. J Dent Res 1993 Mar;72(3):558-65.
2. Barros LO, Magalhães A, Loguercio AD, Saboia VPA. Resin-dentin bonds of etch-and-rinse adhesives to alcohol-saturated acid-etched dentin. J Adhes Dent 2013 Aug;15(4):333-40.
3. Barton AFM. Handbook of solubility parameters and other cohesion parameters. CRC Press: Boca Raton, 1991; 69-156.
4. Bertassoni LE, Orgel JPR, Antipova O, Swain MV. The dentin organic matrix-limitations of restorative dentistry hidden on the nanometer scale. Acta Biomater 2012;8(7):2419–33.
5. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, De Stefano Dorigo E. Dental adhesion review: Aging and stability of the bonded interface. Dent Mater 2008 Jan;24(1):90-101.
6. Cadenaro M, Breschi L, Rueggeberg FA, Agee K, Di Lenarda R, Carrilho M, *et al*. Effect of adhesive hydrophilicity and curing time on the permeability of resins bonded to water vs. ethanol-saturated acid-etched dentin Dent Mater 2009 Jan;25(1):39-47.
7. Carrilho MRO, Geraldeli F, Tay F, De Goes MF, Carvalho RM, Tjäderhane L, *et al*. *In vivo* preservation of the hybrid layer by chlorhexidine. J Dent Res 2007 Jun;86(6):529-33.
8. Carvalho CA, Cantoro A, Mazzoni A, Goracci OC, Breschi L, Ferrari M. Effect of ethanol application on post-luting to intraradicular dentine. Int Endod J 2009 Feb;42(2): 129–35.

9. Carvalho RM, Mendonça JS, Santiago SL, Silveira RR, Garcia FCP, Tay FR, *et al*. Effects of HEMA/solvent combinations on bond strength to dentin. J Dent Res 2003 Aug;82(8):597-601.
10. Carvalho RM, Manso AP, Geraldeli S, Tay FR, Pashley DH. Durability of bonds and clinical success of adhesive restorations. Dent Mater 2012 Jan;28(1):72–86.
11. Chambers ST, Peddie B, Pithie A. Ethanol disinfection of plastic-adherent micro-organisms. J Hosp Infect. 2006;63(2):193-6.
12. De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, Suzuki K, *et al*. Four-year water degradation of total-etch adhesives bonded to dentin. J Dent Res 2003 Feb;82(2):136-140.
13. Embery G, Hall R, Waddington R, Septier D, Goldberg M. Proteoglycans in dentinogenesis. Crit Rev Oral Biol Med 2001;12(4):331-349.
14. Feitosa VP, Leme AA, Sauro S, Correr-Sobrinho L, Watson TF, Sinhoreti MA, *et al*. Hydrolytic degradation of the resin–dentine interface induced by the simulated pulpal pressure, direct and indirect water ageing. J Dent 2012 Dec;40(12):1134–43.
15. Garcia Del Moral R. Coloraciones para fibras colagenas y elasticas del tejido conjuntivo, Coloraciones para sustancia amiloidea, Cap 10. p.175–8. In: Garcıa del Moral R, editor. Laboratorio de Anatomıa Patologica. Madrid: McGraw-Hill/Interamericana de España; 1993.
16. Garcia-Godoy F, Tay FR, Pashley DH, Feilzer A, Tjäderhane L, Pashley EL. Degradation of resin-bonded human dentin after 3 years of storage. Am J Dent 2007 Apr;20(2):109-13.
17. Goldberg M, Takagi M. Dentine proteoglycans: composition, ultrastructure and functions. Histochem J 1983 Nov;25(11):781-806.

18. Guo X, Spencer P, Wang Y, Ye Q, Yao X, Williams K. Effects of a solubility enhancer on penetration of hydrophobic component in model adhesives into wet demineralized dentin. Dent Mater 2007 Dec;23(12):1473-83.
19. Hashimoto M, Ohno H, Kaga M, Endo K, Sano H, Oguchi H. *In vivo* degradation of resin-dentin bonds in humans over 1 to 3 years. J Dent Res 2000 Jun;79(6):1385-1391.
20. Hashimoto M, Ito S, Tay FR, Svierzo NR, Sano H. Fluid Movement across the Resin-Dentin Interface during and after Bonding. J Dent Res 2004;83(11):843-48.
21. Hosaka K, Nishitani Y. Tagami J, Yoshiyama M. Brackett WW, Agee KA, *et al.* Durability of resin-dentin bonds to water- vs. ethanol-saturated dentin. J Dent Res 2009 Feb;88(2):146-51.
22. Huang X, Li L, Huang C, Du X. Effect of ethanol-wet bonding with hydrophobic adhesive on caries-affected dentine. Eur J Oral Sci 2011 Aug;119(4):310-15.
23. Ito S, Hashimoto M, Wadgaonkar B, Svizero N, Carvalho RM, Yiu C, *et al.* Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity. Biomaterials 2005 Nov;26(33):6449-59.
24. Li F, Liu XY, Zhang L, Kang JJ, Chen JH. Ethanol-wet bonding technique may enhance the bonding performance of contemporary etch-and-rinse. J Adhes Dent. 2012 Apr;14(2):113–20.
25. Loguercio AD, Uceda-Gomez N, Carrilho MR, Reis A. Influence of specimen size and regional variation on long-term resin-dentin bond strength. Dent Mater 2005 Mar;21(3):224-231.
26. Loguercio AD, Reis A, Ballester RY. Polymerization shrinkage: effects of constraint and filling technique in composite restorations. Dent Mater 2004 Mar;20(3):236-43.

27. Malacarne J, Carvalho RM, De Goes MF, Svizero N, Pashley DH, Tay FR, *et al*. Water sorption/solubility of dental adhesive resins. Dent Mater 2006 Oct;22(10):973-80.
28. Miller RG, Bowles CQ, Chappelow CC, Eick JD. Application of solubility parameter theory to dentin-bonding systems and adhesive strength correlations. J Biomed Mater Res 1998 Aug;41(2):237-43.
29. Nakabayashi N, Kojima K, Mashuara E. The promotion of adhesions by the infiltration of monomers into tooth substrates. J Biomed Mat Res 1982 May;16(3):265-73.
30. Nishitani Y, Yoshiyama M, Donnelly AM, Agee KA, Sword J, Tay FR, *et al*. Effects of resin hydrophilicity on dentin bond strength. J Dent Res 2006a Nov; 85(11):1016-1021.
31. Osorio E, Toledano M, Aguilera FS, Tay FR, Osorio R. Ethanol wet-bonding technique sensitivity assessed by AFM. J Dent Res 2010 Nov;89(11):1264-69.
32. Oyarzún A, Rathkamp H, Dreyer E. Immunohistochemical and ultra-structural evaluation of the effects of phosphoric acid etching on dentin proteoglycans. Eur J Oral Sci 2000 Dec;108(6):546-54.
33. Pashley EL, Zhang Y, Lockwood PE, Rueggeberg FA, Pashley DH. Effects of HEMA on water evaporation from water-HEMA mixtures. Dent Mater 1998 Jan;14(1):6-10.
34. Pashley DH, Tay FR, Carvalho RM, Rueggeberg FA, Agee KA, Carrilho M, *et al*. From dry bonding to water-wet bonding to ethanol-wet bonding. A review of the interactions between dentin matrix and solvated resins using a macromodel of the hybrid layer. Am J Dent 2007 Feb;20(1):7-20.

35. Pereira PNR, Bedran-de-Castro AKB, Durante WR, Yamauti M (2007). Removal of noncollagenous components affects dentin bonding. J Biomed Mater Res B: Appl Biomater 2007 Jan;80(1):86-91.
36. Reis AA, Loguercio AD, Azevedo CL, Carvalho RM, Julio SM, Grande RH. Moisture spectrum of demineralized dentin for adhesive systems with different solvent bases. J Adhes Dent 2003;5:183-92.
37. Reis AF, Giannini M, Pereira PN. Effects of a peripheral enamel bond on the long-term effectiveness of dentin bonding agents exposed to water in vitro. J Biomed Mater Res B Appl Biomater 2008 Apr;85:10–7.
38. Saboia VP, Silva FC, Nato F, Mazzoni A, Cadenaro M, Mazzotti G, *et al.* Analysis of differential artificial ageing of the adhesive produced by a two-step etch-and-rinse adhesive. Eur J Oral Sci 2009 Oct;117(5):618-24.
39. Sadek FT, Pashley DH, Nishitani Y, Carrilho MR, Donnelly A, Ferrari M, *et al*. Application of the hydrophobic resin adhesive to acid-etched dentine with an alternative wet bonding technique. J Biomed Mater Res 2008 Jan;84(1):19-29.
40. Sadek FT, Castellan CS, Braga RR, Mai S, Tjäderhane L, Pashley DH, *et al*. One-year stability of resin-dentin bonds created with a hydrophobic ethanol-wet bonding technique. Dent Mater 2010 Apr;26(4):380-86.
41. Sadek FT, Mazzoni A, Breschi L, Tay FR, Braga RR. Six-month evaluation of adhesives interface created by a hydrophobic adhesive to acid-etched ethanol-wet bonded dentine with simplified dehydration protocols. J Dent 2010 Apr;38(4):276-83.
42. Spencer P, Wang Y. Adhesive phase separation at the dentin interface under wet bond conditions. J Biomed Mat Res 2002 Dec;62(3):447-56.

43. Tay FR, Pashley DH. Water treeing - A potential mechanism for degradation of dentin adhesives. Am J Dent 2003 Feb;16(1):6-12.

44. Tay FR, Pashley DH, Kapur RR, Carrilho MRO, Hur YB, Garrett LV, *et al.* Bonding BisGMA to dentin - A proof of concept for hydrophobic dentin bonding. J Dent Res 2007 Nov;86(11):1034-39.

45. Tay FR, Pashley DH, Loushine RJ, Weller RN, Monticelli F, Osorio F. Self-etching adhesives increase collagenolytic activity in radicular dentin. J Endod 2006 Sep;32(9):862-68.

46. Toledano M, Osorio R, Perdigão J, Rosales JI, Thompson JY, Cabrerizo-Vilchez MA. Effect of acid etching and collagen removal on dentin wettability and roughness. J Biomed Mater Res 1999 Nov;47(2):198-203.

47. Vaidyanathan TK, Vaidyanathan J. Recent advances in the theory and mechanism of adhesive resin bonding to dentin: A critical review. J Biomed Mater Res B Appl Biomater 2009 Feb;88(2):558-78.

48. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, *et al.* Buonocore Memorial Lecture. Adhesion to enamel and dentin: Current status and future challenges. Oper Dent 2003 May-Jun;28(3):215-35.

Table 1. Materials used in the study.

Comercial brand	Main components	Mode of application	Manufacturer
Adper Single Bond 2	Ethanol, bis-GMA, HEMA, GDMA, polycarboxylic acid copolymer, UDMA, water ter, CQ, EDMAB, DPIHFP	Apply one layer of adhesive, wait for 20 s, air stream for 5 s and polymerize for 10 s	3M/ESPE
Scotchbond Etchant	-35% phosphoric acid, water, silica	Apply a layer for 15 s and wash for 15 s	3M/ESPE
Filtek Z350 XT	-Bis-GMA, UDMA, Bis-EMA, TEGDMA resins, zirconium, silica	Apply increments of 2 mm and polymerize for 20 s.	3M/ESPE

Table 2. Bond strenght and Distribution of Fracture Modes

	μTBS (MPa)*		Failure Modes (%)** 24 h				18 mos			
	24 h	18 mos	CD	CR	M	A	CD	CR	M	A
Control	31.7 ± 3.9 ^{A}	31.5 ± 3.8 ^{A}	9	18	61	12	9	12	65	14
Ethanol	30.3 ± 4.3 ^{A}	21.9 ± 3.2 ^{B}	4	6	83	7	5	6	68	21

***** Bond strength values are means ± standard deviations. Different superscripts indicate statistically significance diference ($p<0.05$).

******Failure modes pattern: CD, cohesive failure in dentin; CR cohesive failure in resin; M - mixed failure; A - adhesive.

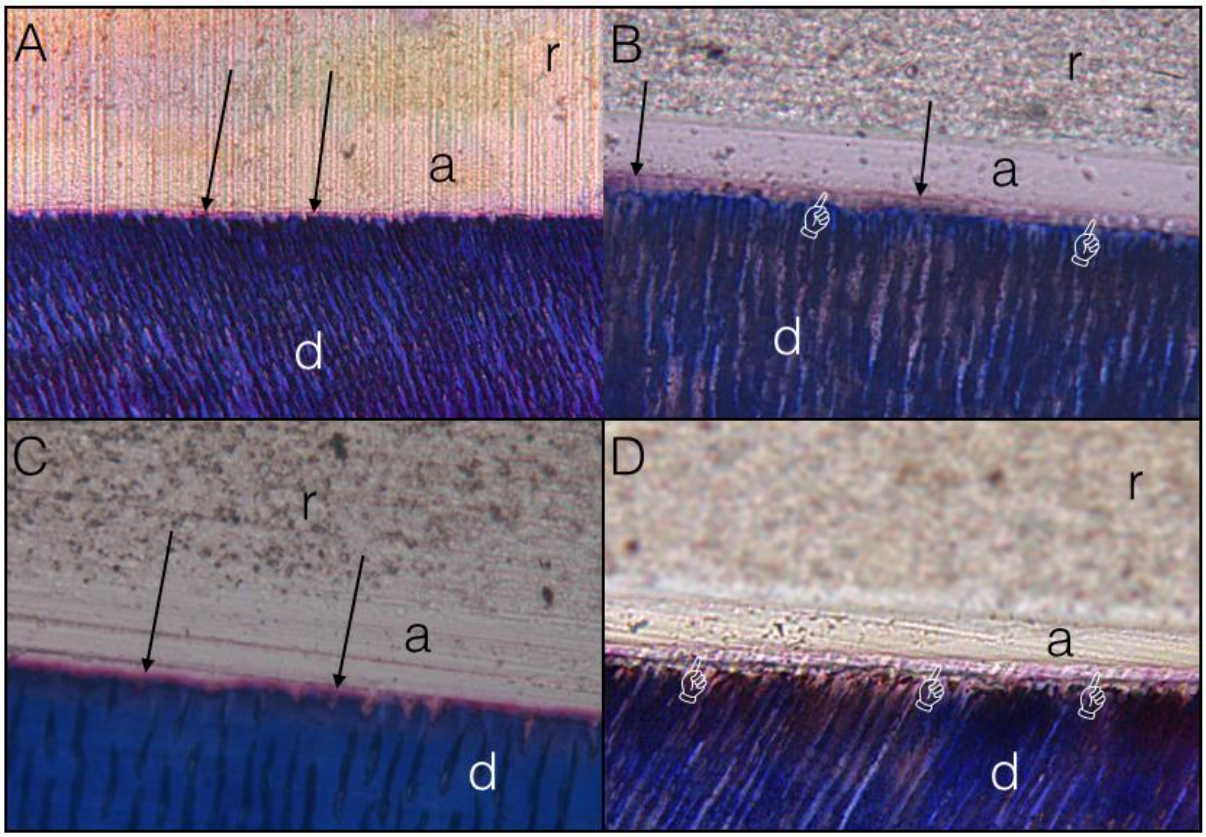


Fig. 1 - Light micrographs Masson's Trichrome of resin-dentin interface: A) Control immediate; B) Control aged; C) Ethanol immediate; D) Ethanol aged. r - resin; a - adhesive; d - dentin. Arrows – red zone, indicating presence of denuded collagen fibrils; Pointers - white zone indicating absence of both polymers or exposed collagen fibrils, suggesting degradation of resin-dentin interface.

4 CONCLUSÃO GERAL

Com base nos resultados dos trabalhos desta tese, podemos concluir que:

- O pré-tratamento com etanol a 70% ou 100% aumenta a resistência de união inicial de um adesivo simplificado de condicionamento total.
- O pré-tratamento da dentina com etanol a 70% *in vivo* diminui a resistência de união à dentina e aumenta a degradação da interface resina-dentina após 18 meses.

REFERÊNCIAS

BUONOCORE, M. G. A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. **J. Dent. Res.,** v. 34, p. 849-853, 1955.

CARVALHO, R. M.; MENDONÇA, J. S.; SANTIAGO, S. L.; SILVEIRA, R. R.; GARCIA, F. C. P.; TAY, F. R. *et al*. Effects of HEMA/solvent combinations on bond strength to dentin. **J. Dent. Res.,** v. 82, p. 597-601, 2003.

DE MUNCK, J.; VAN MEERBEEK, B.; YOSHIDA, Y.; INOUE, S.; VARGAS, M.; SUZUKI, K. *et al*. Four-year water degradation of total-etch adhesives bonded to dentin. **J. Dent. Res.,** v. 82, p. 136-140, 2003.

GARCIA-GODOY, F.; TAY, F. R.; PASHLEY, D. H.; FEILZER, A.; TJÄDERHANE, L.; PASHLEY, E. L. Degradation of resin-bonded human dentin after 3 years of storage. **Am. J. Dent.,** v. 19, p. 109-113, 2007.

GUIMARÃES, L. A.; ALMEIDA, J. C. F.; WANG, L.; D'ALPINO, P. H. P.; GARCIA, F. C. P. Effectiveness of Immediate Bonding of Etch-and-rinse Adhesives to Simplified Ethanol-saturated Dentin. **Braz. Oral Res.,** v. 26, p. 177-182, 2012.

HASHIMOTO, M.; OHNO, H.; KAGA, M.; ENDO, K.; SANO, H.; OGUCHI, H. *In vivo* degradation of resin-dentin bonds in humans over 1 to 3 years. **J. Dent. Res.,** v. 79, p. 1385-1391, 2000.

HOSAKA, K.; NISHITANI, Y.; TAGAMI, J.; YOSHIYAMA, M.; BRACKETT, W. W.; AGEE, K. A.; TAY, F. R.; PASHLEY, D. H. Durability of resin-dentin bonds to water- vs. ethanol-saturated dentin. **J. Dent. Res.,** v. 88, n. 2, p.146-151, 2009.

LIU, Y.; TJÄDERHANE, L.; BRESCHI, L.; MAZZONI, A.; LI, N.; MAO, J.; PASHLEY, D. H.; TAY, F. R. Limitations in bonding to dentin and experimental strategies to prevent bond degradation. **J. Dent. Res.,** v. 90, p. 953-968, 2011.

MAZZONI, A.; PASHLEY, D. H.; NISHITANI, Y.; BRESCHI, L.; TJÄDERHANE, L.; TOLEDANO, M. *et al*. Reactivation of Inactivated Endogenous Proteolytic Activities in Phosphoric Acid-etched Dentin by Etch-and-rinse Adhesives. **Biomaterials,** v. 27, p. 4470-4476, 2006.

NAKABAYASHI, N.; KOJIMA, K.; MASHUARA, E. The promotion of adhesions by the infiltration of monomers into tooth substrates. **J. Biol. Mat. Res.,** v. 16, p. 265-273, 1982.

OSORIO, E.; TOLEDANO, M.; AGUILERA, F. S.; TAY, F. R.; OSORIO, R. Ethanol wet-bonding technique sensitivity assessed by AFM. **J. Dent. Res.,** v. 89, p. 1264-1269, 2010.

PASHLEY, D. H.; TAY, F. R.; CARVALHO, R. M.; RUEGEBERG, F. A.; AGEE, K. A.; CARRILHO, M. *et al.* From dry bonding to water-wet bonding to ethanol-wet bonding. A review of the interactions between dentin matrix and solvated resins using a macromodel of the hybrid layer. **Am. J. Dent.,** v. 20, p. 7-20, 2007.

SADEK, F. T.; BRAGA, R. R.; MUENCH, A.; LIU, Y.; PASHLEY, D. H.; TAY, .F R. Ethanol wet-bonding challenges current anti-degradation strategy. **J. Dent. Res.,** v. 89, n. 12, p. 1499-1504, 2010a.

SADEK, F. T.; CASTELLAN, C. S.; BRAGA, R. R.; MAI, S.; TJÄDERHANE, L.; PASHLEY, D. H.; TAY, F. R. One-year stability of resin-dentin bonds created with a hydrophobic ethanol-wet bonding technique. **Dent. Mater.,** v. 26, n. 4, p. 380-386, 2010.

SAURO, S.; TOLEDANO, M.; AGUILERA, F. S.; MANNOCCI, F.; PASHLEY, D. H.; TAY, F. R.; WATSON, T. F.; OSORIO, R. Resin-dentin bonds to EDTA-treated vs. acid-etched dentin using ethanol wet-bonding. **Dent. Mater.,** v. 26, p. 368-379, 2010.

TAY, F. R.; PASHLEY, D. H. Water treeing - A potential mechanism for degradation of dentin adhesives. **Am. J. Dent.,** v. 16, p. 6-12, 2003.

VAN MEERBEEK, B.; DE MUNCK, J.; YOSHIDA, Y.; INOUE, S.; VARGAS, M.; VIJAY, P. *et al.* Buonocore Memorial Lecture. Adhesion to enamel and dentin: Current status and future challenges. **Oper. Dent.,** v. 28, p. 215-235, 2003.

ANEXOS

ANEXO A – Aprovação no Comitê de Ética em Pesquisa do estudo *in vitro* (Capítulo 1).

Universidade Federal do Ceará
Comitê de Ética em Pesquisa

Of. Nº 107/10 Fortaleza, 28 de maio de 2010
Protocolo COMEPE nº 109/ 10
Pesquisador responsável: Francisco Cláudio Fernandes Alves e Silva
Deptº./Serviço: Departamento de Odontologia/ UFC
Título do Projeto: "Influência do uso do etanol como pré-tratamento da dentina, na resistência de união de adesivos de condicionamento total"

Levamos ao conhecimento de V.Sª. que o Comitê de Ética em Pesquisa da Universidade Federal do Ceará – COMEPE, dentro das normas que regulamentam a pesquisa em seres humanos, do Conselho Nacional de Saúde – Ministério da Saúde, Resolução nº 196 de 10 de outubro de 1996 e complementares, aprovou o protocolo e o TCLE do projeto supracitado na reunião do dia 27 de maio de 2010.

Outrossim, informamos, que o pesquisador deverá se comprometer a enviar o relatório final do referido projeto.

Atenciosamente,

Mirian Parente Monteiro.

Dra. Mirian Parente Monteiro
Coordenadora Adjunta do Comitê
de Ética em Pesquisa
COMEPE/UFC

ANEXO B – aprovação no Comitê de Ética em Pesquisa do estudo *in vivo* (Capítulo 2).

Universidade Federal do Ceará
Comitê de Ética em Pesquisa

Of. Nº 108/10 Fortaleza, 28 de maio de 2010
Protocolo COMEPE nº 110/ 10
Pesquisador responsável: Francisco Cláudio Fernandes Alves e Silva
Deptº./Serviço: Departamento de Odontologia/ UFC
Título do Projeto: "Estudo in vivo do uso do etanol como pré-tratamento da dentina, na resistência de união dos adesivos de condicionamento total"

Levamos ao conhecimento de V.Sª. que o Comitê de Ética em Pesquisa da Universidade Federal do Ceará – COMEPE, dentro das normas que regulamentam a pesquisa em seres humanos, do Conselho Nacional de Saúde – Ministério da Saúde, Resolução nº 196 de 10 de outubro de 1996 e complementares, aprovou o protocolo e o TCLE do projeto supracitado na reunião do dia 27 de maio de 2010.

Outrossim, informamos, que o pesquisador deverá se comprometer a enviar o relatório final do referido projeto.

Atenciosamente,

Mirian Parente Monteiro

Dra. Mirian Parente Monteiro
Coordenadora Adjunta do Comitê
de Ética em Pesquisa
COMEPE/UFC

ANEXO C - **TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO**

(Referente ao capítulo 1)

Título da pesquisa:

Influência do uso do etanol como pré-tratamento da dentina, na resistência de união de adesivos de condicionamento total.

Nesta pesquisa serão utilizados dentes humanos extraídos, nos quais serão realizados testes mecânicos para avaliar o comportamento de materiais usados em restaurações, ou seja, será observado se o material usado colou firmemente ou não no dente. A pesquisa será realizada na Faculdade de Odontologia da Universidade Federal do Ceará, e se você quiser colaborar com o pesquisador, basta ceder o dente que for extrair para que possa ser utilizado e assinar o termo de Consentimento Livre e Esclarecido. Porém, se não quiser doar seu dente, o seu tratamento não será prejudicado. Para maiores esclarecimentos pode entrar em contato com o pesquisador responsável – Francisco Cláudio Fernandes Alves e Silva – à Rua Afonso Celso, 625, Ap. 101/B, Aldeota, Tel. 3461.1620 / 9983.9019 ou com o Comitê de Ética em Pesquisa da Universidade Federal do Ceará-UFC, pelo fone: 3366-8338.

Eu,.....

após ter sido devidamente esclarecido(a) dos objetivos da pesquisa acima mencionada, aceitei voluntariamente doar o(s) dente(s) que extraí para o pesquisador Francisco Cláudio Fernandes Alves e Silva. Fui ainda esclarecido que minha identidade não será divulgada por qualquer meio, que os procedimentos serão realizados no(s) meu(s) dente(s) após sua indicada remoção e que, portanto não me trará nenhum risco à saúde e que o material recolhido será utilizado unicamente para a presente pesquisa.

Fortaleza, .../...../.....

.....
Assinatura do doador

.....
Assinatura da testemunha

.....
Assinatura do pesquisador

Impressão digital do dador

ANEXO D - **TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO**

(Referente ao capítulo 2)

Título da pesquisa:

Estudo *in vivo* do uso do etanol como pré-tratamento da dentina, na resistência de união dos adesivos de condicionamento total.

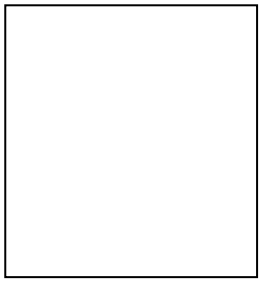
Nesta pesquisa serão utilizados dentes humanos que serão restaurados e extraídos para serem realizados testes mecânicos com a finalidade de avaliar o comportamento de materiais usados em restaurações, ou seja, será observado se o material usado colou firmemente ou não no dente. A pesquisa será realizada na Faculdade de Odontologia da Universidade Federal do Ceará, e se você quiser colaborar com o pesquisador, basta submeter-se a realizar a restauração, extrair e ceder o dente para que possa ser utilizado e assinar o termo de Consentimento Livre e Esclarecido. Porém, se não quiser doar seu dente, o seu tratamento não será prejudicado. Para maiores esclarecimentos pode entrar em contato com o pesquisador responsável – Francisco Cláudio Fernandes Alves e Silva – à Rua Afonso Celso, 625, Ap. 101/B, Aldeota, Tel. 3461.1620 / 9983.9019 ou com o Comitê de Ética em Pesquisa da Universidade Federal do Ceará-UFC, pelo fone: 3366-8338.

Eu,.....
.....após ter sido devidamente esclarecido(a) dos objetivos da pesquisa acima mencionada, aceitei voluntariamente restaurar, extrair e doar o(s) dente(s) para o pesquisador Francisco Cláudio Fernandes Alves e Silva. Fui ainda esclarecido que minha identidade não será divulgada por qualquer meio, que os procedimentos serão realizados no(s) meu(s) dente(s) após sua indicada remoção e que, portanto não me trará nenhum risco à saúde e que o material recolhido será utilizado unicamente para a presente pesquisa.

Fortaleza, .../...../.....

.....
Assinatura do doador

.....
Assinatura da testemunha



ANEXO E - **JERD - Journal of Esthetic and Restorative Dentistry**

Author Guidelines

Information for Authors

Editorial Office Contact Information

Dr. Harald Heymann – Editor in Chief Department of Operative Dentistry University of North Carolina CB#7450, 302 Brauer Hall Chapel Hill, NC, USA 27599-7450

Betty Cates – Editorial Assistant Email: betty_cates@dentistry.unc.edu Phone: (919) 537-3438 Fax: (919) 537-3990

MANUSCRIPT PREPARATION

Getting Started • Launch your web browser (supported browsers include Internet Explorer 6 or higher, or 7.2, Safari 1.2.4, or Firefox 1.0.4) and go to the journal's online Submission Site: http://mc.manuscriptcentral.com/jerd • Log-in or click the "Create Account" option if you are a first-time user. • If you are creating a new account: - After clicking on "Create Account," enter your name and e-mail information and click "Next." Your e-mail information is very important. - Enter your institution and address information as appropriate, and then click "Next." - Enter a user ID and password of your choice (we recommend using your e-mail address as your user ID), and then select your area of expertise. Click "Finish." • If you have an account, but have forgotten your log in details, go to Password Help on the journals online submission system http://mc.manuscriptcentral.com/jerd and enter your email address. The system will send you an automatic user ID and a new temporary password. • Log-in and select "Author Center"

Submitting Your Manuscript

• After you have logged in, click the "Submit a Manuscript" link in the menu bar.
• Enter data and answer questions as appropriate. You may copy and paste directly from your manuscript and you may upload your pre-prepared covering letter.
• Click the "Next" button on each screen to save your work and advance to the next screen.
• You are required to upload your files. - Click on the "Browse" button and locate the file on your computer. - Select the designation of each file in the drop down next to the Browse button. - When you have selected all files you wish to upload, click the "Upload Files" button.
• Review your submission (in HTML and PDF format) before sending to the Journal. Click the "Submit" button when you are finished reviewing.

Manuscript Types Accepted ***Original Research Articles*** are related to laboratory research or clinical research.

Clinical Technique Articles describe significant achievements and improvements in clinical practice such as comprehensive interdisciplinary dental treatment, introduction of new technology or practical approaches to recognized clinical challenges. They should conform to the highest scientific and clinical practice standards with supporting references where indicated.

Case Reports must represent new or novel approaches to dealing with specific clinical problems. Proper qualifying and/or disclaiming statements should be included if inadequate research is available to validate the techniques being presented.

Review Articles may be submitted independently or invited by the Editor and include systematic literature reviews of topics related to esthetic and restorative dentistry, as well as more general, comprehensive reviews or updates of a given topic.

General Guidelines

Product trade names cited in the text must be accompanied by a generic term, and followed by the manufacturer, city, and state/country in parentheses. References in the text and figure legends to teeth illustrated in a figure should be identified by name (eg, upper right central incisor), not by number. The manuscripts submitted to the Journal must be written in appropriate English. It is the author's responsibility to ensure this by either having sufficient English language skills or by obtaining the services of an English-as-second-language expert.

The same general headings and sections should be used in the articles as used in the abstract. See below for the specific headings and sections indicated for the various types of articles.

Title Page The title page must include all authors' full names, academic degrees, and institutional affiliations and locations. If the manuscript was originally presented as part of a meeting, please state the name, date, and location of the meeting. Sources of support in the form of grants, equipment, products, drugs, or all of these must be disclosed. A corresponding author must be designated and full details of the correspondent's address provided: name, address, telephone and fax numbers, and e-mail address. Unless specified otherwise, the corresponding author's address also will be used for reprint requests.

Abstract A structured abstract of no more than 250 words must be provided for each article. Footnotes, references, and abbreviations are not used in the abstract. For ***original research articles***, the abstract should include the following headings and sections: (1) Objective. This section includes a statement of the problem and the purpose of the study, (2) Materials and Methods. This section should include materials, methods and statistical analyses employed in the study. (3) Results. (4) Conclusions. For ***clinical technique articles and case reports***, the abstract should include the following headings and sections: (1) Objective. This section includes a statement of the problem and a general description of the topic or treatment to be addressed. (2) Clinical Considerations. This section should include a brief description of the clinical materials and techniques employed. (3) Conclusions.

For ***systematic literature review articles***, the abstract should include the following headings and sections: (1) Objective. This section should include a statement of the topic to be reviewed and a description of the search strategy of relevant literature (search terms and databases), (2) Materials and Methods. This section should contain inclusion criteria (language, type of studies *i.e.* randomized controlled trial or other, duration of studies and

chosen endpoints). (3) Results. This section should include evaluation of papers and level of evidence. (4) Conclusions.

For ***general review articles*** the abstract should include the following headings and sections: (1) Objective. This section should include a statement of the topic to be reviewed. (2) Overview. This section should include a brief summary of the findings of the review. (3) Conclusions.

Clinical Significance In a few sentences, please indicate the clinical importance and implications of the research or clinical technique discussed, and if applicable, its relevance to esthetic dentistry.

References References should be numbered consecutively in the order in which they are first mentioned in the text, and listed at the end of the text in numeric, not alphabetic, order. Identify references in text, tables, and legends by Arabic numerals in superscript. References cited only in tables or figure legends should be numbered subsequent to the numbering of references cited in the text. Unpublished sources, such as manuscripts in preparation and personal communications, are not acceptable as references. Only sources cited in the text should appear in the reference list. List all authors when four or fewer; when more than four, list the first three and add "*et al*."

CITATION FORMAT

Journal Articles Donnelly PV, Miller C, Ciardullo T, *et al*. Occlusion and its role in esthetics. J Esthet Dent 1996; 8:111-8.

Books Hickey JC, Zarb GA. Boucher's prosthodontic treatment for edentulous patients. 9th ed. St. Louis (MO): CV Mosby; 1985.

Tables Type or print out each table with double spacing on a separate page. Ensure that each table is cited in the text, number tables consecutively in the order of their first citation in the text, and provide a brief title for each. Give each column a brief, descriptive heading. No table should contain data that could be included in the text in several sentences.

Illustrations Images must be submitted electronically according to the following specifications: • COLOR photographs should be saved as TIF files in CMYK at a minimum of 12.5 cm (5 in.) in width at 300 dpi. • BLACK AND WHITE photographs should be saved as TIF files in grayscale at a minimum of 12.5 cm (5 in.) in width at 300 dpi. • Line drawings should be prepared in Microsoft Word or PowerPoint, or in Adobe Illustrator without embedded images from other sources. Existing line drawings should be scanned at 1,200 dpi at a minimum of 12.5 cm (5 in.) in width and saved as EPS files. • All images must be labeled clearly in consecutive order with the figure number and part. Hard copies of images are no longer accepted. Photomicrographs must feature internal scale markers. Symbols, arrows, or letters used in these should contrast with the background. Original magnification must be provided.

Figure reproduction cannot improve on the quality of the originals. It does not correct the exposure, sharpen the focus, or improve the contrast of the original print. Any special instructions about sizing, placement, or color should be clearly noted. Electronic submissions are not returned to the authors. Figure legends must be numbered to correspond with the

figures and typed or printed on a separate page. Symbols, arrows, or letters used to identify parts of the illustration must be explained clearly in the legend. If a figure has been previously published, the legend must acknowledge the original source.

PERMISSIONS Written permission must be obtained for material that has been published in copyrighted material; this includes tables, figures, and quoted text that exceeds 150 words.

Photographs of People The Journal of Esthetic and Restorative Dentistry follows current HIPAA guidelines for the protection of patient/subject privacy. If an individual pictured in a digital image or photograph can be identified, his or her permission is required to publish the image. The corresponding author may submit a letter signed by the patient authorizing the Journal of Esthetic and Restorative Dentistry to publish the image/photo. Or, a form provided by the Journal of Esthetic and Restorative Dentistry (available by clicking the "Instructions and Forms" link in Manuscript Central) may be downloaded for your use. This approval must be received by the Editorial Office prior to final acceptance of the manuscript for publication. Otherwise, the image/photo must be altered such that the individual cannot be identified (black bars over eyes, etc).

Manipulation of Digital Photos Authors should be aware that the Journal considers digital images to be data. Hence, digital images submitted should contain the same data as the original image captured. Any manipulation using graphical software should be identified in either the Disclosure and Acknowledgements section or the caption of the photo itself. Identification of manipulation should include both the name of the software and the techniques used to enhance or change the graphic in any way. Such a disclaimer ensures that the methods are repeatable and ensures the scientific integrity of the work.

No specific feature within an image may be enhanced, obscured, moved, removed, or introduced. The grouping of images from different SEMS, different teeth, or the mouths of different patients must be made explicit by the arrangement of the figure (*i.e.*, by using dividing lines) and in the text of the figure legend. Adjustments of brightness, contrast, or color balance are acceptable if they are applied to the whole image and as long as they do not obscure, eliminate, or misrepresent any information present in the original, including backgrounds.

The removal of artifacts or any non-integral data held in the image is not allowed. For instance, removal of papillae or "cleaning up" of saliva bubbles is not allowed.

Cases of deliberate misrepresentation of data will result in rejection of a manuscript, or if the misrepresentation is discovered after a manuscript's acceptance, revocation of acceptance, and the incident will be reported to the corresponding author's home institution or funding agency.

DISCLOSURE AND ACKNOWLEDGMENTS Please provide any information you wish to include acknowledging contributions from individuals such as for statistical support, labwork, etc. Also it is imperative that you provide a disclosure statement if you have any financial interest in any of the companies whose products or devices are included in the paper. If no financial interest exists, the following statement must be used: "The authors do not have any financial interest in the companies whose materials are included in this article."

SUBMISSION CHECKLIST

• Letter of submission

• An electronic copy of the manuscript (typed, double-spaced), which includes:
• Title page - (1) title of article; (2) full name, academic degrees/affiliations of authors; (3) name, address, telephone and fax numbers, e-mail of corresponding author
• Abstract
• Article proper (clinical or research)
• References (on separate sheet)
• Tables (on separate sheets)
• Legends (on separate sheet)
• Illustrations properly labeled
• Signed permissions and patient releases
• Disclosure Statement

For authors signing current licensing/copyright agreement

Note to Contributors on Deposit of Accepted Version

Funder arrangements

Certain funders, including the NIH, members of the Research Councils UK (RCUK) and Wellcome Trust require deposit of the Accepted Version in a repository after an embargo period. Details of funding arrangements are set out at the following website: http://www.wiley.com/go/funderstatement. Please contact the Journal production editor if you have additional funding requirements.

Institutions

Wiley has arrangements with certain academic institutions to permit the deposit of the Accepted Version in the institutional repository after an embargo period. Details of such arrangements are set out at the following website: http://www.wiley.com/go/funderstatement.

If you do not select the OnlineOpen option you will follow the current licensing signing process as described above.

For authors choosing OnlineOpen If you decide to select the OnlineOpen option, please use the links below to obtain an open access agreement to sign [this will supersede the journal's usual license agreement]. By selecting the OnlineOpen option you have the choice of the following Creative Commons License open access agreements:

Creative Commons Attribution License OAA

Creative Commons Attribution Non-Commercial License OAA

Creative Commons Attribution Non-Commercial -NoDerivs License OAA

To preview the terms and conditions of these open access agreements please click the license types above and visit
http://www.wileyopenaccess.com/details/content/12f25db4c87/Copyright--License.html.

NEW: Online production tracking is now available for your article through Wiley-Blackwell's Author Services. Author Services enables authors to track their article - once it has been accepted - through the production process to publication online and in print. Authors can check the status of their articles online and choose to receive automated e-mails at key

stages of production. The author will receive an e-mail with a unique link that enables them to register and have their article automatically added to the system. Please ensure that a complete e-mail address is provided when submitting the manuscript. Visit http://authorservices.wiley.com/bauthor/ for more details on online production tracking and for a wealth of resources including FAQs and tips on article preparation, submission and more. Vers. 8-15-13

ANEXO F - **The Journal of Adhesive Dentistry**

GUIDELINES FOR AUTHORS

The Journal of Adhesive Dentistry is a quarterly 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. Review articles – to topics related to adhesive dentistry.

3a. Invited focus articles – presenting a position or hypothesis on a basic science or clinical subject of relevance 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.

3b. Invited commentaries – critiquing a focus article by addressing the strong and weak points of the focus article 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 a same issue of the Journal.

4. Invited guest editorials – may periodically be solicited by the Editorial Board.

5. Proceedings of symposia, workshops, or conferences - covering topics of relevance to adhesive dentistry related topics.

6. Letters to the Editor – may be submitted to the editor-in-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.jad.dk) Manuscript texts should be uploaded as PDF or 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 (pc@quintessence.dk). 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 be opened using Adobe Photoshop, (TIF, GIF, JPG, PSD, EPS etc.) or as a Microsoft PowerPoint Documents (ppt).

Mailing address:

Manuscript Editor

The Journal of Adhesive Dentistry Quintessence Copenhagen

European Editorial Office

Fuglevadsvej 27, DK-2800 Lyngby, Denmark

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 prepared 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, title, professional affiliation, 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.

• 3-5 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 whenever possible, but trade names and manufacturer should be included parenthetically at first mention.

REFERENCES

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

• The reference list should appear at the end of the article, numbered in alphabetical 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 (up to six). If the reference is to 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 patterns 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.

Paper version:

• The figure number and first author's last name should be indicated on the back of each photograph or on the mount of each slide. Also indicate the top edge lightly in pencil.

Do not mark author's name on duplicates!

• Do not bend, fold, or use paper clips. Do not mount slides in glass.

• For protection against damage or loss, authors should retain duplicate slides and illustrations.

• All illustrations are returned after publication.

• Original artwork must be provided with original submission.

Black & white – submit one set of high-quality glossy prints. Should the quality prove inadequate, negatives will be requested as well. Photographs should be unmounted and untrimmed. Radiographs – submit the original radiograph as well as two sets of prints.

Color – Original slides (35 mm transparencies) must be submitted, plus two sets of prints made from them. When instruments and appliances are photographed, a neutral background is best; structured fabrics are unsuitable.

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). Lines within graphs should be of a single weight unless special emphasis is needed. Legends – Figure legends should be grouped as a file on a separate sheet and typed double-spaced.

TABLES

• Each table should be logically organized, as a file 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 AND 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 ethical committee must be presented.

REPRINTS

The corresponding author is given 50 free reprints of the article. If additional reprints are desired, they must be ordered from the publisher when the page proofs are reviewed by the authors. The publisher does not stock reprints; however, back issues can be purchased.