UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS ODONTOLÓGICAS

Bruna Gabrielle da Silva Sutil

EFEITO DOS MÉTODOS DE ENVELHECIMENTO ARTIFICIAL SOBRE A RESISTÊNCIA DE UNIÃO DE SISTEMAS ADESIVOS APLICADOS À DENTINA

Santa Maria, RS
2021

Bruna Gabrielle da Silva Sutil

EFEITO DOS MÉTODOS DE ENVELHECIMENTO ARTIFICIAL SOBRE A RESISTÊNCIA DE UNIÃO DE SISTEMAS ADESIVOS APLICADOS À DENTINA

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

Orientador: Prof. Dr. Alexandre Henrique Susin

Santa Maria, RS
2021

Sutil, Bruna Gabrielle da Silva
EFEITO DOS MÉTODOS DE ENVELHECIMENTO ARTIFICAL SOBRE A RESISTÊNCIA DE UNIÃO DE SISTEMAS ADESIVOS APLICADOS À DENTINA / Bruna Gabrielle da Silva Sutil.- 2021.
72 p.; 30 cm

Orientador: Alexandre Henrique Susin
Tese (doutorado) - Universidade Federal de Santa Maria, Centro de Ciências da Saúde, Programa de Pós Graduação em Ciências Odontológicas, RS, 2021

1. Degradação 2. Dentina 3. Envelhecimento artificial 4. Resistência adesiva 5. Sistemas adesivos I. Susin, Alexandre Henrique II. Título.

Sistema de geração automática de ficha catalográfica da UFSM. Dados fornecidos pelo autor(a). Sob supervisão da Direção da Divisão de Processos Técnicos da Biblioteca Central. Bibliotecária responsável Paula Schoenfeldt Patta CRB 10/1728.

Endereço: Rua André Marques , n.718, ap.505, Bairro Centro, Santa Maria, RS. CEP: 97010-040
Fone (055) 99106 7975; E-mail: brunasutil@hotmail.com

Bruna Gabrielle da Silva Sutil

EFEITO DOS MÉTODOS DE ENVELHECIMENTO ARTIFICIAL SOBRE A RESISTÊNCIA DE UNIÃO DE SISTEMAS ADESIVOS APLICADOS À DENTINA

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

Aprovado em 21 de julho de 2021:

Alexandre Henrique Susin, Dr. (UFSM)
(Presidente da Banca/Orientador)

Luana Severo Alves, Dra. (UFSM)

Bruno Lopes da Silveira, Dr. (UFSM)

Carlos Eduardo Agostini Balbinot, Dr. (UFN)

Marciano de Freitas Borges, Dr. (UFN)

Santa Maria, RS
2021

AGRADECIMENTOS

Agradeço a ***Deus*** *por permitir que eu chegasse até aqui, mantendo-me em condições necessárias para alcançar esse objetivo. Sem Ele, essa jornada não poderia ser cumprida. A lição que Ele nos passa é que com fé, dedicação, esforço e as bênçãos do Altíssimo, tudo é possível.*

Aos meus pais, ***Beatriz Pires da Silva*** *e* ***Alair Quadros Sutil****, por terem me abençoado com a vida, por me ensinarem que o estudo é o melhor caminho a seguir, e por terem formado a pessoa que hoje eu sou. Obrigada por toda ajuda, cuidado e carinho mãe; saiba que tenho muito amor por vocês e que sou muito grata. Ainda irei retribuir tudo o que fizeram por mim. Aos meus irmãos* ***Jannes Miranda****,* ***Rafael Sutil****,* ***Paola Sutil*** *e* ***Pollyana Sutil*** *que de uma forma ou de outra, sempre estiveram me apoiando e torcendo por mim, mesmo que à distância; um agradecimento especial à* ***Priscila Sutil****, minha mana e primeira grande amiga, pela cumplicidade, fraternidade, amor e pelos momentos felizes e inesquecíveis compartilhados na infância e adolescência; e ao meu maninho caçula,* ***Lucas Miranda****, que independente de estar passando por uma fase complicada, jamais deixou de me incentivar, de me mostrar que não devemos perder a fé e de demonstrar o quanto me ama. Vocês são muito importantes para mim. Amo vocês!*

À minha madrinha, tia e segunda mãe, ***Carmem Rosane Coden Carnelosso*** *e a minha avó* ***Olímpia Quadros Subtil*** *que foram essenciais em minha adolescência, um período que foi importante para definir o estudo como prioridade em minha vida. Obrigada tia por ter me ajudado e obrigada vó por ter me abrigado e dado o suporte nesse período. Sou grata pela atenção e carinho oferecidos a mim nessa fase de minha vida. De forma igual, agradeço com muitas saudades, à minha vozinha* ***Maria Alisse Gassen da Silva****, que infelizmente não está mais entre nós, pelo amor e afeto doados, fundamentais durante minha infância.*

Aos meus amigos ***Mari Teresinha Maia de Amarante*** *e* ***Gilmar Martins de Amarante*** *por terem me acolhido nos momentos que mais precisei, por todo o suporte e carinho que me foi dado em algumas das situações mais difíceis da minha trajetória. Obrigada* ***Mari****, minha mãezinha de coração, pelo convívio, por todos os conselhos, pelo amor e ajuda quando mais necessitei e por me manter em pé, impulsionando-me a conquistar meus objetivos. Agradeço por me mostrar que não devemos perder a fé de que algo melhor sempre nos espera mais adiante, e por me fazer acreditar em minha capacidade de vencer e superar todas as etapas. Sou muito grata por você ter se feito presente nos momentos em que me senti só e impotente; foi um alento para meu coração. Deus foi muito generoso ao permitir que vocês cruzassem* *meu caminho. Vocês são como uma família para mim; sempre os levarei em meu coração, com todo o amor, como uma filha tem por seus pais. A vocês, apenas gostaria de expressar minha eterna gratidão.*

À minha amiga ***Bárbara Maia****, que sempre me apoiou e incentivou para que eu conseguisse concluir essa etapa. Não tenho palavras para lhe agradecer, minha irmã de coração, por ter permanecido ao meu lado, mostrando-me que eu era capaz e que não deveria desistir de tudo. Mesmo nos momentos em que eu própria duvidei de mim, você estava lá, colocando-me para cima, fazendo-me enxergar a pessoa que eu sou e me mostrando que sim, é possível seguir em frente, superar todas as adversidades que a vida me impôs e obter êxito em todas as áreas da minha vida. Obrigada por ser minha confidente, pelo nosso bom convívio diário e principalmente, por me aturar naqueles momentos em que nem eu mesma me suportava. Você não faz ideia de como sua companhia me salvou de muitos dos meus dias ruins. Agradeço todos os dias por Deus ter colocado uma amiga como você em minha vida. Saiba que mesmo que algum dia a distância nos separe fisicamente, estarei com você sempre em pensamento, pois você tem um lugarzinho eterno em meu coração. Amo você, maninha!*

Ao meu orientador, professor ***Alexandre Henrique Susin*** *pela oportunidade de dividir comigo seus conhecimentos. Não tenho palavras para agradecer todo o suporte, compreensão e pela confiança em mim depositada. Agradeço por todas às vezes que o senhor me incentivou, sempre demostrando paciência, empatia e carinho pelos seus orientados. É um exemplo de profissional e de ser humano; amigo que qualquer pessoa iria querer ter por perto. Com certeza, tomarei o senhor como modelo a ser seguido, tanto no aspecto profissional, como no quesito ser humano, pois é uma pessoa de coração enorme, generosa e digna de admiração. Meus mais sinceros agradecimentos!*

Aos meus ***colegas de pós-graduação*** *que tive o prazer de conviver durante o doutorado. Obrigada pelos momentos de descontração e pela ajuda quando solicitada.*

A todos os meus ***amigos*** *e* ***familiares*** *que, de alguma forma estiveram presentes comigo nessa trajetória, acompanhando-me e torcendo por mim.*

À ***Universidade Federal de Santa Maria****, ao* ***Programa de Pós-Graduação em Ciências Odontológicas*** *e ao* ***Curso de Odontologia*** *por todo o suporte e estrutura fornecida, possibilitando uma formação de qualidade, abrangendo graduação, mestrado e doutorado. Tenho muito orgulho de fazer parte dessa instituição.*

Aos ***Professores da Graduação e do Programa de Pós-Graduação em Ciências Odontológicas*** *por todo o conhecimento compartilhado.*

À ***Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)*** *pela concessão da minha bolsa, a qual viabilizou a execução deste trabalho.*

Ao ***Banco de Dentes Humanos da Universidade Federal de Santa Maria*** *pela doação do material biológico utilizado nesta pesquisa.*

A ***todos****, os meus sinceros agradecimentos!*

A tarefa não é tanto ver aquilo que ninguém viu,
mas pensar o que ninguém ainda pensou,
sobre aquilo que todo mundo vê.
(Arthur Schopenhauer)

RESUMO

EFEITO DOS MÉTODOS DE ENVELHECIMENTO ARTIFICIAL SOBRE A RESISTÊNCIA DE UNIÃO DE SISTEMAS ADESIVOS APLICADOS À DENTINA

AUTORA: Bruna Gabrielle da Silva Sutil
ORIENTADOR: Alexandre Henrique Susin

No presente trabalho serão apresentados dois estudos acerca dos métodos de envelhecimento artificial mais comumente utilizados, bem como relacionar o método de degradação que possui maior influência sobre as diferentes classes de adesivos. O primeiro estudo, *in vitro*, avaliou qual protocolo de envelhecimento tem maior efeito deletério sobre a resistência de união ao microcisalhamento (μSBS), de quatro classes diferentes de sistemas adesivos. 150 terceiros molares (n=5) foram preparados e divididos aleatoriamente de acordo com os métodos de envelhecimento (armazenamento em água – EA por 24 horas ou 6 meses, em hipoclorito de sódio – NaOCl por 1 ou 5 horas, termociclagem – TC, em regime de 10.000 ou 30.000 ciclos) e abordagem/classe adesiva (adesivo de condicionamento ácido prévio de 3 passos – Scotchbond Multi- Purpose (SBMP), adesivo de condicionamento ácido prévio de 2 passos – Single Bond 2 (SB2), adesivo autocondicionante de dois passos – Clearfil SE Bond (CSE) e adesivo universal – Scotchbond Universal nas estratégias autocondicionante (SBU/SE) e de condicionamento ácido prévio (SBU/ER). Os resultados de μSBS foram submetidos à análise de variância a dois fatores e ao teste de Bonferroni (p = 0,05), além da análise de Kaplan-Meier para produzir as curvas de sobrevivência. Os resultados demonstraram que os fatores método de envelhecimento e adesivo, bem como a interação dos fatores foram estatisticamente significantes (p = 0,00). O protocolo que teve o maior efeito deletério sobre a resistência adesiva foi a TC sob o regime de 30.000 ciclos para os adesivos SBMP, CSE, SBU/SE. A análise de Kaplan-Meier revelou que os métodos de envelhecimento influenciaram de forma diferente cada sistema adesivo. Conclui-se que os métodos de envelhecimento promovem efeitos distintos para cada uma das classes de adesivos, exceto para o armazenamento em água por 6 meses, que não afetou negativamente a resistência de união dos adesivos testados. No segundo estudo, foi executada uma revisão metodológica da literatura, abrangendo o período de 2011 a 2021, para estabelecer o método de envelhecimento artificial mais frequente ao se realizar testes de resistência adesiva, e também verificar se há alguma relação entre o protocolo escolhido e o sistema adesivo testado. Para esse estudo, foram aplicados os seguintes critérios de inclusão: estudos originais publicados em língua inglesa, estudos *in vitro* apresentando algum método de envelhecimento artificial, com experimentos realizados em dentina e que apresentasse algum teste de resistência de união. Dos 5.248 artigos encontrados na busca, 387 estudos foram selecionados depois da avaliação preliminar e remoção de duplicatas. Dois examinadores independentes aplicaram os critérios de inclusão, resultando em 27 artigos selecionados para serem lidos na íntegra. Os dados da metodologia e dos resultados dos estudos foram revisados e extraídos, para identificar o método de envelhecimento e sistemas adesivos mais frequentemente utilizados. Observou-se que a degradação em água, com períodos de armazenamento variados, e a termociclagem com diferentes regimes de ciclos foram os métodos de envelhecimento mais frequentes na literatura selecionada. Dentre os adesivos testados, o mais utilizado foi o sistema adesivo universal. No entanto, não foi encontrada uma correlação entre o protocolo de envelhecimento e o sistema adesivo testado.

Palavras-chave: Degradação. Dentina. Envelhecimento artificial. Resistência adesiva. Sistemas adesivos.

ABSTRACT

EFFECT OF ARTIFICIAL AGING METHODS ON THE BOND STRENGTH OF ADHESIVE SYSTEMS APPLIED TO DENTIN

AUTHOR: Bruna Gabrielle da Silva Sutil
ADVISOR: Alexandre Henrique Susin

In the present work, two studies on the most commonly used methods of artificial aging will be presented, as well as relating the degradation method that has the greatest influence on the different classes of adhesives. The first paper, an *in vitro* study, evaluated which artificial aging method more negatively affects the microshear bond strength (µSBS) of four categories of adhesive systems. 150 caries-free human third molars (n=5) were prepared and randomly divided according to the aging method (water storage for 24 hours or 6 months - WS, sodium hypochlorite storage for 1 or 5 hours – NaOCl, thermocycling with 10,000 or 30,000 thermal cycles – TC), and adhesive/approach (a three-step, etch-and-rinse - Scotchbond Multi-Purpose (SBMP); a two-step, etch-and-rinse - Single Bond 2 (SB2); a two-step, self-etch - Clearfil SE Bond (CSE) and an universal adhesive- Scotchbond Universal applied in self-etch (SBU/SE) and etch-and-rinse (SBU/ER) mode. µSBS results were subjected to two-way ANOVA and post hoc Bonferroni tests ($p<0.05$), in addition to the Kaplan-Meier analysis to produce the survival curves. The results showed that the aging method and adhesive factors, as well as the interaction of the factors were statistically significant ($p = 0.00$). The more deleterious aging method was 30,000 thermo cycles which affected the SBMP, CSE and SBU/SE adhesives. Kaplan-Meier survival curves revealed that aging methods influenced each adhesive system differently. It is concluded that the aging methods promote distinct effects for each of the adhesive classes, except for storage in water for 6 months, which did not affect the bond strength of the adhesives negatively. In the second study, a methodologic review was carried out, covering the literature from 2011 to 2021, to establish the most frequent artificial aging method when performing adhesive strength tests, and also to verify if there is any correlation between the chosen protocol and the adhesive system tested. For this study, the following inclusion criteria were applied: original study, published in English, in vitro studies showing some method of artificial aging, with experiments performed on dentin and that presented some microbond test. From a total of 5,248 articles found in the search, 387 studies were selected after preliminary evaluation and removal of duplicates. Two independent reviewers applied the inclusion criteria, resulting in 27 articles screened to be read in full. Methodology and results data were reviewed and extracted to identify the most frequently used aging method and adhesive systems. It was observed that aging in water, with varied storage periods, and thermocycling with different number of cycles were the most frequent aging methods in the selected literature. Among the tested adhesives, the most used was the universal adhesive system. However, no correlation was found between the aging protocol and adhesive system tested.

Keywords: Adhesive system. Artificial aging. Bonding Strength. Degradation. Dentin.

SUMÁRIO

1 INTRODUÇÃO

A constante evolução dos materiais restauradores, bem como o aumento da demanda por procedimentos estéticos adesivos permitiram que novos sistemas adesivos fossem introduzidos no mercado, sendo aprimorados em relação às técnicas adesivas e a sua composição química. De modo geral, os sistemas adesivos são responsáveis pela união do material restaurador às estruturas dentárias (CHEN et al., 2015; FREEMAN et al., 2012; LAWSON et al., 2015; LOGUERCIO et al., 2015). É através da interação micromecânica entre o agente de união e o esmalte e dentina, que ocorre essa união aos substratos dentais. Esse processo, que envolve a substituição de minerais dos tecidos duros por monômeros resinosos, chamado hibridização, cria em termos moleculares, uma mistura de polímeros adesivos e tecidos dentais, resultando na camada híbrida. Nesse processo, as fibras colágenas expostas da dentina desmineralizada são impregnadas pelos componentes monoméricos do adesivo (NAKABAYASHI et al., 1982; NAKABAYASHI et al., 1996).

Por ter constituintes hidrofílicos em sua composição, a camada híbrida é suscetível à atração de água e, consequentemente, à degradação da sua interface (DE MUNCK et al., 2005). Em virtude disso, apesar da adequada resistência de união imediata dos adesivos contemporâneos, a interface adesiva continua sendo um ponto crítico para a manutenção da estabilidade adesiva ao longo do tempo e, dessa forma, ela é responsável pelo sucesso clínico das restaurações (FRANÇA et al., 2007; OKUDA et al., 2002)

Dessa forma, muitos fatores podem afetar a qualidade e durabilidade dessa interface, influenciando o comportamento mecânico das restaurações. Assim, observa-se que a maior resistência adesiva ocorre nas primeiras 24 horas após aplicação do adesivo e, à medida que o tempo passa, vai reduzindo sua resistência, influenciada por fatores como tempo em si, meio de armazenamento e tipo de adesivo (LELOUP et al. 2001, SANO et al., 2006; RINASTITI et al., 2011). O ambiente oral constitui um desafio à integridade da interface de união, pois a presença de fluidos, saliva, bebidas e alimentos que alteram fisica e quimicamente o meio, bem como as alterações de temperatura, fazem com que o processo de degradação hidrolítica dessa interface seja facilitado; consequentemente, diminuindo a adesão e produzindo deteriorações marginais que poderão levar à falha da restauração. (AMARAL et al., 2007; ANCHIETA et al., 2015; BRESCHI et al., 2008; DE MUNCK et al., 2003; DE MUNCK et al., 2005; RINASTITI et al., 2011).

Em virtude disso, a durabilidade de união deve ser testada a longo prazo (OKUDA et al., 2002). Métodos de envelhecimento *in vitro* que simulam as condições do ambiente oral são adequados em prever o comportamento de materiais, e funcionam como filtros no momento de selecionar quais materiais merecem seguir adiante com testes *in vivo* (DENG et al. 2014). Dessa forma, protocolos experimentais padronizados de testes *in vitro* podem prever as mudanças que acontecem a nível estrutural e mecânico das restaurações, fornecendo informações importantes acerca da durabilidade de união (CARRILHO et al., 2004; DE MUNCK et al., 2005; DENG et al., 2014; HEINTZE et al., 2015; MAKISHI et al., 2016; MORRESI et al., 2014; SAI et al., 2016; YAMAUTI et al., 2003). Dentre os protocolos experimentais de envelhecimento *in vitro*, os mais comumente utilizados são o envelhecimento em água, ciclagem térmica e armazenamento em solução de hipoclorito de sódio (AMARAL et al., 2007; DE MUNCK et al., 2005; DE MUNCK et al., 2007; DENG et al., 2014; GALE et al., 1999; TOLEDANO et al., 2006; YAMAUTI et al., 2003).

O envelhecimento em água é o método de degradação artificial mais comum. Nessa técnica, os espécimes são imersos em água, a uma temperatura de 37º C por um período de tempo que varia entre os estudos, de alguns meses a anos (AMARAL et al., 2007; DE MUNCK et al., 2005). O efeito do armazenamento em água sobre a camada híbrida se dá por meio da degradação hidrolítica de suas fibras colágenas, contribuindo para a redução da resistência adesiva ao longo do tempo (AMARAL et al., 2007; ANCHIETA et al., 2015; HASHIMOTO et al., 2003; HASHIMOTO et al., 2009; MASARWA et al., 2016; RINASTITI et al., 2011). O processo de envelhecimento água, também pode aumentar a sorção de água de materiais poliméricos (AMARAL et al., 2007; REIS et al., 2003; REIS et al., 2010). Maior sorção de água tem sido relacionada aos monômeros hidrofílicos e dessa forma, nos adesivos polimerizados, a sorção pode comprometer as propriedades mecânicas do adesivo, pois a água absorvida com facilidade causa um efeito plastificador de suas moléculas (BAHROLOLUMI et al., 2017; HOSAKA et al., 2010; MORTIER et al., 2004; MUSANJE et al., 2001; REIS et al., 2013).

No protocolo de ciclagem térmica, os espécimes são submetidos a mudanças de temperatura cíclicas através da imersão em água, simulando as condições orais. Além do efeito da degradação da água sobre os espécimes, a alternância cíclica de temperatura (quente e frio) gera tensões de contração e expansão repetitivas na interface adesiva, levando a gaps na interface; por outro lado, as altas temperaturas podem acelerar a deterioração hidrolítica da camada híbrida (AMARAL et al., 2007; DE MUNCK et al., 2005; GALE et al., 1999; KITASAKO et al., 2000; MORRESI et al., 2014). Embora haja a sugestão de que 10.000 ciclos corresponderiam a um ano de função *in vivo*, o regime de termociclagem varia consideravelmente entre os estudos, o que poderia dificultar uma padronização do teste

(AMARAL et al., 2007; CHEN et al., 2015; DE MUNCK et al., 2005; HARIRI et al, et al., 2012; KAMEL et al., 2014; MAKISHI et al., 2016; SAI et al., 2016; TSUJIMOTO et al., 2016; TSUJIMOTO et al., 2016a; TSUJIMOTO et al.,2017 ;WAGNER et al., 2014; WANG et al., 2017).

O hipoclorito de sódio (NaOCl), um agente proteolítico não específico também vem sendo utilizado como método de envelhecimento (DENG et al., 2014). Alguns estudos demonstraram que o envelhecimento em solução de NaOCl a 10%, por 5h, foi eficiente em dissolver a camada híbrida e diminuir os valores de resistência de união, tanto para adesivos etch-and-rinse quanto self-etch (MONTICELLI et al., 2007; TOLEDANO et al., 2006 ; YAMAUTI et al., 2003). A solução de NaOCl a 10% atua como um agente desproteinizante, removendo componentes orgânicos da interface adesiva como as fibras colágenas não infiltradas pelo adesivo, mimetizando o que ocorreria no condicionamento ácido, levando à degradação da camada híbrida e consequentemente, a diminuição da resistência de união (AMARAL et al., 2007; DE MUNCK et al., 2007; DENG et al., 2014; TOLEDANO et al., 2006; YAMAUTI et al., 2003).

Embora haja na literatura diversos estudos em que se utilizam os métodos de envelhecimento artificial com o intuito de diminuir a resistência mecânica (CUEVAS-SUÁREZ et al., 2019; DENG et al., 2014; NICOLOSO et al., 2019; TEIXEIRA et al., 2021), ainda não há um consenso sobre qual protocolo específico possui maior efeito deletério sobre a resistência de união, para as diferentes classes de adesivos e estratégias adesivas. Nesse contexto, a presente tese foi delineada e dividida em dois estudos, com o intuito de tentar responder a questão acima.

O primeiro artigo, intitulado **“Artificial aging methods: Effects on the bond strength of one, two and three-step adhesive systems”**, é um estudo *in vitro*, em que se avaliou qual método de envelhecimento artificial tem maior influência negativa sobre a resistência de união de quatro classes diferentes de sistemas adesivos.

O segundo artigo, que trata de uma revisão metodológica, está intitulado **“Is there a correlation between the aging method and adhesive system used influencing the bond strength? A methodologic review”**. A partir da literatura atual, esse estudo propôs-se a identificar, através da revisão de outros estudos *in vitro*, os métodos de envelhecimento que estão sendo utilizados ao se avaliar a resistência adesiva, e tentar estabelecer o protocolo mais utilizado para cada tipo de sistema adesivo.

2 ARTIGO 1 – ARTIFICIAL AGING METHODS: EFFECTS ON THE BOND STRENGTH OF ONE, TWO AND THREE-STEP ADHESIVE SYSTEMS

Este artigo será submetido à publicação no periódico *Operative Dentistry*, ISSN: 0361-7734, Fator de Impacto: 2.390, Qualis CAPES: A1. As normas para publicação estão descritas no Anexo A.

Artificial aging methods: Effects on the bond strength of one, two and three-step adhesive systems

BGS Sutil[a], GS Teixeira[b], DZ Righes[c], AH Susin[d]

[a] Bruna Gabrielle da Silva Sutil, DDS, MS, PhD student, Post-Graduate Program in Dentistry Sciences, Federal University of Santa Maria
Postal address: Roraima Avenue, 1000, 26F Building, Room 2248, Santa Maria RS 97105-900, Brazil.
E-mail address: brunasutil@hotmail.com
Telephone number: +55 55 99105 7975
ORCID ID: https://orcid.org/0000-0003-2709-5620

[b] Gabriela Simões Teixeira, DDS, MS, PhD student, Post-Graduate Program in Dentistry Sciences, Federal University of Santa Maria
Postal address: Roraima Avenue, 1000, 26F Building, Room 2248, Santa Maria RS 97105-900, Brazil.
E-mail address: gsimoesteixeira@gmail.com
Telephone number: +55 55 99958 0022
ORCID ID: https://orcid.org/0000-0003-2167-2204

[c] Danielle Zorzo Righes, DDS, MS, PhD student, Post-Graduate Program in Dentistry Sciences, Federal University of Santa Maria
Postal address: Roraima Avenue, 1000, 26F Building, Room 2248, Santa Maria RS 97105-900, Brazil.
Telephone number: +55 55 99613 6521
E-mail address: righesdz@gmail.com
ORCID ID: https://orcid.org/0000-0002-7552-7224

[d] Alexandre Henrique Susin, DDS, MS, PhD, Department of Restorative Dentistry, Federal University of Santa Maria
Postal address: Roraima Avenue, 1000, 26F Building, Room 2248, Santa Maria RS 97105-900, Brazil.
Telephone number: +55 55 98138 4096
E-mail address: alexandre.susin@ufsm.br
ORCID ID: https://orcid.org/0000-0002-7083-6028

[a] **Corresponding author:**
Bruna Gabrielle da Silva Sutil, DDS, MS, PhD student, Post-Graduate Program in Dentistry Sciences, Federal University of Santa Maria
Postal address: Roraima Avenue, 1000, 26F Building, Room 2248, Santa Maria RS 97105-900, Brazil.
E-mail address: brunasutil@hotmail.com
Telephone number: +55 55 99105 7975
ORCID ID: https://orcid.org/0000-0003-2709-5620

Artificial aging methods: Effects on the bond strength of one, two and three-step adhesive systems

Running title: Effect of the artificial aging protocols on the adhesive strategies

Summary

To determine which artificial aging method more negatively affects the dentin bond strength on each studied adhesive system/approach tested under µSBS. A total of 150 caries-free human third molars were prepared and randomly divided according to the factors (n = 5): aging method (water storage for 24 hours or 6 months, subjected to 10,000 or 30,000 thermal cycles; and sodium hypochlorite [NaOCl] storage for 1 or 5 hours, adhesive/approach (a three-step, etch-and-rinse - Scotchbond Multi-Purpose; a two-step, etch-and-rinse - Single Bond 2; a two-step, self-etch - Clearfil SE Bond and a universal adhesive, which was applied under the protocols of etch-and-rinse (two-step) and self-etch (one step) - Scotchbond Universal. The microshear bond strength test results (in MPa) were subjected to two-way analysis of variance (ANOVA) with post hoc Bonferroni tests ($p<0.05$) and Kaplan-Meier analysis to produce survival curves. The failure pattern had results expressed in percentages. Two-way ANOVA revealed that the "aging method", "adhesive/approach" factors, and the "adhesive/approach*aging method" interaction had a significant effect on the bond strength (all of them with $p = 0.00$). The aging method affected the bond strength of each adhesive differently. The more deleterious aging method was 30,000 thermo cycles which affected the three-step, the two-step self-etch and the one-step adhesive. Kaplan-Meier survival curves confirmed the different influence of aging methods on each adhesive. The more frequent failure pattern was the adhesive in any adhesive/approach and aging method. Artificial aging methods affect the bond strength of an adhesive system differently. The 6-month water aging method did not affect the performance of any of the adhesives, and the other methods studied promoted distinct effects on the bond strength of each adhesive.

Clinical significance: The effectiveness of bonding techniques may decrease with aging. Thus, there are different protocols for artificial aging which simulate oral conditions; however, there is still no consensus on which one has the most deleterious effect on the different adhesive strategies.

Keywords: Artificial aging; Interface degradation; Bond strength; Hydrolysis; Adhesive systems.

INTRODUCTION

The stress that the hybrid layer (HL) suffers due to thermal and mechanical variations in the oral environment associated to the ester bond hydrolysis promote a continuous degradation process which contributes to reduce the bond strength over time,[1] making the resin/dentin interface critical to debonding.

Mechanical forces from chewing, thermal variations, volumetric alterations, and the pH from food and beverages cause stresses in the adhesive interface which result in a gradual loss of the bond strength, since the HL is a micrometric structure formed by a mixture of dentin organic matrix, residual hydroxyapatite crystallites, resin monomers, solvents and collagen fibrils which are intimately joined to the dental substrate and the resin composite.[2,3]

Clinical evaluations are the most accurate way to evaluate the performance of restorative materials, however they require long-term studies and can be difficult to standardize and to control bias.[4] In vitro studies of mechanical properties such as micromechanical tests performed after the specimens are submitted to an artificial aging process can be used to predict the performance of the bonded interface between resin composite and dental substrate in the long-term as a way to reach faster, more controlled and reliable tests regarding bonding stability.[5,6]

The most frequent artificial aging methods mentioned in the literature are: thermocycling (from 10,000 to 30,000 cycles) which uses intermittent changing of cold and hot water to promote hydrolysis simulation of unprotected collagen fibrils by contraction and expansion stress of the adhesive interface;[7-9] long-term water storage at 37°C (from 3 to 12 months) which simulates the oral environment conditions;[10] and immersion in sodium-hypochlorite solution [1 to 10%] from 1 to 5 hours, in which the proteolytic properties degrade the organic resin and unprotected collagen fibrils.[11]

In a previous study was evaluated the effect of different artificial aging methods on the bond strength of a universal adhesive.[6] Although the literature has shown different studies regarding the effect of aging methods on the bond strength,[6,9,11,12] none of them have determined which one was the most effective method to decrease the bond strength to a specific adhesive. Thus, to the best of the authors' knowledge, this issue has not been previously addressed.

The aim of the present study was to determine which artificial aging method more negatively affects the dentin bond strength in each studied adhesive system/approach tested under µSBS. The null hypothesis is that the aging methods do not affect the µSBS of the tested adhesive systems differently.

MATERIAL AND METHODS

Ethics and experimental design

This study *in vitro* was submitted to and approved by an Institutional Ethics Committee. The 150 caries-free third molars were obtained from an Institutional tooth bank up to three months after extraction by clinical indications and were stored in a 5% chloramine T solution at 4°C before being used in this study.

The adhesive systems were selected to fulfill the following prerequisites:

- a three-step, etch-and-rinse (SBMP);
- a two-step, etch-and-rinse (SB2);
- a two-step, self-etch (CSEB), and
- a universal adhesive, which was applied under the etch-and-rinse (two-step) – SBU (ER) and self-etch (one step) – SBU (SE) protocols.

The artificial methods were:

- water storage for 24 hours as control group, and for 6 months (W24 h and W6 m);
- thermocycling for 10,000 and for 30,000 cycles (T10,000 and T30,000),
- storage in 10% sodium hypochlorite solution for 1 hour and for 5 hours (SH1 h and SH5 h).

The representation of the experimental design and the details of the artificial aging methods are in Table 1. Table 2 shows the materials, manufacturer, batch number, chemical composition and approach of the materials used in this study.

Specimen preparation

The teeth roots were cut off and the crown was mesio-distally sectioned with a low-speed water cooled diamond-saw disc in a cutting machine (Labcut 1010, Extec Inc, Enfield, Connecticut, United States). Both sections of each tooth were embedded in PVC rings with self-curing acrylic resin (JET, Classico Art Od, São Paulo, Brazil) to expose the dentin surface. The smear layer was standardized in thickness by using a 600-grit silicon carbide paper for 1 minute under water irrigation in a mechanical polishing machine[13] (EcoMet 250; Buehler, Lake Bluff, Illinois, United States). The samples were randomly divided into 30 groups (n=5) according to the factors: “adhesive/approach” (5) and aging method (6), as mentioned above (Table 1).

Bonding and restoration procedures

The adhesive systems were applied according to the manufacturer’s instructions (Table 2). Next, 3 starch tubes (Renata; Pastificio Selmi, Londrina, Parana, Brazil) of 1 mm height and 0.96 mm internal diameter hole were positioned on the mesial, central, and distal thirds over dentin before adhesive photocuring[14]. A light-emitting diode (Emitter D— Shuster Eq. Od. Ltd, Santa Maria, Brazil) with 900 mW/cm^2 light output was used to photocure the adhesive for 10 seconds, and then a resin composite (Filtek Z350 XT shade A2E; 3M ESPE Dental Products) was used to fill in the starch tubes, followed by light activation for 20 seconds.

Finally, the samples were stored for 24 hours in distilled water at 37°C, and the starch matrix was removed with air-water spray. The dentin/resin interfaces were examined using a stereomicroscope at 10x magnification (Discovery V20, Zeiss; Oberkochen, Germany) to detect any defects such as bubbles or voids. The specimens with defects were replaced.

Microshear bond strength test

A wire-loop technique was chosen for performing the µSBS tests after the aging process.[15] A 0.2-mm stainless steel wire was attached to a specific device in a universal testing machine (EMIC DL 1000; Instron, S.J. Pinhais, Brazil) and the shear load was applied at a crosshead speed of 1.0 mm/min with cell of 1 kN until failure of the bonded restoration. Parallelism between the wire, the load cell, and the adhesive-resin interface were carefully performed during the test, as well as to make the load application as close as possible to the bond interface.

Failure analysis

After the test, the interface was evaluated under stereomicroscopy (Discovery V20, Carl-Zeiss, Oberkochen, Germany) at 30x magnification to classify the failure mode according to the following characteristics: Adhesive (**A**) – failure only occurred at the adhesive line; Mixed (**M**) – failure occurred at the adhesive line and involved a portion of dentin or resin; Cohesive in dentin (**CD**) – failure occurred exclusively in dentin; and Cohesive in resin (**CR**) – failure occurred exclusively in resin. All restorative procedures and mechanical tests were performed by a previously trained operator.

Statistical analysis

This study considered the tooth as the statistical unit, and the mean bond strength of the pins of each tooth was considered as the result for each unit. Normal distribution of the bond strength data was assumed after Kolmogorov-Smirnov tests, and the µSBS means were analyzed by Two-Way analysis of variance (adhesive versus aging method). The Bonferroni's post hoc test at a significance level of 0.05 was performed for multiple comparisons.

A Kaplan-Meier test was additionally performed to produce survival curves and to analyze the significance of the aging methods on each adhesive by p-values from the Log Rank[16,17]. It was used to determine the isolated effects of the aging tests over each adhesive and not to test more ample interactions (i.e. adhesive x adhesive or aging x aging). Censored data were not considered as an event because the survival curves were from the data of the successfully tested specimens, since the specimens pre-testing failed were discarded and substituted. All the statistical tests were performed with the Statistical Package for Social Sciences v.25 program (SPSS, IBM Inc., Armonk, NY).

RESULTS

The µSBS results (in MPa) and standard deviations of experimental groups are summarized in Table 3. Table 4 shows a complimentary interpretation using arrows of the up-down of the bond strength. The Two-Way Analysis of variance showed that the adhesive and aging factors and the adhesive*aging interaction presented statistically significant differences (p=0.00). The comparisons in columns show the effects of the aging methods on each adhesive (i.e. it shows the performance of each of the adhesives submitted to each of the aging methods). The CI values were extracted from the Kaplan-Meier analysis referring to the "survival time". The left and right values respectively represent the lower and upper 95% confidence levels.

Since the aim of this study is to define the effects of aging methods on the bond strength, the W24 h group of each adhesive was assumed as the control group (since the specimens were submitted to the µSBS test 24h after the restoration was built). The horizontal comparisons show the effect of each aging method posted on each column for the same adhesive in the line. The most deleterious aging methods to the SBMP were SH1 h and SH5 h (16.39 MPa to 10.56 and 10.96 MPa, respectively). The T30,000 aging was more deleterious to CSEB compared to the control (21.13 MPa to 14.38 MPa, respectively) or to the SBU(SE) group (20.21 MPa to 15.98 MPa). The SBU with the ER approach had better bond strength to the SH1 h group compared with the thermocycled groups and the control group. The same adhesive showed to be more negatively influenced by aging in water and in thermocycling than the SH5 h group.

The effect of all aging methods applied to each adhesive analyzed by the Kaplan-Meier test showed that they significantly affected the survival distribution (Mantel-Cox Log Rank, p=0.00 (Figure 1).

The predominant failure pattern was the adhesive for all the adhesives used in any of the aging methods, with all patterns similarly distributed in percentage ranging from: A, 71 to 80; M, 8 to 13; CR, 6 to 11, and CD, 4 to 9 (Figure 2).

DISCUSSION

In a previous study[6], the aging methods were focused on defining the most deleterious method concerning maintenance of the bond strength of a universal adhesive (Scotchbond Universal) under two approaches (i.e., self-etch and etch-and-rinse to enamel and dentin substrates). In that study, it was found that the T20,000 and T30,000 groups significantly reduced the bond strength to enamel substrate using the self-etch approach. It was also found that the storage in sodium hypochlorite for 5 hours provided an increase in bond strength to dentin for both approaches.

The focus herein was to show the influence of the aging methods on the bond strength of 4 commercial brands of adhesive systems (1 of them used in a self-etch and in the etch-and-rinse approach) to dentin. They were chosen to cover the approaches of etch-and-rinse adhesive systems of two steps (SBU used as etch-and-rinse and SB2); etch-and-rinse adhesives of three steps (SBMP) and self-etch adhesives of one step (SBU as self-etch) and a self-etch adhesive of two steps (CSEB).

The natural aging process by hydrolysis of the HL can be explained by the mass loss promoted by the water addition to the ester bonds resulting in the breaks of covalent bonds between the polymers.[18-21] It is also expected that the artificial aging methods are responsible for decreasing the bond strength since they directly influence the degradation of the resin/dentin interface by affecting each individual component of HL or by their synergic effects. The aging processes that the groups were submitted to in this study affected the adhesives' performance differently, regardless of the number of the steps and approach. Although the literature has reported decrease in bonding to SB2 after water aging[22,23], our study found that it was stable for all aging processes applied. The substantial difference between SB2 and SBMP is the presence of a co-polymer of itaconic and acrylic acid in SB2;

in addition, a different number of steps were implemented, namely SB2 with two-steps and SBMP had three-steps. The current literature presents a gap in investigations about the level of influence of the number of steps in the immediate and long-term bond strength. Studies concerning bonding stability of adhesives based on the number of steps normally discuss chemical variations, protocol alterations and the influence of the dental substrate.[24-26] We found that the SBMP had its bond strength affected in the T30,000, SH1 h and SH5 h groups, however it was not affected in the W6 m nor in the T10,000 groups. It was also found that the different aging protocols showed differences from the control, T30,000 and SH1 1 groups which were affected in different levels, since the SH1 h group was also significantly different compared to T30,000, reinforcing the statement that each aging method acts on a specific property of the HL resulting in distinct outcomes[3].

The CSEB is considered as a "gold standard" between the self-etch adhesives since it performed "excellent" in the *in vivo* and *in vitro* tests.[27] Even so, it showed that the bond strength was affected by both thermocycling times tested. Regarding the HL micromorphology, even though it was demonstrated that adhesives which present methacryloyloxi-decyl-dihydrogen-phosphate (MDP) showed superior stability after 12 months in water storage[28] as they are able to react with the hydroxyapatite from the dentin to form an interfacial nano-layered bonding structure in the adhesive interface, the CSEB showed that this particular characteristic did not eliminate the possibility of the HL degradation by hydrolysis of the polymer[29] chain, reinforcing that the presence of water may also have caused a reduction in the frictional forces between the polymer chains, as well as hydrolysis of the filler-matrix interfaces, thus decreasing the bonding and the mechanical properties of the resin.[30] As the SBU also contains MDP, similar behavior with CSEB would be expected, but the SBU generally showed be more affected by thermocycling in both approaches.

The self-etch approach in the sodium hypochlorite storage times performed similarly to the control, but an unexpected result was found for the etch-and-rinse approach; it showed an increase of bond strength for both (1 and 5 h) times. In a direct comparison between approaches to SBU for each aging, it was noticed that the etch-and-rinse had lower performance than the self-etch approach, indicating that the acid conditioning was responsible for worse performance[31-33], even though it has been reported that thermocycling also did not affect the bonding stability[32], in addition to the approach.[34]

The Log Rank (p=0.00) showed that all aging methods affected the adhesives. Estimation of the cumulative failure distribution function runs in statistics under a general name of survival analysis.[35] In the case of this study, the Kaplan-Meier methodology was used to estimate the primary outcome represented by the stress limit applied to the specimen. The survival analysis determines the isolated effects of the aging tests over each adhesive and not to test more ample interactions (i.e. adhesive x adhesive or aging x aging). Censored data were not considered for any event because the estimation was calculated for the data which was successfully tested; therefore, specimens with pre-testing failures were discarded and substituted in this study. The 95% confidence interval shown in Table 3 has the lower confidence level in the left value, while the right one represents the upper confidence level. This means that 95% of the specimens would fracture between these MPa values.

In view of the above, this study found that the artificial aging methods affected the bond strength differently, regardless of the adhesive system, thereby rejecting the null hypothesis. Our study also showed that the distribution of fracture patterns was not a relevant factor in all groups in which the bond strength was affected by aging. The distribution was similar in percentages compared with the control group, corroborating previous studies[36], although it is not in accordance with other findings which have been demonstrated in increasing the cohesive in resin and mixed failures between adhesive and composite resin for aged groups.[37] It could be speculated that these discrepancies may be in consequence of the use of non-standardized criterion of pattern classification or due to different devices to examine the interface, such as: scanning electron microscopy[36] or stereomicroscope at different magnification levels.[6]

Several artificial aging methods should be considered to simulate the stresses which occur with the restoration. It seems clear that there are discrepancies in the effects among the artificial methods applied in *in vitro* tests. Moreover, the velocity that each aging needs to promote the deleterious effects in bond strength could be further investigated. In view of the limitations to achieve better similarity between in *in vitro* and clinical studies, artificial aging methods must be more explored to seek out the best alternative in aging to be applied to each specific adhesive and/or to each adhesive approach.

CONCLUSION

The artificial aging methods affected each one of the adhesive systems differently. An influencing pattern was not demonstrated. The W6 m aging did not affect the performance of the adhesives compared to the control group, although the other methods promoted distinct effects on the bond strength of each adhesive.

REFERENCES

1. Frassetto A, Breschi L, Turco G, Marchesi G, Di Lenarda R, Tay FR, Pashley DH & Cadenaro M. (2016) Mechanisms of degradation of the hybrid layer in adhesive dentistry and therapeutic agents to improve bond durability–a literature review. *Dental Materials* **32(2)** e41–e53.
2. Nakabayashi, N, Nakamura, M, & Yasuda, N (1991) Hybrid layer as a dentin-bonding mechanism. *Journal of Esthetic Dentistry*. **3(34)** 133-138.
3. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, & Dorigo ES (2008) Dental adhesion review: Aging and stability of the bonded interface *Dental Materials*, **24(1)** 90-101.
4. Van Meerbeek B, Peumans M, Poitevin A, Mine A, Van Ende A, Neves A, & De Munck J (2010) Relationship between bond-strength tests and clinical outcomes. *Dental Materials* **26(2)** 100-121.
5. Cardoso MV, Neves AA, Mine A, Coutinho E, Van Landuyt K, De Munck, J & Van Meerbeek B (2011) Current aspects on bonding effectiveness and stability in adhesive dentistry. *Australian Dental Journal* **56(S 1)** 31-44.
6. Teixeira GS, Pereira GKR, & Susin AH (2021) Aging Methods-An Evaluation of Their Influence on Bond Strength. *European Journal of Dentistry* 28(Online ahead of print).
7. Gale MS & Darvell BW (1999) Thermal cycling procedures for laboratory testing of dental restorations *Journal of Dentistry* **27(2)** 89–99.
8. Amaral FL, Colucci V, Palma-Dibb RG, & Corona SA (2007) Assessment of in vitro methods used to promote adhesive interface degradation: a critical review. *Journal of Esthetic and Restorative Dentistry* **19(6)** 340–353.
9. Deng D, Yang H, Guo J, Chen X, Zhang W, & Huang C (2014) Effects of different artificial ageing methods on the degradation of adhesive-dentine interfaces *Journal of Dentistry* **42(12)** 1577–1585.
10. Fukuoka A, Koshiro K, Inoue S, Yoshida Y, Tanaka T, Takatsumi I, Suzuki K, Sano H, & Van Meerbeek B (2011) Hydrolytic stability of one-step self-etching adhesives bonded to dentin *The Journal of Adhesive Dentistry* **13(3)** 243–248.
11. Nicoloso GF, Carvalho MP, Soares FZM, Susin AH, & Rocha RO (2019) Short-term aging and the dentin bond strength of adhesive systems *General Dentistry* **67(3)** 71-76.

12. Cuevas-Suárez CE, Ramos TS, Rodrigues SB, Collares FM, Zanchi CH, Lund RG, Da Silva AF, & Piva E. (2019) Impact of shelf-life simulation on bonding performance of universal adhesive systems *Dental Materials* **35(9)** 204-219.
13. Tay FR & Pashley DH (2001) Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers *Dental Materials* **17(4)** 296–308.
14. Tedesco TK, Montagner AF, Skupien JA, Soares FZ, Susin AH, & Rocha RO (2013) Starch tubing: an alternative method to build up microshear bond test specimens *The Journal of Adhesive Dentistry* **15(4)** 311–315.
15. Foong J, Lee K, Nguyen C, Tang G, Austin D, C Ch'ng C, Burrow MF, & Thomas DL (2006) Comparison of microshear bond strengths of four self-etching bonding systems to enamel using two test methods *Australian Dental Journal* **51(3)** 252–257.
16. Afshar H, Nakhjavani YB , Taban SR , Baniameri Z, & Azam Nahvi A (2015) Bond Strength of 5th, 6th and 7th Generation Bonding Agents to Intracanal Dentin of Primary Teeth *Journal of Dentistry (Tehran)* **12(2)** 90-98.
17. Sonoda H, Banerjee A, Sherriff M, Tagami J, & T.F. Watson TF (2005) An in vitro investigation of microtensile bond strengths of two dentine adhesives to caries-affected dentine *Journal of Dentistry* **33(4)** 335–342.
18. Tay FR & Pashley DH. Have dentin adhesives become too hydrophilic? (2003) *Journal Canadian Dental Association* **69(11)** 726–731.
19. Watanabe I & Nakabayashi N (1993) Bonding durability of photocured phenyl-P in TEGDMA to smear layer-retained bovine dentin. *Quintessence International* **24(5)** 335–342.
20. Tay FR, Hashimoto M, Pashley DH, Peters MC, Lai SC, Yiu CK, & Cheong C (2003) Aging affects two modes of nanoleakage expression in bonded dentin. Journal of Dental Research 82(7) 537–541.
21. Betancourt DE, Baldion PA, & Castellanos JE (2019) Resin-Dentin Bonding Interface: Mechanisms of Degradation and Strategies for Stabilization of the Hybrid Layer *International Journal of Biomaterials* ***2019(eCollection)***
22. De Munck J, Shirai K, Yoshida Y, Inoue S, Van Landuyt K, Lambrechts P, Suzuki K, Shintani H, & Van Meerbeek B (2006) Effect of Water Storage on the Bonding Effectiveness of 6 Adhesives to Class I Cavity Dentin *Operative Dentistry* **30(1)** 456-465.

23. Martins GC, Calixto AL, Gomes OMM, Loguercio AD, D'Alpino PHP, & Reis A (2009) Effect of water storage on resin-dentin bond strengths formed by different bonding approaches *Indian Journal of Dental Research* **20(4)** 431-436.
24. Gunaydin Z, Yazici AR, & Cehreli ZC (2016) In Vivo and In Vitro Effects of Chlorhexidine Pretreatment on Immediate and Aged Dentin Bond Strengths *Operative Dentistry* **41(3)** 258-267.
25. Courson F, Bouter D, Ruse ND, & Degrange M (2005) Bond strengths of nine current dentine adhesive systems to primary and permanent teeth *Journal of Oral Rehabilitation* **32(4)** 296-303.
26. Loguercio AD, Luque-Martinez I, Muñoz MA, Cuadros-Sanchéz J, & Reis A (2014) A comprehensive laboratory screening of three-step etch-and-rinse adhesives *Operative Dentistry* **39(6)** 652-662.
27. Mine, A, De Munck J, Cardoso MV, Van Landuyt KL, Poitevin A, Kuboki T, Yoshida Y, Suzuki K, Lambrechts P, & Van Meerbeek B (2009) Bonding effectiveness of two contemporary self-etch adhesives to enamel and dentin *Journal of Dentistry,* **37(11)** 872-883.
28. Anchieta RB, Machado LS, Martini AP, Dos Santos PH, Giannini M, Janal M, Tovard N, Sundfeld RH, Rocha EP, & Coelho PG (2015) Effect of long-term storage on nanomechanical and morphological properties of dentin–adhesive interfaces *Dental Materials* **31(2)** 141-153
29. Yoshida Y, Yoshihara K, Nagaoka N, Hayakawa S, Torii Y, Ogawa T, Osaka A, & Meerbeek BV (2012) Self- assembled nano-layering at the adhesive interface *Journal of Dental Research* **91(4)** 376-381.
30. Makishi P, André CB, Ayres APA, Martins AL, & Giannini M (2016) Effect of Storage Time on Bond Strength and Nanoleakage Expression of Universal Adhesives Bonded to Dentin and Etched Enamel *Operative Dentistry* **41(3)** 305-317.
31. Vermelho PM, Reis AF, Ambrosiano GMB, & Giovanini M (2017) Adhesion of multimode adhesives to enamel and dentin after one year of water storage *Clinical Oral Investigations* **21(5)** 1707–1715.
32. Wagner A, Wendler M, Petschelt A, Belli R, & Ulrich L (2014) Bonding performance of universal adhesives in different etching modes *Journal of Dentistry* **42(7)** 800-807.

33. Zheng-yi Z, Fu-Cong, T, Li-na N, Ochalad K, Chene, C, F Fua, Xiao-yan W, David H. Pashleyd DH, Tay, FR (2016) Defying ageing: An expectation for dentine bonding with universal adhesives? *Journal of Dentistry* **45**(Epub) 43–52.
34. Muñoz MA, Luque-Martinez I, Malaquias P, Hass V, Reis A, Campanha NH, & Loguercio AD (2015) In Vitro longevity of bonding properties of universal adhesives to dentin *Operative Dentistry* **40(3)** 282-292.
35. Stawarczyk B, Tufall S, Roos M, Schmidlin PR, & Lümkemann.N (2018) Bonding of composite resins to PEEK: the influence of adhesive systems and air-abrasion parameters *Clinical Oral Investigations* **22(2)** 763–771.
36. Dacic S, Milan Miljkovic M, Mitic A, Radenkovic G, Marija AA, & Jovanovi M (2020) Influence of etching mode and composite resin type on bond strength to dentin using universal adhesive system *Microscopy Research and Technique* **23(**Online ahead of print) 1–8.
37. Pedrosa VO, França FMG, Turssi CP, & Do Amaral FLB (2018) Effects of caffeic acid phenethyl ester application on dentin MMP-2, stability of bond strength and failure mode of total-etch and self-etch adhesive systems *Archives of Oral Biology* **94** 16–26.

TABLES

Table 1. *Study design: overview of all tested groups. Artificial aging methods (AAM) – characteristics and name, materials, n and performed test.*

	AAM characteristics	AAM name	ADHESIVE / APPROACH	n	TEST
150 human third molars	Samples prepared and immediately stored in distilled water at 37°C for 24 h or 6 months before testing. Water was changed weekly for the longer period (6 months).	W24 h	SBMP	5	wire-loop microshear bond strength
			SB2		
			CSEB		
			SBU (SE)		
			SBU (ER)		
		W6 m	SBMP	5	
			SB2		
			CSEB		
			SBU (SE)		
			SBU (ER)		
	Samples thermocycled 10,000 or 30,000 times at 5–55°C for 30 s each batch, 5 s of transfer time, before testing	T10,000	SBMP	5	
			SB2		
			CSEB		
			SBU (SE)		
			SBU (ER)		
		T30,000	SBMP	5	
			SB2		
			CSEB		
			SBU (SE)		
			SBU (ER)		
	Samples kept immersed in 10% NaOCl solution at room temperature for 1 or 5 h, before testing	SH1 h	SBMP	5	
			SB2		
			CSEB		
			SBU (SE)		
			SBU (ER)		
		SH5 h	SBMP	5	
			SB2		
			CSEB		
			SBU (SE)		
			SBU (ER)		

Table 2. ***Materials used in this study – Manufacturer, chemical composition and approaching.***

Material/ Manufacturer an batch number and approaching	**Composition**	**Bonding Procedure**
Adper Scotchbond Multi Purpose **(3M ESPE, St Paul, MN, EUA)** **(05433)** **Three-step etch-and-rinse**	Scotchbond Universal Etchant: 34% phosphoric acid 2. Primer: HEMA, poli-alkenolic acid, water 3. Adesive: Bis-GMA, HEMA, dimetacrilates, photoinitiator.	1.Applying phosphoric acid, 2.Let undisturbed for 15 s, 3.Rinsing with air-water spray for 5 s, 4.Drying with absorbent paper 5.Applying of primer and immediately air jet for 5 s, 6.Applying of adhesive and immediately air-jet for 5 s, 7.Photocuring for 10 s.
Adper Single Bond 2 **(3M ESPE, St Paul, MN, EUA)** **(190769)** **Two-step etch-and-rinse**	1. Scotchbond Universal Etchant: 34% phosphoric acid 2. Adesive: Bis-GMA, HEMA, nanoparticles of sílica, silane, dimethacrilates, co-polymer of itaconic and acrilic acid (copolimer Vitrebond™), water, ethanol	1.Applying phosphoric acid, 2.Let undisturbed for 15 s, 3.Rinsing with air-water spray for 5 s, 4.Drying with absorbent paper 5.Applying two layers of adhesive and immediately air-jet for 5 s, 6.Photocuring for 10 s.
Clearfil SE Bond **(Kuraray, Co., Osaka, JP)** **(00995)** **Two-step self-etch**	1. Primer: MDP, HEMA, hydrophilic dimethacrylate, camphorquinone, N,N-diethanol p-toluidine, water 2. Adhesive: MDP, BIS-GMA, HEMA, hydrophobic dimethacrylate, camphorquinone, N,N-diethanol p-toluidine, colloidal SiO2.	1.Applying of primer and let undisturbed for 20 s, 2.Applying a gently air –jet for 5 s, 3.Applying of adhesive 4.Photocuring for 10 s.
Scotchbond Universal **(3M ESPE, St Paul, MN, EUA)** **(610588)** **Universal – two-step etch-and-rinse / one-step self-etch**	1. Scotchbond Universal Etchant: 34% phosphoric acid 2. Adhesive: methacryloyloxydecyl dihydrogen phosphate, phosphate monomer, dimethacrylate resins, hydroxyethyl methacrylate, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane	**Etch-and-rinse approach:** 1.Applying phosphoric acid, 2.Let undisturbed for 15 s, 3.Rinsing with air-water spray for 5 s, 4.Drying with absorbent paper 5.Applying of adhesive with agitation for 20 s and immediately air-jet for 5 s, 7.Photocuring for 10 s. **Self-etch approach:** 1.Applying of adhesive with agitation for 20 s and immediately air-jet for 5 s,

		2.Photocuring for 10 s.
Filtek™ Z350 XT **(3M ESPE, St Paul, MN, EUA)** **(187359)** **Nanohybrid resin composite**	Bis-GMA, Bis-EMA, UDMA, TEGDMA, zirconia/silica filler	**Restorative Procedure:** Applying in increments of 2 mm, Photocuring for 20 s.
Abreviations: Bis-GMA: bisphenol-A glycidyldimethacrylate, TEGDMA: triethyleneglycol dimethacrylate; UDMA: urethane dimethacrylate; Bis-EMA: bisphenol-A ethoxylated dimethacrylate; MDP =10-methacryloyloxydecyl dihydrogen phosphate; HEMA: 2-hydroxyethyl methacrylate.		

Table 3. *Overview of descriptive statistics including mean and standard deviation (SD) and Confidence Interval (CI) (α=0.05) for μSBS. Adhesive/aging values in MPa.*

ADHESIVE / AGING	W24 h	*95% CI*	W6 m	*95% CI*	T10,000	*95% CI*	T30,000	*95% CI*	SH1 h	*95% CI*	SH5 h	*95% CI*
SBMP	16.39 (1.40) $^{\text{B a}}$	*15.80 - 6,97*	14.52 (1.20) $^{\text{B ab}}$	*13.92-15.13*	15.80 (1.26) $^{\text{Ba}}$	*13.75 - 17.85*	12.64(2.31$^{\text{A Bb}}$	*11.14 - 14.15*	10.56 (0.82) $^{\text{C c}}$	*9.83 - 11.29*	10.96(1.99)$^{\text{C bc}}$	*10.04 - 11.88*
SB2	14.41 (1.05) $^{\text{B a}}$	*13.64- 15.18*	13.74 (1.07) $^{\text{Ba}}$	*13.17 - 14.30*	13.53 (3.20) $^{\text{Ba}}$	*11.82 - 15.23*	11.45 (2.30)$^{\text{B a}}$	*9.72 - 13.18*	11.10 (2.06) $^{\text{C a}}$	*10.25- 11.96*	10.60 (1.31)$^{\text{C a}}$	*9.66 - 11.54*
CSEB	21.13 (1.11) $^{\text{A b}}$	*20.41- 21.85*	24.03 (1.34) $^{\text{A a}}$	*23.20 - 24.85*	19.63 (1.96) $^{\text{Ab}}$	*18.64 - 20.61*	14.38 (2.98)$^{\text{A c}}$	*12.85 - 15.92*	25.38 (1.20) $^{\text{A a}}$	*24.44- 26.32*	23.04(2.11)$^{\text{A ab}}$	*21.96 -24.12*
SBU(SE)	20.21 (2.36) $^{\text{A b}}$	*19.18- 21.23*	25.20 (1.25) $^{\text{A a}}$	*24.26 - 26.13*	20.49 (3.56) $^{\text{Ab}}$	*18.27 - 22.70*	15.98 (3.69)$^{\text{A c}}$	*14.26 - 17.70*	27.90 (1.81) $^{\text{A a}}$	*27.00- 28.81*	25.14 (2.70)$^{\text{A a}}$	*23.79 - 26.49*
SBU(ER)	13.52 (1.24) $^{\text{B b}}$	*12.7 - 14.29*	12.61 (1.68) $^{\text{B bc}}$	*11.84 - 13.38*	13.15 (1.86)$^{\text{Bbc}}$	*12.30 - 14.01*	10.02 (2.48)$^{\text{B c}}$	*8.45 - 11.60*	17.03 (1.80) $^{\text{B a}}$	*15.92- 18.14*	16.14(2.58)$^{\text{B ab}}$	*14.37 - 17.91*

The different uppercase letters (columns) and the different lowercase letters (lines) indicate statistically significant differences. Bonferroni's Post Hoc test, ($p < 0.05$).

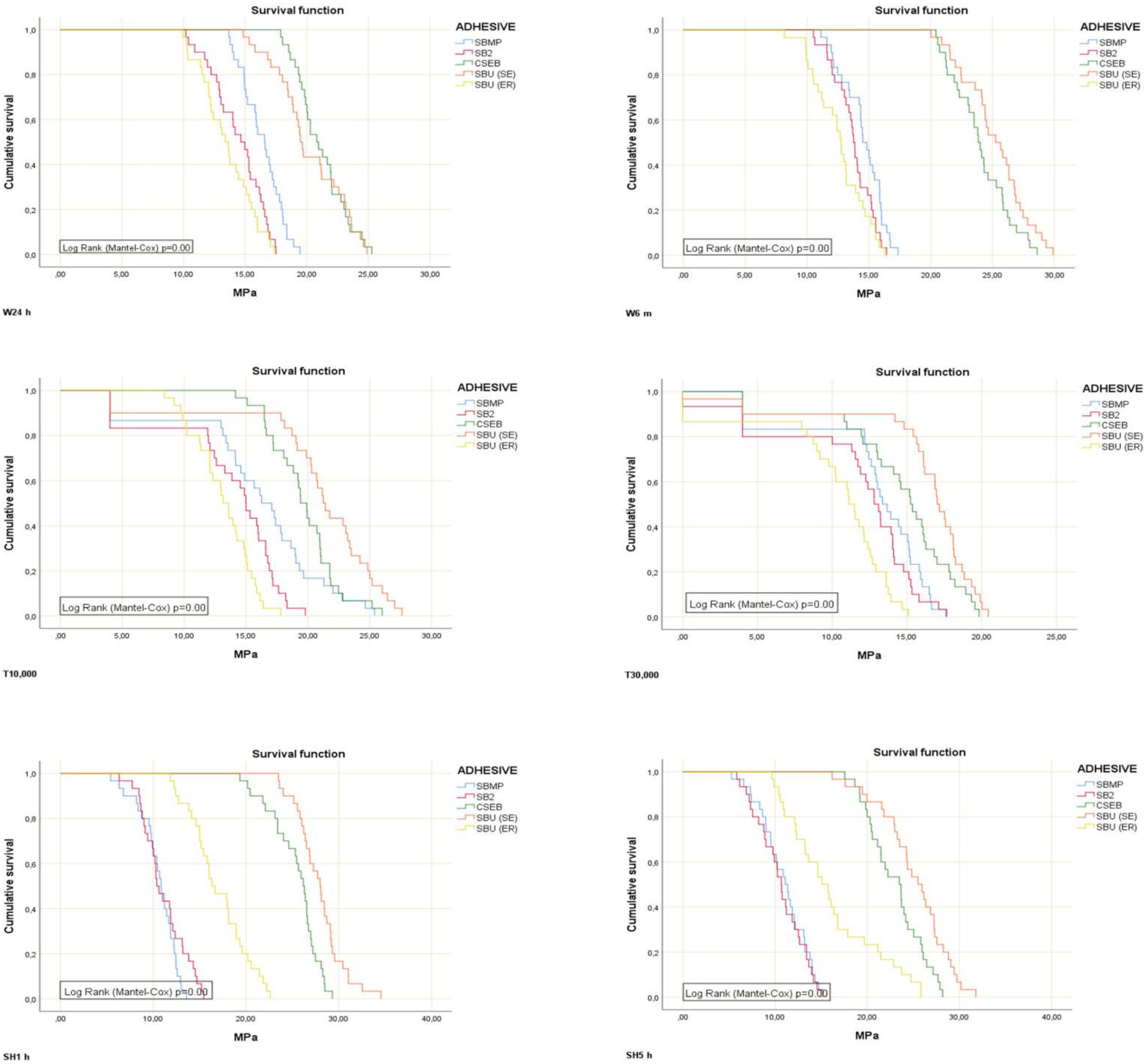
Table 4. *The complimentary interpretation (using arrows) for up-down µSBS of aging methods for each adhesive system/step based on the W24 h (control group).*

		Aging methods					
Adhesive	**Number of steps/approach**	**W24 h**	**W6 m**	**T10,000**	**T30,000**	**SH1 h**	**SH5 h**
SBMP	3 steps - etch-and-rinse	↔	↔	↔	↓ ^{b}	↓ ^{c}	↓ ^{bc}
SB2	2 steps - etch - and-rinse	↔	↔	↔	↔	↔	↔
CSEB	2 steps - self-etch	↔	↔	↓ ^{b}	↓ ^{c}	↔	↔
SBU (SE)	1 step – self-etch	↔	↔	↓ ^{b}	↓ ^{c}	↔	↔
SBU (ER)	2 steps – etch-and-rinse	↔ ^{bc}	↔	↔	↔	↑ ^{a}	↑ ^{a} ↔ ^{b}

In line comparisons to the W24 h (control group): the neutral arrows mean that comparisons presented no statistical differences; the down arrows mean there are statistically significant lower results than the others, and the up arrows mean that there are statistically significant higher results than the others. Alphabetical order means statistical differences, higher to lower bond strength, respectively.

FIGURES

Figure 1. Kaplan-Meier survival distribution curves. Each graph represents an aging method and the distinct curves in the graphs represent the adhesives. The Log Rank compares the aging effect on the adhesive. All have p=0.00, demonstrating to be significantly different regarding the aging effect.



W24 h

W6 m

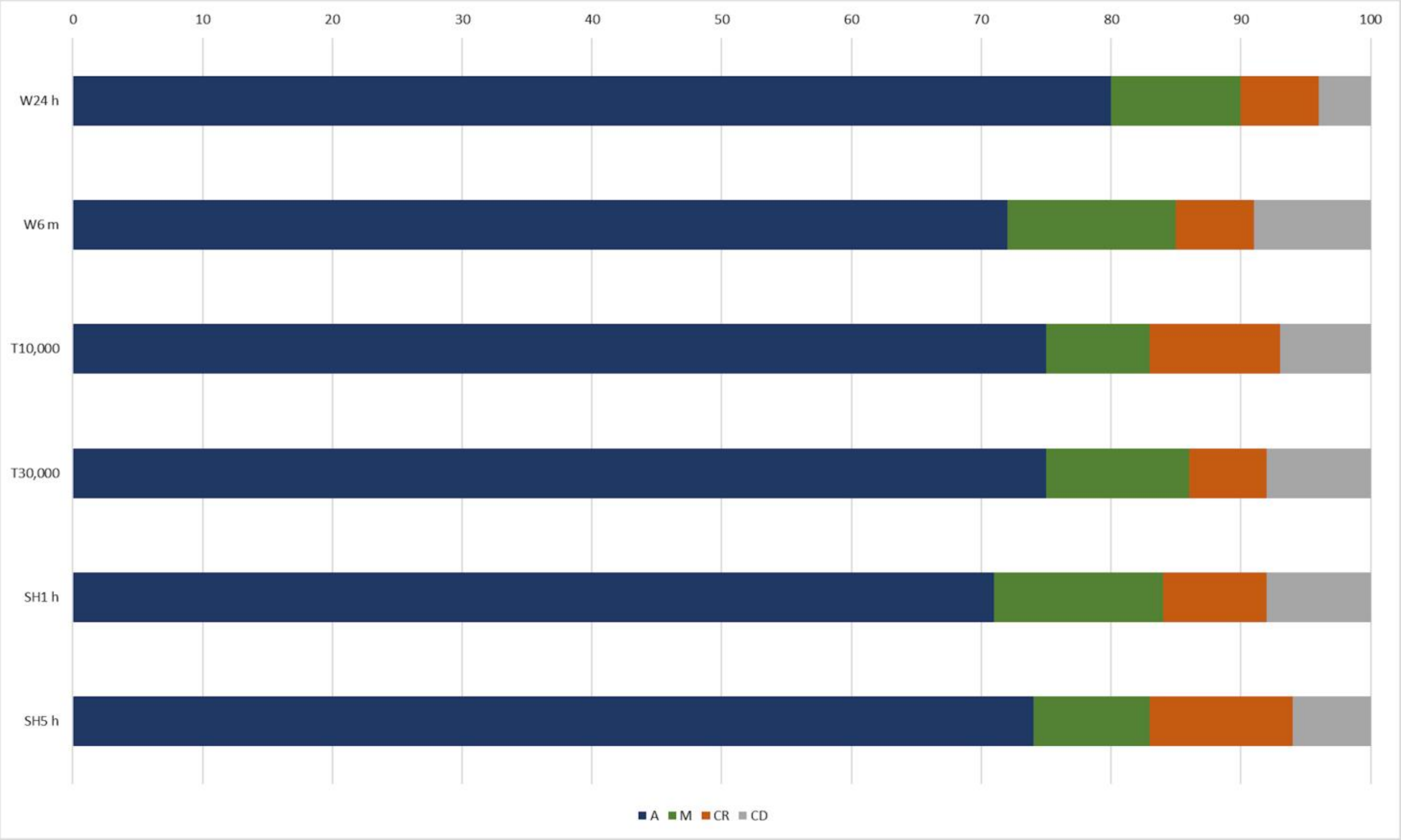
T10,000

T30,000

SH1 h

SH5 h

Figure 2. Overview of failure pattern distribution for all groups in % (ranging). Adhesive (A); Mixed (M); Cohesive in resin (CR) and Cohesive in dentin (CD).



3 ARTIGO 2 – IS THERE A CORRELATION BETWEEN THE AGING METHOD AND ADHESIVE SYSTEM USED INFLUENCING THE BOND STRENGTH? A METHODOLOGICAL REVIEW

Este artigo será submetido à publicação no periódico *Operative Dentistry*, ISSN: 0361-7734, Fator de Impacto: 2.390, Qualis CAPES: A1. As normas para publicação estão descritas no Anexo A.

Is there a correlation between the aging method and adhesive system used influencing the bond strength? A methodological review

BGS Sutil[a], GS Teixeira[b], DZ Righes[c], ET Chaves[d], AH Susin[e]

[a] Bruna Gabrielle da Silva Sutil, DDS, MS, PhD student, Post-Graduate Program in Dentistry Sciences, Federal University of Santa Maria
Postal address: Roraima Avenue, 1000, 26F Building, Room 2248, Santa Maria RS 97105-900, Brazil.
E-mail address: brunasutil@hotmail.com
Telephone number: +55 55 99105 7975
ORCID ID: https://orcid.org/0000-0003-2709-5620

[b] Gabriela Simões Teixeira, DDS, MS, PhD student, Post-Graduate Program in Dentistry Sciences, Federal University of Santa Maria
Postal address: Roraima Avenue, 1000, 26F Building, Room 2248, Santa Maria RS 97105-900, Brazil.
E-mail address: gsimoesteixeira@gmail.com
Telephone number: +55 55 99958 0022
ORCID ID: https://orcid.org/0000-0003-2167-2204

[c] Danielle Zorzo Righes, DDS, MS, PhD student, Post-Graduate Program in Dentistry Sciences, Federal University of Santa Maria
Postal address: Roraima Avenue, 1000, 26F Building, Room 2248, Santa Maria RS 97105-900, Brazil.
Telephone number: +55 55 99613 6521
E-mail address: righesdz@gmail.com
ORCID ID: https://orcid.org/0000-0002-7552-7224

[d] Eduardo Trota Chaves, DDS, MS student, Post-Graduate Program in Dentistry Sciences, Federal University of Santa Maria
Postal address: Roraima Avenue, 1000, 26F Building, Room 2248, Santa Maria RS 97105-900, Brazil.
Telephone number: +55 53 99969 2474
E-mail address: eduardo.trota@yahoo.com
ORCID ID: http://orcid.org/0000-0002-5313-4980

[e] Alexandre Henrique Susin, DDS, MS, PhD, Department of Restorative Dentistry, Federal University of Santa Maria
Postal address: Roraima Avenue, 1000, 26F Building, Room 2248, Santa Maria RS 97105-900, Brazil.
Telephone number: +55 55 98138 4096
E-mail address: alexandre.susin@ufsm.br
ORCID ID: https://orcid.org/0000-0002-7083-6028

[a] **Corresponding author:**

Bruna Gabrielle da Silva Sutil, DDS, MS, PhD student, Post-Graduate Program in Dentistry Sciences, Federal University of Santa Maria

Postal address: Roraima Avenue, 1000, 26F Building, Room 2248, Santa Maria RS 97105-900, Brazil.

E-mail address: brunasutil@hotmail.com

Telephone number: +55 55 99105 7975

ORCID ID: https://orcid.org/0000-0003-2709-5620

Is there a correlation between the aging method and adhesive system used influencing the bond strength? A methodological review

Running title: Effect of the artificial aging protocols on the bond strength. A review

Summary

This study aimed to conduct a methodological review of the literature on laboratory studies assessing the most frequent artificial aging method described concerning micro bond tests in dentin from 2011 to 2020, and to verify any correlation between the chosen method and the adhesive system used. This review was conducted in accordance with the PRISMA statement. A total of 27 studies were screened and the material and methods and results sections were reviewed to identify the most frequently used artificial aging methods and adhesive systems.It was found that the water storage at 37°C for different times, and thermocycling with a variable number of cycles are the most frequent presented aging methods. The universal adhesives were the most used material, but a correlation between the proposed method x chosen adhesive was not identified. Efforts to reach an effective bonding degradation compatible with the long-term clinical service of each adhesive used should be made to establish criteria for choosing the most reliable aging method in *in vitro* studies, with the goal of offering researchers the best options for aging samples closer to the long-term clinical conditions to which adhesive restorations are submitted.

Clinical significance: Bond strength tests after the samples are submitted to aging methods are commonly performed to verify the effectiveness of adhesive systems to simulate the clinical performance of bonded restorations.

Keywords: Artificial aging; Bond strength; Dentin.

INTRODUCTION

The resin/dentin interface is an area impregnated by hydrophobic and hydrophilic contents of adhesive systems. It was conceptually considered as a “new biocompatible material” and described as a “hybrid layer” (HL) formed by the involvement of monomers around the collagen fibrils from the surface of demineralized dentin.[1,2] This occurs because the hydrophilic components of the hybrid layer act as semi-permeable membranes, attract water, and consequently degrade.[3] It is reinforced by the “water treeing” concept[4] which reveals the nanoleakage phenomenon and its clinical significance due to the hydrolytic degradation by self-propagation of the water channels throughout the HL.

The mechanisms of degradation of the resin/dentin interface is mediated by biological or oral responses starting with the failure at the junction between adhesive and the underlying dental tissue[5] reinforced by interferences which also act to decrease the longevity of bonded restorations, such as mechanical forces by chewing, volumetric alterations as consequence of thermal variations, and pH defy from the food and beverage.[6] There is consensus that the degree and velocity of the bonding degradation is material dependent[7], however other conditions such as bonding procedure and quality of dentin recognizably affect the integrity of the interface over time.[8–10]

The adhesives currently used perform favorably on immediate tests considering the bond strength[11–13] and micromorphology[14], however the same effectiveness is rarely observed when they are tested in clinical trials.[15–18]Thus, the artificial aging methods (AAM) are recommended in *in vitro* studies to partially simulate the effects of the oral environmental in the long-term, although the variety, concurrency and the intensity of deleterious interferences involved in the bonding decrease in clinical conditions, and may not be properly simulated to be applied in *in vitro* studies of bond strength.[19]

Storage in distilled water at 37°C from 3 to 12 months, 10,000 to 30,000 cycles of thermocycling and immersion in NaOCl solution from 1 to 5 hours are the most common AAM reported in the literature.[6,19–21] Researchers have commonly submitted specimens to one of these methods to compare to the baseline (with no AAM), expecting to decrease the bond strength regardless of the adhesive composition, number of steps and approach. However, the aging method which more pronouncedly decreases the bond strength for each type of adhesive should be defined in order to be used as standard in future studies.

Therefore, the aim of this study is to perform a 10-year literature search collecting data regarding artificial aging methods used in *in vitro* studies of bond strength of adhesive systems and to identify which is the most used method for each type of adhesive system. Additionally, to critically analyze their effects in decreasing the bond strength.

METHODS

As this review focuses on methodological aspects concerning the proceedings and application of AAM on the samples to perform bond strength tests in dentin, a search was carried out for the years between 2011 to 2020 to identify *in vitro* studies in the PubMed and in the Web of Science databases.

This review was conducted in accordance with the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA statement).[22] The question to be answered for this review was as follows: Which AAM are used in bond strength tests of adhesive systems? As well as: Are researchers opting for a different AAM for specific adhesive systems?

The Population, Intervention, Control, Outcome and Study (PICOS) used concerning this methodological study was: Population - artificial aging methods; Intervention - bonded restorations; Control - use of adhesive systems for micro bond strength tests on dentin; Outcome - bond strength test after samples are submitted to an artificial aging method; and Studies - only *in vitro* studies.[23]

Based on the PICOS structure, the common terms frequently presented as keywords in *in vitro* studies of bond strength were applied in both electronic databases follows adhesive system, dentin adhesive, aging, aging methods, artificial aging methods, bond strength, dentin bonding, and dentin bond strength. The mentioned sequence of terms was assumed as the search strategy for the topic “all fields” for the “advanced search” in the PubMed database and as author keywords and topic for the Web of Science database. The search strategy is shown in Table 1.

The retrieved data from selected articles were compiled to produce a table concerning relevant aspects of AAM to be discussed and reach a conclusion regarding the aim of this review.

RESULTS

Figure 1 shows a flowchart summarizing the applied study selection process which resulted in 27 articles included in this review. A total of 5,248 manuscripts were presented in the databases search. The title and running abstract were considered for preliminary selection of the manuscripts. After removing the duplicates and the preliminary evaluation, 387 manuscripts were selected to be submitted to the inclusion criteria by two independent examiners with substantial agreement inter-examiner Kappa Coefficient (KC = 0.79).

The applied inclusion criteria were: 1. Manuscript as an original study; 2. Manuscript published in English; 3. Manuscript with *in vitro* experiment presenting AAM; 4. Manuscript with experiments performed in dentin; and 5. Manuscript performing dentin microbond tests. In this stage, any manuscripts which presented no experiment in dentin or undefined AAM applied were then excluded.

After applying these criteria, 27 manuscripts remained to have their data extracted for this study. The material and methods and the results sections of the selected manuscripts were read in order to extract the necessary data to compile information to produce tables and graphs concerning the author/year of the published manuscript; artificial aging method, adhesive(s) system(s) used, microbond strength test; statistical decrease/increase or the same bond strength after applying AAM and predominant failure.

Table 2 summarizes the retrieved data of the 27 selected studies and Figures 2 and 3 show the AAM and the adhesive systems which were mentioned in the selected articles. Figure 4 shows the changes in the bond strength of each adhesive system.

DISCUSSION

Aging the adhesive interface of micro-specimens of bonded restorations is currently the most validated method to assess adhesion durability, since all adhesive classes exhibit evidence of degradation after 3 months of AAM which resembles *in vivo* aging effects.[24] The AAM are frequently incorporated into laboratory research to simulate the clinical conditions to which the bonded restorations are submitted.[25] They should act over the tooth/restoration interface similarly to what is expected when the restoration is in function and suffering stresses from chewing forces, differences in pH and temperature from consuming acidic, hot and cold foods and beverages.[26] According to Teixeira et al. (2021)[19], the aging processes affect the adhesive performances differently, regardless of the adhesive system used and also different from the natural aging process which affects the interface by synergic effects of several interferences[6,19,27]; the AAM recursively affects the HL by one main deleterious effect. For example, water storage promotes degradation by hydrolysis of the hydrophilic resin components[28]; 10% NaOCl solution as AAM and is considered an extreme aging condition used to guarantee the organic component degradation[29] and the thermocycling is used to promote the hydrolysis of the unprotected collagen fibrils by repetitive contraction/expansion stress through intermittently being submitted to cold and hot water.[30,31]

The current literature presents a gap regarding investigations about the level of influence of the number of steps and aging processes on bond strength. Studies concerning bonding stability of adhesives under aging do not mention the cause-and-effect correlation between the factors. Several studies of durability and stability of adhesion testing bond strength and micro and nanoleakage on direct restorations only highlight the blend of monomers, cross-linking monomers, dentin pretreatment, alternative adhesive approaches and protocols[19,20,26,27], with no mention to the possible influence of the choice of aging method relative to any specific adhesive or compound molecule.

Based on the questions suggested for this study, our aim is to identify if researchers have preferences for one or more kinds of AAM, as well as if there is a preference for a specific AAM and intensity according to the adhesive system used. Finding answers to these questions could help researchers to submit the specimens to the most effective AAM and with more similarity to clinical conditions. From the view that water storage and thermocycling have the most prevalence as AAM in the base literature in this study, both methods will be considered in this discussion.

Water storage was the most frequently used method to age specimens with a frequency of 42% of the studies. The long-term exposure of dentin-resin interface in water at 37°C is a recognized way of degrading the hybrid layer.[32] The exact mechanism which promote the bonding decrease is not completely known and it is generally attributed to "hydrolysis", however it is certainly associated to a deterioration of collagen fibrils, nanoleakage and degradation of the hydrophilic resin components by host-derived proteases with collagenolytic activity.[19,28,33] The water-dependance of the degradation mechanism is reinforced by the fact of the oil stored samples keeping the dentin-resin bonds stable, whereas the others which were stored in water had decreased bond strength.[34] The interference in bond strength of this aging process was better understood through sample geometry and regional bond introduced in a study by Shono et al. (1999)[33], in which the authors found a significant decrease in the bond strength of the samples immersed in water for 3 months with increasing deleterious effects according to the bonding interface migrated from superficial to deep dentin.

The data found show that there are differences in the performance of the bond strength after the samples are aged in water, regardless of the storage time. Although this method presented no studies in which the increase in bond strength was detected, a decrease was more frequently observed[35–38] than for those in which the bond strength was kept stable.[39] In both studies, the authors did not mention how it is expected that water storage will influence the bond strength nor the degradation mechanism that each specific adhesive could be affected by. The literature shows no consensus concerning the performance of *in vitro* bond strength tests of bonded restorations submitted to water aging, regardless of the storage time[7,40].

The thermocycling method to age specimens from 2,500 to 12,000 cycles in water baths of temperature ranging from 5°C to 55°C was observed in 19% of the selected studies. It represented the second highest frequency of AAM. Findings in studies which reviewed AAM[41] report an ample variation in the temperature between the water baths ranging from 1 to 50,000 cycles at temperatures varying from 0°C to 80°C. The variation in the number of cycles used in the selected studies confirms that the method does not have a previous standard, even though it is considered that a specimen must be submitted to 10,000 cycles to simulate 1 year of *in vivo* bond degradation, as based on a study by Gale & Darvell (1999)[41] which reported an *in vivo* thermal cycle frequency of 20 to 50 times per day.

The alternate immersion of the specimens in cold and hot water simulating the thermal changes of temperature promoted by food and drinks in the oral cavity stimulate the hydrolysis of unprotected collagen fibrils by repetitive contraction/expansion stress of the interfacial components, thus accelerating the deleterious effect of water.[3] The expected effect is a decrease in the bond strength since the crack propagation promotes forming gaps along the adhesive interface which allow water and oral pathogenic fluid permeation through it[42], in turn generating bond degradation. Since the purpose of submitting the specimens to an aging method is to add a deleterious factor to stress the bonding interface, the employed method needs to be effective in this regard; therefore, specimens with a low number of cycles could not be sufficiently stressed for the purpose of simulating the action of the oral environment.

The authors who opted to perform a low number of cycles probably based this on the ISO guidance for testing adhesion to tooth structures from 1994, which recommends 500 thermal cycles as enough to simulate the long-term effects. This study found contradictory bond strength performances after submitting specimens to a different number of thermal cycles at the same temperature in the baths. While the bond strength in one study[43] decreased after 10,000 cycles, studies performed with 12,000[44], 5,000[45] and 2,500 cycles[46] presented no differences between aged groups versus the baseline. Although all of the above mentioned studies use temperatures of 5°C and 55°C, they had other methodological similarities and differences regarding the performed tests and adhesives, such as: µTBS and Clearfil SE Bond, Adper Single Bond 2 and XP Bond up to 12,000 cycles; µTBS and Prime&Bond Universal and Optibond FL up to 10,000 cycles; µSBS and Optibond FL and Optibond All-in-One up to 5,000 cycles; and µSBS and Adper Single Bond 2 to 2,500 cycles. It seems that the "thermocycling factor" did not act independently on increasing, decreasing, or maintaining the bond strength. This agrees with other studies in which the thermocycling affected the bond strength differently[21,31,47,48]. The wide variation in the number of cycles also contributes to unexpected results after submitting specimens to the deleterious effects of aging, and the adhesive could be responsible for these results. Solvated adhesives based on HEMA are the most frequently presented in studies with aged groups and they are responsible for most of the discrepant results.[49] This is in agreement with Hashimoto et al. (2011)[50] who concluded that the bonding performance is material dependent, meaning that the adhesive system formulation and approach definitively reveals implications when the specimens are submitted to any aging process.

The evolution of the adhesive systems shows that the current bonding techniques to the direct resin composite restorations tend to use universal adhesives. They are the latest class of bonding agents introduced in dentistry and they are amply discussed in the literature[51–54]. Furthermore, it is not uncommon that the microbond tests after aging show an increase in the bond strength. In this study, universal adhesives represented 43% of the options of tested materials distributed into the diversity of tests and aging methods. Contemporary adhesives such as Universal adhesives containing 10-methacryloyldecyl dihydrogen phosphate (MDP) are cited as the most promising in the literature due to better results in immediate tests and less sensibility to the aging methods[19] in preserving the integrity of the interface longer than the others which contain other acidic functional monomers.[55]

In vitro studies of bond strength test a wide variety of adhesive systems, including changes in their formulation, different application protocols, use of pretreatment on substrates and different tests and their variations. The most commonly used aging methods are water storage at 37°C for different times, and thermocycling with a variable number of cycles. It could be plausible to determine a more reliable method to perform aging of the samples to reach an effective bonding degradation compatible to the long-term clinical service of each adhesive used.

CONCLUSION

Based on the results of this study, it was found that the aging method choice is random and does not consider adhesive characteristics or not even a specific criterion which defines the type of AAM to be used in each study. Efforts should be concentrated to establish criteria for choosing the aging method in *in vitro* studies, with the goal of offering researchers the best options for aging of samples closer to the long-term clinical conditions to which adhesive restorations are submitted. *In vitro* studies performing different aging methods for each adhesive could define if any method promotes more intense deleterious effects than others for a specific adhesive or molecule.

REFERENCES

1. Nakabayashi N, Saimi Y. (1996) Bonding to intact dentin. *Journal of Dental Research* **75(9)** 1706-1715.
2. Nakabayashi N, Kojima K & Masuhara E. (1982) The promotion of adhesion by the infiltration of monomers into tooth substrates. *Journal of Biomedical Material Research* **16(3)** 265-273.
3. De Munck J, Van Landuyt K, Peumans M, Lambrechts P, Braem M & Van Meerbeek B. (2005) A critical review of the durability of adhesion to tooth tissue: Methods and results. *Journal of Dental Research* **84(2)** 118-132.
4. Tay F & Pashley D. (2003) Water treeing-a potential mechanism for degradation of dentin adhesives. *Americam Journal of Dentistry* **16(1)** 6-12.
5. Bedran-Russo A, Leme-Kraus A, Vidal CMP & Teixeira EC. (2017) An Overview of Dental Adhesive Systems and the Dynamic Tooth – Adhesive Interface Dental adhesives Dentin Enamel Bond strength Biodegradation Technology. *Dental Clinic of North America* **61(4)** 713-731.
6. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di R & De Stefano E. (2007) Dental adhesion review : Aging and stability of the bonded interface. 24(1) 90-101.
7. Fukuoka A, Koshiro K, Inoue S, Yoshida Y, Tanaka T, Ikeda T, Suzuki K, Hidehiko Sano & Van Meerbeek B. (2011) Hydrolytic stability of one-step self-etching adhesives bonded to dentin. **13(3)** 2-3.
8. Arrais C, Giannini M, Nakajima M & Tagami J. (2004) Effects of additional and extended acid etching on bonding to caries-affected dentine. *European Journal of Oral Sci*ence **112(5)** 458-464.
9. Cavalli V, Sebold M, Shinohara M, Pereira P &, Giannini M. (2018) Dentin bond strength and nanoleakage of the adhesive interface after intracoronal bleaching. *Microscopy Reserach and Technique* **81(4)** 428-436.
10. Muñoz M, Luque-Martinez I, Malaquias P, Hass V, Reis A, Campanha N, & Loguercio A. (2015) In Vitro Longevity of Bonding Properties of Universal Adhesives to Dentin. **40(3)** 282-292.
11. Dačić S, Miljković M, Mitić A, Radenković G, Anđelković-Apostolović M & Jovanović M. (2021) Influence of etching mode and composite resin type on bond strength to dentin using universal adhesive system. *Microscroscopy Research and Technique* **84(6)** 1212-1219.

12. Perdigão J, Muñoz M, Sezinando A, Luque-Martinez I, Staichak R, Reis A & Loguercio. (2014) A Immediate Adhesive Properties to Dentin and Enamel of a Universal Adhesive Associated With a Hydrophobic Resin Coat. *Operative Dentistry* **39(5)** 489-499.

13. Felemban N, Ebrahim M & Huwaya A. (2017) Effect of adhesive layers on microshear bond strength of nanocomposite resin to dentin. **9(2)** 3-7.

14. Sartori N, Peruchi LD, Phark J & Duarte Jr S. (2016) The Influence of Intrinsic Water Permeation on Different Dentin Bonded Interfaces Formation. *Journal of Dentistry 48 46-54.*

15. Favetti M, Schroeder T, Montagner A, Correa M, Cenci T & Cenci M. (2017) Effectiveness of pre-treatment with chlorhexidine in restoration retention: A 36-month follow-up randomized clinical trial. *Journal of Dentistry* 60 44-49.

16. Mena-Serrano A, Kose C, De Paula E, Tay L, Reis A, Loguercio A, & Perdigão J. (2013) A new universal simplified adhesive: 6-month clinical evaluation. *Journal of Esthetic and Restororative Dentistry* **25(1)** 55-69.

17. Ram D, Mamber E, Fuks B. (2005) Clinical performance of a non-rinse conditioning sealant in three paediatric dental practices: a retrospective study. *International Journal of Paediatric Dentistry* **15(1)** 61-66.

18. Söderholm K-JM, Ottenga M & Nimmo S. (2013) Four-year clinical evaluation of two self-etching dentin adhesives of different pH values used to restore non-retentive cervical lesions. *American Journal of Dentistry* **26(1)** 28-32.

19. Teixeira G, Pereira G, & Susin A. (2021) Aging Methods — An Evaluation of Their Influence on Bond Strength. *European Journal of Dentistry* **28** 1-6

20. Reis A, Martins GC, de Paula E, Davila A, & Loguercio A. (2015) Alternative Aging Solutions to Accelerate Resin-Dentin Bond Degradation. Journal of Adhesive Dentistry **17(4)** 321-329.

21. Cetin A & Dink H (2020) Effects of Artificial Aging on the Bond Strengths of Universal Dental Adhesives. *Nigerian Journal of Clinical Pratice* **23(8)** 1148-1154.

22. Liberati A, Altman D, Tetzlaff J, Mulrow C, Gøtzsche P, Loannidis J, Clarke M, Devereaux P, Kleijnen J & Moher D. (2009) *The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of Studies That Evaluate Health Care Interventions: Explanation and Elaboration*. *PLoS Medicine Guidelines* 7(6) 1-28.

23. Amir-Behghadami M & Janati A. (2020) Population, Intervention, Comparison, Outcomes and Study (PICOS) design as a framework to formulate eligibility criteria in

systematic reviews. *Emergency Medicine Journal*. **37(6)** 387.

24. De Munck J, Landuyt V & Peumans M. (2020) A Critical Review of the Durability of Adhesion to Tooth Tissue: Methods and Results. *Critical Reviews in Oral Biology and Medicine* **84(2)** 118-132.
25. Hariri I, Shimada Y, Sadr A, Ichinose S & Tagami J. (2012) The effects of aging on shear bond strength and nanoleakage expression of an etch-and-rinse adhesive on human enamel and dentin. *Journal of Adhesive Dentistry*. **14(3)** 235-243.
26. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R & De Stefano D. (2008) Dental adhesion review: aging and stability of the bonded interface. *Dental Materials* **24(1)** 90-101.
27. Tay F, Hashimoto M, Pashley D, Peters M , Lai S, Yiu C & Cheong C (2003) Aging affects two modes of nanoleakage expression in bonded dentin. *Journal of Dental Research* **82(7)** 537-541.
28. Frassetto A, Breschi L, Turco G, Marchesi G, Di Lenarda R , Tay F , Pashley D & Cadenaro M. (2016) Mechanisms of degradation of the hybrid layer in adhesive dentistry and therapeutic agents to improve bond durability - A literature review. *Dental Materials*. **32(2)** e41-e53.
29. Pascon F, Kantovitz K, Sacramento P, Nobre-dos-Santos M, & Puppin-Rontani R. (2009) Effect of sodium hypochlorite on dentine mechanical properties. A review. *Journal of Dentistry* **37(12)** 903-908.
30. Asaka Y , Amano S, Rikuta A, Kurokawa H, Miyazaki M , Platt J & Moore B. (2007) Influence of Thermal Cycling on Dentin Bond Strengths of single-step Self-Etch Adhesive Systems. *Operative Dentistry* **32(1)** 73-78.
31. Daneshkazemi A, Davari A , Akbari M, Davoudi A & Badrian H (2015) Effects of Thermal and Mechanical Load Cycling on the Dentin Microtensile Bond Strength of Single Bond-2. *International Journal of Oral Health* **7(8)** 9-13.
32. Gwinnett A & Yu S. (1995) Effect of long-term water storage on dentin bonding. *American Journal of Dentistry* **8(2)** 109-111.
33. Shono Y, Terashita M, Shimada J, Kozono Y, Carvalho R, Russel C & Pashley D (1999) Durability of resin-dentin bonds. J*ournal of Adhesive Dentistry* **1(3)** 211-218
34. García-Godoy F, Tay F, Pashley D, Feilzer A, Tjäderhane L & Pashley E. (2007) Degradation of resin-bonded human dentin after 3 years of storage. *American journal of Dentistry* **20(2)** 109-113.
35. Porto I, Rocha A, Ferreira I, Barros B, Avila E, Silva M, Oliveira M, Lobo T, Oliveira

J, Nascimento T, Freitas J & Freitas, JD. (2021) Polyphenols and Brazilian red propolis incorporated into a total-etching adhesive system help in maintaining bonding durability. *Heliyon* **7(2)** 1-12.

36. Firouzmandi M, Mohaghegh M & Jafarpisheh M. (2020) Effect of silver diamine fluoride on the bond durability of normal and carious dentin. *Journal of Clinical and Experimental Dentistry*. **12(5)** e468-e473.
37. Jowkar Z, Shafiei F, Asadmanesh E & Koohpeima F. (2019) Influence of silver nanoparticles on resin-dentin bond strength durability in a self-etch and an etch-and-rinse adhesive system. *Restororative Dentistry and Endodontics*.**44(2)** 1-9.
38. Fallahinejad-Ghajari M, Ghasemi A, Badiee M, Abdolazimi Z & Akbarzadeh A. (2019) Microshear Bond Strength of Scotchbond Universal Adhesive to Primary and Permanent Dentin: A Six-Month In Vitro Study. *Frontier in Dentistry* **16(3)** 173-180.
39. Manso AP, Helena R, Grande M, Bedran-Russo, A, Reis A, Loguercio A, Pashley D & Carvalho R. (2014) Can 1% chlorhexidine diacetate and ethanol stabilize resin-dentin bonds? *Dental Materials* **30(7)** 735-741.
40. Breschi L, Cammelli F, Visintini E, Mazzoni A, Vita F, Carrilho M, Cadenaro M, Foulger S, Mazzoti G, Tay F, Di Lenarda R & Pashley D. (2009) Influence of chlorhexidine concentration on the durability of etch-and-rinse dentin bonds: a 12-month in vitro study. *Journal of Adhesive Dentistry* 11(3) 191-198.
41. Gale M & Darvell W. (1999) Thermal cycling procedures for laboratory testing of dental restorations. *Journal of Dentistry* **27(2)** 89-99.
42. Amaral F, Colucci V, Palma-Dibb R & Corona S. (2007) Assessment of in vitro methods used to promote adhesive interface degradation: A critical review. *Journal of Esthetic and Restorative Dentistry* **19(6)** 340-353.
43. Al-Nabulsi M, Daud A, Yiu C, Omar H, Sauro S , Fawzy A , Daood U (2019) Co-blend application mode of bulk fill composite resin. *Materials (Basel)* **12(16)** 1-15.
44. Galafassi D, Colucci V, Cecchin D, Scatena C, Nascimento &, Corona S. (2013) Effect of endodontic irrigants on microtensile bond strength to dentin after thermocycling and long-term water storage. *Journal of Dentistry (Tehran)*. **10(5)** 426-42635.
45. Bumrungruan C & Sakoolnamarka R. (2016) Microshear bond strength to dentin of self-adhesive flowable composite compared with total-etch and all-in-one adhesives. *Journal of Dental Science*. **11(4)** 449-456.
46. Sharma A , Aggarwal N , Rastogi S , Choudhury R, Tripathi S (2017) Effectiveness of

platelet-rich fibrin in the management of pain and delayed wound healing associated with established alveolar osteitis (dry socket)
European Journal of Dentistry 11(4) 508-513

47. Wang R, Shi Y, Li T, Pan Y, Cui Y & Xia W. (2017) Adhesive interfacial characteristics and the related bonding performance of four self-etching adhesives with different functional monomers applied to dentin. *Journal of Dentistry* **62** 72-80.
48. Sai K, Shimamura Y, Takamizawa T, Tsujimoto A, Imai A, Endo H, Barkmeier W, Latta M & Miyazaki M (2016) Influence of degradation conditions on dentin bonding durability of three universal adhesives. *Journal of Dentistry* **54** 56-61.
49. Anchieta B, Machado L, Martini A, dos Santos P, Giannini M, Janal M, Tovar N, Sundfeld R, Rocha E & Coelho P. (2015) Effect of long-term storage on nanomechanical and morphological properties of dentin–adhesive interfaces. *Dental Materials* **31(2)** 141-153.
50. Hashimoto M, Nagano F, Endo K & Ohno H. (2011) A review: Biodegradation of resin — dentin bonds. *Japanese Dental Science Review* **47** 5-12.
51. Yoshihara K, Nagaoka N, Hayakawa S, Okihara T, Yoshida Y & Van Meerbeek B (2018) Chemical interaction of glycero-phosphate dimethacrylate (GPDM) with hydroxyapatite and dentin. *Dental Materials* **34(7)** 1072-1081.
52. Loguercio AD, Andrade E, Paula D, Hass V, Luque-Martinez I, Reis A & Perdigão J (2015)A new universal simplified adhesive : 36-Month randomized double-blind clinical trial. *Journal of Dentistry* **43(9)** 1083-1092.
53. Perdigão J, Kose C, Mena-Serrano AP, De Paula EA, Tay LY, Reis A, Loguercio AD (2014) A New Universal Simplified Adhesive: 18-Month Clinical Evaluation. *Operative Dentistry* **39(2)** 113-127.
54. Tsujimoto A, Barkmeier W, Takamizawa T, Wilwerding T, Latta M, & Miyazaki M. (2017) Interfacial Characteristics and Bond Durability of Universal Adhesive to Various Substrates Interfacial Characteristics and Bond Durability of Universal Adhesive to Various Substrates. *Opererative Dentistry* **42** e59-e70.
55. Tsuchiya K, Takamizawa T, Barkmeier W, Tsubota K , Tsujimoto A , Berry T, Erickson R, Latta M & Miyazaki M. (2016) Effect of a functional monomer (MDP) on the enamel bond durability of single-step self-etch adhesives. *European Journal of Oral Science* **124(1)** 96-102.

TABLES

Table 1. The search strategy.

Based terms / Records	Presented term to search
Adhesive system	adhesive systems OR adhesive system OR dentin adhesives OR dentin bonding agents
Aging	aging OR aging methods OR artificial aging methods
	AND
Bond strength	bond strength OR dentin bonding OR dentin bond strength
Date of publication	2011/01/01 to 2020/12/31
	Queries
Pub Med Query presentation / 4393	((((((adhesive systems) OR (adhesive system)) OR (dentin adhesives)) OR (dentin bonding agents)) OR (((aging) OR (aging methods)) OR (artificial aging methods))) AND (((bond strength) OR (dentin bonding)) OR (dentin bond strength))) AND (("2011/01/01"[Date - Publication] : "2020/12/31"[Date - Publication]))
Web of Science Query presentation / 855	(AK=(adhesive systems OR adhesive system OR dentin adhesives OR dentin bonding agents) OR TS=(aging OR aging methods OR artificial aging methods) AND TS=(bond strength OR dentin bonding OR dentin bond strength) AND PY=(2011-2020)) AND IDIOMA: (English) AND KIND OF DOCUMENT: (Article) TIME: 2011-2020. Index: SCI-EXPANDED.

Table 2. Study design data of the included studies.

Study Author/Year	Artificial Aging Methods	Adhesive System	Bond Strength Test	Bond Strength Test Values	Predominant failure mode
Saffarpour, 2020	10,000 thermocycles between 5 and 55 °C (dwell time was 20 s and the transfer time was 10 s)	Adper Single Bond 2 (3M ESPE, St. Paul, MN, USA)	µTBS	Decreased (28.165 to 16.593 MPa)	No data
Porto, 2021	24 h of storage in distilled water at 37°C 1 year of storage in distilled water at 37°C	Adper Single Bond 2 (3M ESPE, St. Paul, MN, USA)	µTBS	Decreased Values	Mixed/Adhesive
Kazemi-Yazdi, 2020	24 h of storage in distilled water at 37°C 3 months of water storage associated to 3000 thermocycles between 5-55°C with 20-second dwell times)	Clearfil SE Bond (Kuraray Noritake, Okayama, Japan) Peak Universal Bond (Ultradent, South Jordan, UT, USA)	µTBS	Clearfil SE Bond: did not significantly decrease (18.00 to 16.71 MPa) Peak Universal Bond: decreased (15.33 to 6.47 MPa)	No data
Baena, 2020	24 h of storage in artificial saliva at 37°C 10,000 thermocycles between 5 and 55 °C (dwell time was 20 s)	Optibond FL (Kerr, Orange, CA, USA) Scotchbond Universal (3M ESPE, St. Paul, MN, USA)	µTBS	Optibond FL: decreased (41.3 to 32.2 MPa) Scotchbond Universal: increased (25.0 to 30.4 MPa)	Adhesive
Firouzmandi, 2020	24 h of storage in artificial saliva at 37°C 6 months of storage in distilled water at 37°C	Adper Single Bond 2 (3M ESPE, St. Paul, MN, USA)	µSBS	Decreased (18.37 to 14.72 MPa)	Mixed/Adhesive
Valizadeh, 2019	10,000 thermocycles between 5 and 55 °C (dwell time was 20 s and the transfer time was 10 s)	Scotchbond Universal in self-etch and etch-and-rinse mode (3M ESPE, St. Paul, MN, USA) Adper Single Bond 2 (3M ESPE, St. Paul, MN, USA) Clearfil SE Bond (Kuraray Co. Ltd., Osaka, Japan)	µSBS	There was no comparison with the immediate group (24 h)	No data
Zumstein, 2018	24 h of storage at 37°C 1 year of storage in 100% relative humidity at 37°C	Clearfil SE Bond (Kuraray Co. Ltd., Osaka, Japan) Scotchbond Universal in self-etch and etch-and-rinse mode (3M ESPE, St. Paul, MN, USA)	µTBS	The pooled means and standard deviations were 30.0 ± 9.2 MPa (24 h) and 21.7 ± 7.8 MPa (1 year)	Adhesive
Manso, 2014	24 h of storage in distilled water at 37°C 6 months of storage in distilled water at 37°C 15 months of storage in distilled water at 37°C	All Bond 3 (Bisco Inc., Schaumburg, IL, USA) Excite (Ivoclar Vivadent, New York, USA)	µTBS	All Bond 3: no statistical differences (51.07 MPa - 24h 57.13 MPa – 6 months 47.29 MPa – 15 months) Excite: no statistical differences	Mixed/Adhesive

				(49.51 MPa - 24h 42.10 MPa – 6 months 45.51 MPa – 15 months)	
Al-Nabulsi, 2019	24 h of storage in distilled water at 37°C 10,000 thermocycles between 5 and 55 °C	Prime & Bond Universal (Dentsply Sirona—Dentsply Caulk, Milford, DE, USA) Optibond FL (Kerr Corporation, Orange, CA, USA)	µTBS	Prime & Bond Universal: decreased (36.4 to 31.7 MPa) Optibond FL: decreased (32.1 to 29.9 MPa)	Mixed
Jowkar, 2019	24 h of storage in distilled water at 37°C 6 months of storage in distilled water at 37°C	Adper Single Bond 2 (3M ESPE, St. Paul, MN, USA) Clearfil SE Bond (Kuraray Co. Ltd., Osaka, Japan)	µSBS	Adper Single Bond 2: decreased (20.7 to 14.1 MPa) Clearfil SE Bond: decreased (21.3 to 15.7 MPa)	Mixed
Bumrungruan, 2016	24 h of storage in distilled water at 37°C 5,000 thermocycles between 5 and 55 °C (dwell time was 30 s and the transfer time was 10 s)	OptiBond FL (Kerr Corporation, Orange, CA, USA) OptiBond all-in-one (Kerr Corporation, Orange, CA, USA)	µSBS	OptiBond FL: no statistical differences (32.2 to 31.8 MPa) OptiBond all-in-one: no statistical differences (24.4 to 23.9 MPa)	Adhesive
Miranda, 2020	24 h of storage in distilled water at 37°C 1 year of storage in distilled water at 37°C	Adper Single Bond 2 (3M ESPE, St. Paul, MN, USA) Experimental adhesive	µTBS	The µTBS value after 1 year of storage was similar to the µTBS value found in the immediate time in all groups	Adhesive
Galafassi, 2013	24 h of storage in deionized water at 37°C 12,000 thermocycles between 5°C to 55°C (30 s dwell time, 500 cycles per week for 6 months)	Clearfil SE Bond (Kuraray Co. Ltd., Osaka, Japan) Adper Single Bond 2 (3M ESPE, St. Paul, MN, USA) XP Bond (Dentsply, York, PA, USA)	µTBS	No statistically significant differences were found between the specimens stored for 24 h and those which were thermocycled	Mixed
Ghajari, 2019	24 h of storage in distilled water at 37°C 6 months of storage in distilled water at 37°C	Scotchbond Universal in self-etch and etch-and-rinse mode (3M ESPE, St. Paul, MN, USA)	µSBS	µSBS significantly decreased in all groups	No data
Sabatini, 2015	24 h, 6 months and 1 year of storage at 37°C in artificial saliva solution	All Bond Universal-ABU (Bisco, Schaumburg, IL, USA) Experimental adhesive blends of ABU were prepared with conventional benzalkonium chloride) or with benzalkonium methacrylate	µTBS	All Bond Universal: significantly decreased (29.4 MPa - 24h 16.4 MPa – 6 months 15.3 MPa – 1 year)	No data
Lenzi, 2015	24 h of storage in distilled water at 37°C	Adper Single Bond 2-ASB	µTBS	Overall, aging resulted in a	Mixed/Adhesive

	1 year of storage in distilled water at 37°C	(3M ESPE, St. Paul, MN, USA) Clearfil SE Bond – CSE (Kuraray Co. Ltd., Osaka, Japan)		significant reduction in µTBS values (decreasing ranged of 42.3 % to 61.6 %), except to CSE applied to sound dentin	
Scheffel, 2015	24 h of storage in deionized water at 37°C 6 months and 1 year of storage at 37°C in artificial saliva solution	Adper Single Bond 2 (3M ESPE, St. Paul, MN, USA)	µTBS	Significantly decreased (24.6 MPa - 24h 21.1 MPa – 6 months 18.5 MPa – 1 year)	Mixed/Adhesive
Yeganeh, 2015	24 h of storage in distilled water at 37°C 6 months in distilled water at 37°C	Scotchbond Multi-Purpose (3M ESPE, Seefeld, Germany) Single Bond 2 (3M ESPE, Seefeld, Germany Clearfil SE Bond (Kuraray Co. Ltd., Okayama, Japan) All-Bond SE (BISCO, Schaumburg, Irving Park Rd., IL, USA)	µTBS	Scotchbond Multi-Purpose: Significantly decreased (21.48 – 24 h 14.39 MPa – 6 months) All-Bond SE: Significantly decreased (11.77 – 24 h 9.33 MPa – 6 months)	No data
Tuncer,2015	24 h of storage in distilled water at 37°C 1 year in distilled water at 37°C	Clearfil SE Bond (Kuraray Co. Ltd., Okayama, Japan)	µTBS	Significantly decreased (37.31 MPa to 24.78 MPa)	Mixed/Adhesive
Martins, 2012	24 h of storage in distilled water at 37°C 6 months in distilled water at 37°C	Adper Single Bond 2 (3M/ESPE, St. Paul, MN, USA) AdheSE (Ivoclar Vivadent, Schaan, Liechtenstein) Adper Prompt L-Pop (3M/ESPE, St. Paul, MN, USA)	µTBS	Significant reduction was only found for Adper Single Bond 2 (49.1 to 40.3 MPa)	No data
Castellan, 2013	24h, 3, 6 months and 1 year of storage at 37°C in artificial saliva solution	Adper Single Bond Plus (3M/ESPE, St. Paul, MN, USA) One Step Plus (Bisco, Inc., Schaumburg, IL, USA)	µTBS	Significant reduction was found for One Step Plus (49.66 to 36.85 MPa)	No data
Zabeu, 2018	24 h of storage in distilled water at 37°C 1 year in distilled water at 37°C	Scotchbond Multi-Purpose (3M ESPE, Seefeld, Germany) Clearfil SE Bond (Kuraray Co. Ltd., Okayama, Japan)	µTBS	Scotchbond Multi-Purpose: Significantly decreased (46.55 – 24 h 37.65 MPa – 1 year) Clearfil SE Bond: Significantly decreased (31.47 – 24 h 25.91 MPa – 1 year)	Adhesive
Fugolin, 2019	24 h of storage in distilled water at 37°C	Adper Single Bond 2 (3M/ESPE, St. Paul,	µTBS	Except for one experimental	No data

	3 weeks in distilled water at 37°C 6 months in distilled water at 37°C	MN, USA) Experimental adhesives with (meth)acrylamides		adhesive, all other versions maintained constant bond strength, even after 6 months.	
Alaghehmad, 2018	24 h of storage in distilled water at 37°C 2,500 thermocycles between 5 and 55 °C (dwell time was 30 s and the transfer time was 30 s)	Adper Single Bond 2 (3M/ESPE, St. Paul, MN, USA)	µSBS	No statistically significant differences in the mean microshear bond strength values between the subgroups with and without thermocycling.	Cohesive
Alamoudi, 2018	24 h, 1 week and 2 months of storage in distilled water at 37°C	Adper Single Bond 2 (3M/ESPE, St. Paul, MN, USA)	µTBS	A significant reduction for both chlorhexidine and control groups was found in the three storage times	Adhesive
Venigalla, 2016	24 h of storage at 37°C in artificial saliva solution 6 months of storage at 37°C in artificial saliva solution	Adper Single Bond 2 (3M/ESPE, St. Paul, MN, USA)	µTBS	No significant reduction for all groups	Adhesive
Sampaio, 2013	24 h of storage in distilled water at 37°C 6 months in distilled water at 37°C	Adper Single Bond 2 (3M/ESPE, St. Paul, MN, USA) Clearfil SE Bond (Kuraray Co. Ltd., Okayama, Japan) Adper Se Plus (3M ESPE, St. Paul, MN, USA) P90 LS System (3M ESPE, St. Paul, MN, USA)	µTBS	All systems were stable in terms of bond strength up to 6 month of water storage.	

FIGURES

Figure 1. PRISMA flow diagram.

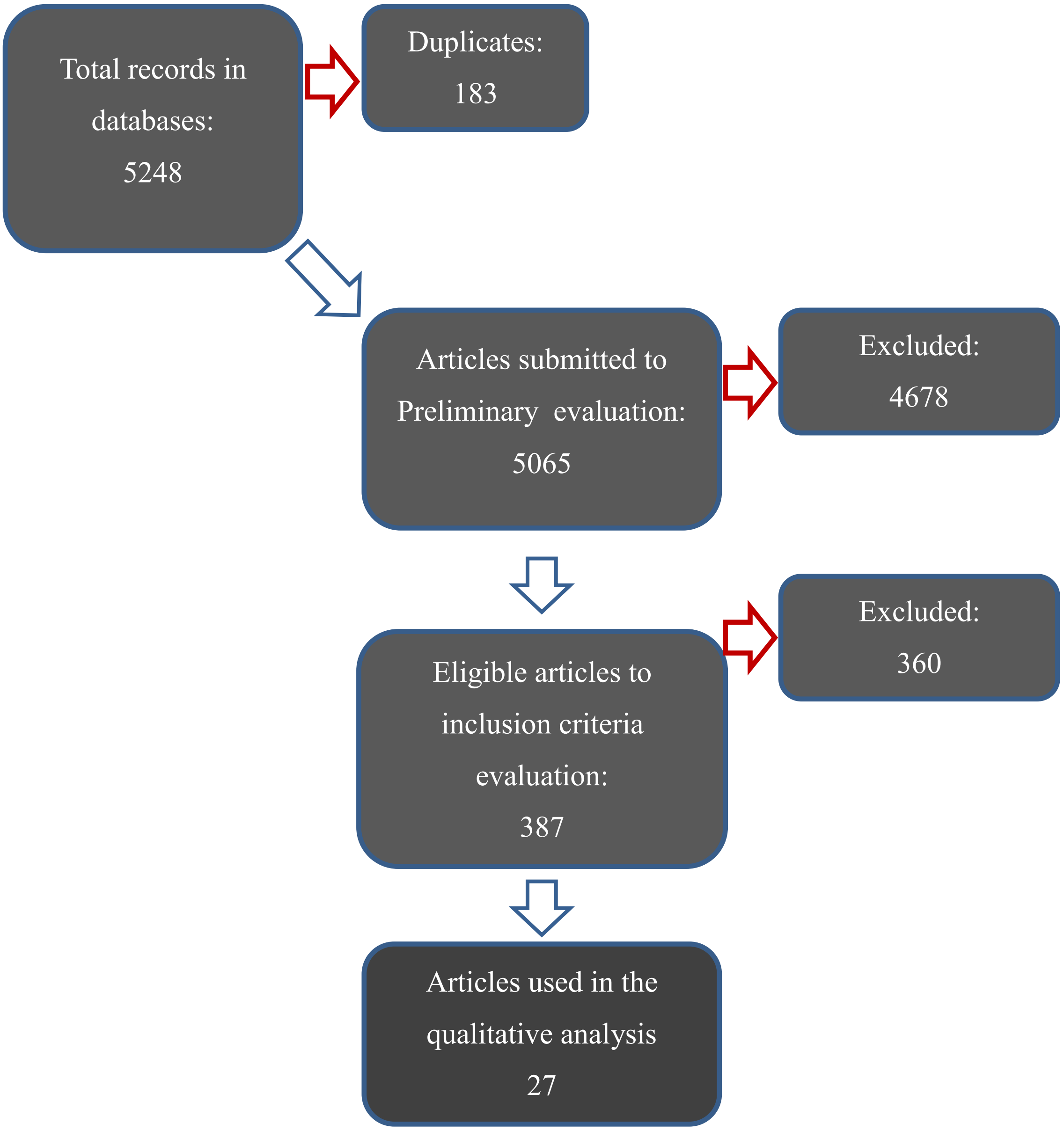


Figure 2. The frequency that the AAM are the choose in the literature (%).

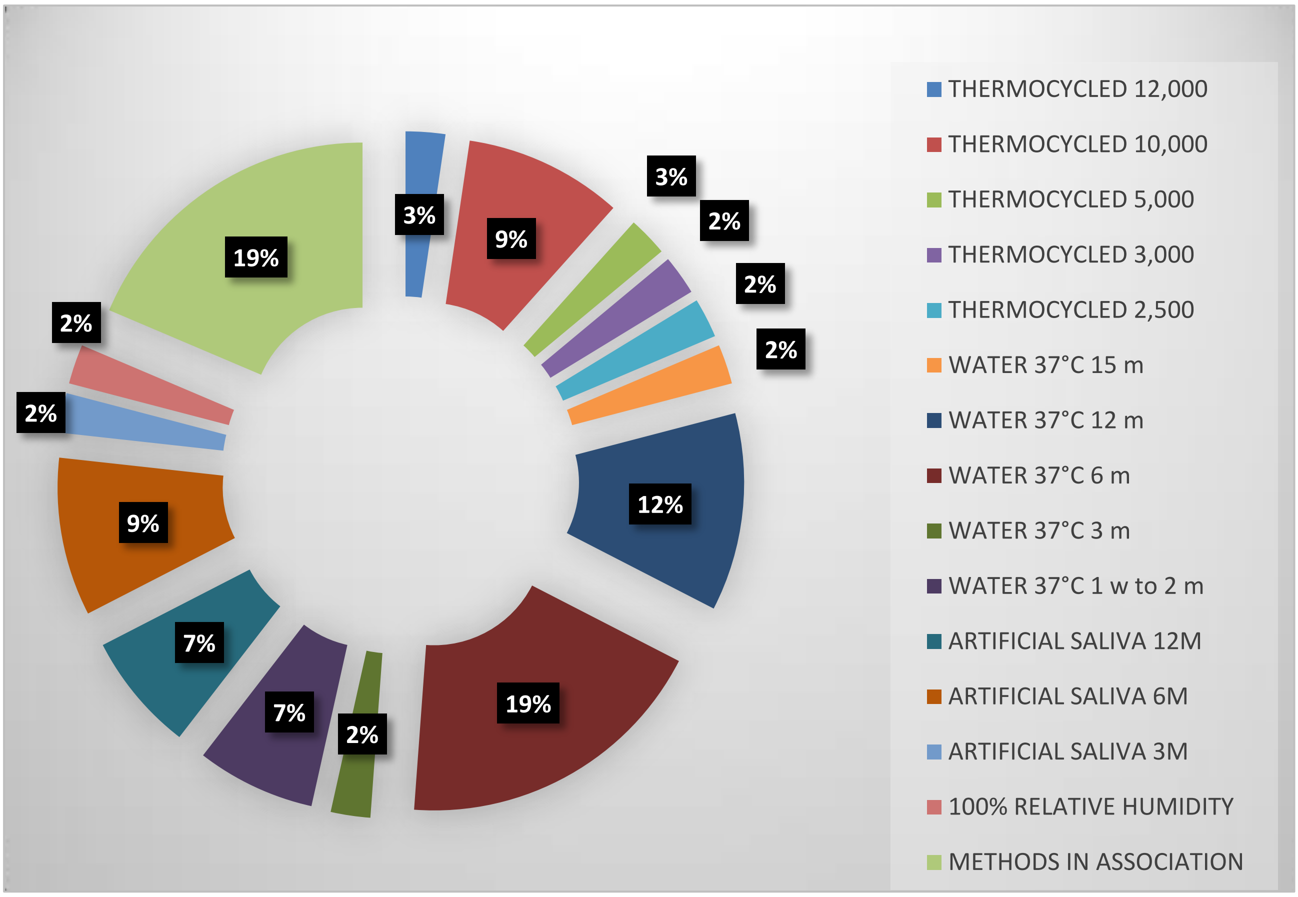


Figure 3. The adhesive system frequency that was used in the literature (%).

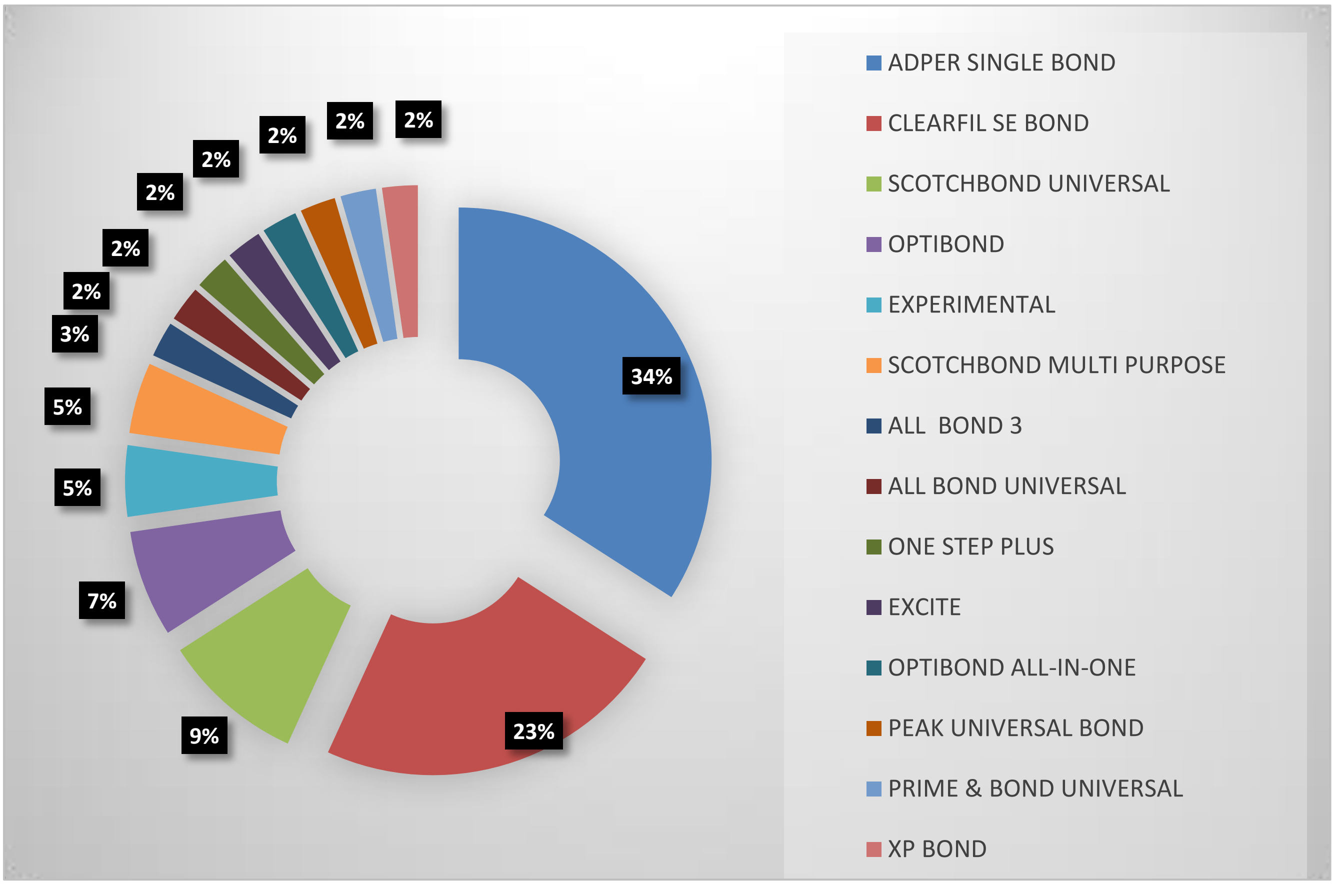
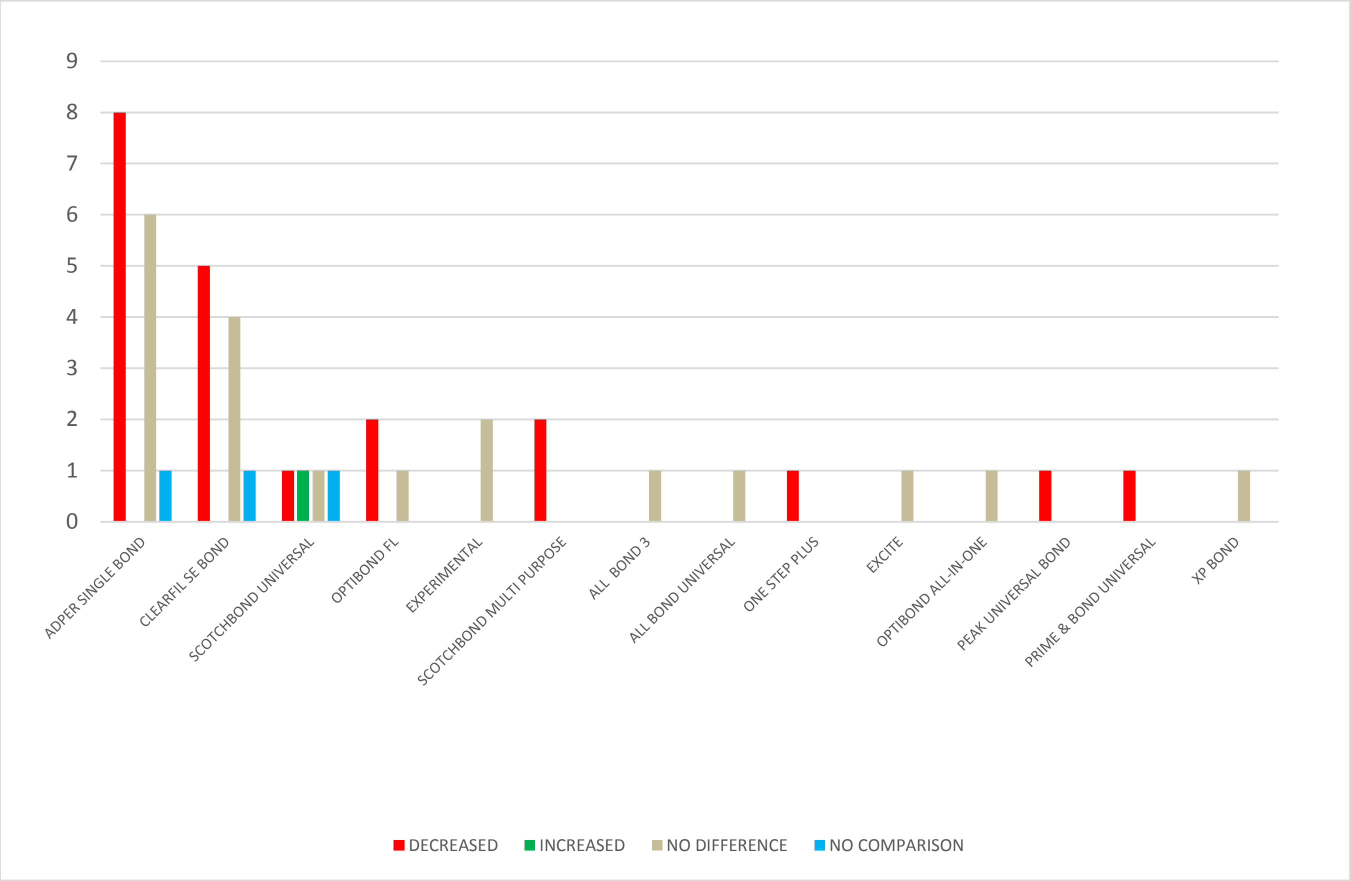


Figure 4. Changes in bond strength results by adhesive system (n).



4 DISCUSSÃO

Ambos os artigos apresentados analisaram os métodos de envelhecimento artificial mais comumente utilizados em estudos laboratoriais e, assim, tentaram identificar qual protocolo tem maior efeito deletério sobre a resistência de união, para as diferentes classes de sistemas adesivos. No artigo 2 constatou-se que o meio de degradação em água (com diferentes períodos de armazenamento), bem como o método de termociclagem (com variados regimes de ciclos), foram os protocolos de escolha para simular as condições de degradação que ocorre no ambiente oral. Assim como no artigo 1, observou-se que esses dois métodos promoveram efeitos distintos na resistência de união das diferentes classes de adesivos, independente do sistema adesivo utilizado. No entanto, a revisão dos estudos *in vitro* no artigo 2, revelou que não há uma padronização entre os métodos, uma vez que tanto o período de armazenamento em água, quanto o regime de ciclos e temperatura na termociclagem variou entre os estudos analisados. Isso está de acordo com outros estudos, em que se confirma que não há um consenso quanto ao desempenho dos testes de resistência de união para restaurações adesivas que são submetidas aos diferentes protocolos de envelhecimento, independente do regime e tempo de degradação (BRESCHI et al., 2008; BRESCHI et al., 2009; CUEVAS-SUÁREZ et al., 2019; DENG et al., 2014.; FUKOKA et al., 2011; NICOLOSO et al., 2019).

Os valores distintos na resistência adesiva podem ser explicados tanto pela intensidade de degradação do método de envelhecimento quanto pela composição do adesivo (COURSON et al., 2005; GUNAYDIN et al., 2016; LOGUERCIO et al., 2014). Em relação à composição química, Anchieta e colaboradores (2015) concluíram que sistemas adesivos contendo o monômero metacriloiloxidecil dihidrogeniofosfato (MDP) apresentam comportamento superior aos demais adesivos que não contém essa molécula, mantendo a resistência adesiva estável mesmo após 12 meses de armazenamento. Esse monômero funcional, que é capaz de modificar as superfícies e produzir adesão química à estrutura dental, está presente em alguns adesivos universais disponíveis no mercado. Essa classe de adesivo é a mais recente do mercado e parece ser tendência nos estudos que avaliam a durabilidade de união (HANABUSA et al., 2012; MAKISHI et al., 2016; MARCHESI et al., 2014; WANG et al., 2017; ZHANG et al., 2016). Isso está de acordo com os resultados do artigo 2, em que a maioria dos estudos revisados utilizou algum tipo de adesivo universal, com diferentes testes de adesão e de envelhecimento.

No artigo 1 da presente tese, apesar de os valores de resistência adesiva não apresentarem alterações significativas após 6 meses de envelhecimento em água, quando comparado ao grupo controle (24h), os diferentes adesivos testados não foram capazes de manter estáveis a resistência de união após a termociclagem, mesmo para aqueles sistemas adesivos contendo a molécula MDP. Isso pode ser influenciado pelo tipo de método de envelhecimento e pelo regime mais intenso de degradação (AMARAL et al., 2007; CHEN et al., 2015; DE MUNCK et al., 2005; HARIRI et al., 2012; KAMEL et al., 2014; MAKISHI et al., 2016; SAI et al., 2016; TSUJIMOTO et al., 2016a; TSUJIMOTO et al.,2017 ;WAGNER et al., 2014; WANG et al., 2017).

Na termociclagem, além do efeito hidrolítico da água sobre a camada híbrida, temos a alternância cíclica de temperatura (quente e frio), simulando as condições do ambiente oral (AMARAL et al., 2007; DE MUNCK et al., 2005). A água tem a capacidade de permear a interface adesiva após a polimerização do adesivo, em virtude da característica hidrofílica da camada híbrida, aumentando a sorção de água e contribuindo para maior hidrólise dessa interface, por meio da degradação das fibras colágenas da dentina e dos monômeros resinosos hidrofílicos do adesivo (FRASSETTO et al., 2016; HOSAKA et al., 2010; MORTIER et al., 2004; REIS et al., 2007; REIS et al., 2013; SHONO et al., 1999;. TEIXEIRA et al., 2021;; TEZVERGIL-MUTLUAY; PASHLEY; MUTLUAY; 2015). Na presença de monômeros hidrofílicos, a água do meio externo é absorvida com facilidade, exercendo um efeito plastificador nas moléculas, determinando alterações em algumas propriedades mecânicas do adesivo (BAHROLOLUMI et al., 2017; BRESCHI et al., 2008; HILGERT et al., 2008). Dessa forma, alguns estudos laboratoriais demonstram que o comprometimento da interface adesiva ocorre, em parte, por meio da degradação dos componentes do adesivo que absorvem quantidades significativas de água, tornando-se cada vez mais permeáveis e suscetíveis à hidrólise (FERRACANE; 2006; ITO et al., 2006; MALACARNE et al., 2006; YIU et al., 2004). Já as mudanças de temperatura do teste de termociclagem induzem tensões de contração e expansão repetitivos na interface dente/material, causando a propagação de trincas, que por sua vez, acarreta gaps na interface, facilitando a infiltração de água na camada híbrida. (AMARAL et al., 2007; DE MUNCK et al., 2005; GALE et al., 1999; KITASAKO et al., 2000; MORRESI et al., 2014).

Estudos realizados in vitro demostraram que adesivos autocondicionantes simplificados, incluindo os universais, comportam-se como membranas semipermeáveis após a polimerização, pois são susceptíveis à sorção de água e se degradam mais rapidamente que os adesivos que apresentam o passo de aplicação da resina hidrofóbica (adesivos convencional de 3 passos e autocondicionante de 2 passos). Uma vez polimerizados, os adesivos simplificados permitem a passagem de fluido dentinário até a superfície, o que contribui para a degradação da interface adesiva e diminuição da resistência de união (BRESCHI et al., 2008; SEZINANDO et al., 2015). Essas conclusões corroboram alguns de nossos resultados, uma vez que os adesivos que representam a categoria autocondicionante e universal, contemplados em nosso estudo, Clearfil SE Bond e Scotchbond Universal, respectivamente, reduziram seus valores de resistência adesiva após a termociclagem.

De modo geral, constatou-se que os métodos de envelhecimento artificial afetaram a resistência de união de forma diferente, independente do adesivo utilizado. Por outro lado, há uma ampla variedade de adesivos disponíveis, com diferentes formulações e protocolos de utilização e, por isso, faz-se necessário avaliar o comportamento desses materiais a longo prazo. Protocolos experimentais padronizados dos testes *in vitro*, como o envelhecimento artificial, fornecem informações valiosas acerca da durabilidade de união e podem servir como filtro para prosseguimento em ensaios clínicos (DE MUNCK et al., 2005; DENG et al., 2014; HEINTZE et al., 2015; MAKISHI et al., 2016; SAI et al., 2016; YAMAUTI et al., 2003). No entanto, parece claro que não há consenso sobre a padronização dos métodos de envelhecimento apresentados nos estudos, uma vez que além dos diferentes testes possíveis, há variação dentro do mesmo método.

Portanto, para conseguir estabelecer uma similaridade mais confiável entre os testes *in vitro* e ensaios clínicos de longo prazo, mais estudos direcionados para os métodos de envelhecimento deveriam ser realizados, a fim de que se possa determinar o protocolo de envelhecimento mais efetivo em degradar a adesão para cada adesivo específico.

5 CONCLUSÃO

Com base nos resultados dos artigos que compõem a presente tese, pode-se concluir que os métodos de envelhecimento artificial afetam de forma distinta cada sistema adesivo. Além disso, observou-se que, entre os estudos *in vitro* analisados, a escolha do protocolo de envelhecimento se dá de forma aleatória, sem levar em consideração o tipo de adesivo testado, sua composição química ou técnica adesiva. Novos estudos são necessários para que se estabeleça um método padronizado de envelhecimento, que se aproxime de forma mais similar ao que ocorre no ambiente oral, assim como, determinar àquele que afeta negativamente de forma mais intensa uma determinada classe de adesivo.

REFERÊNCIAS

AMARAL, F. L. et al. Assessment of in vitro methods used to promote adhesive interface degradation: a critical review. **Journal of Esthetic and Restorative Dentistry**, v. 19, n. 6, p. 340-353, 2007.

ANCHIETA, R. B. et al. Effect of long-term storage on nanomechanical and morphological properties of dentin–adhesive interfaces. **Dental Materials**, v. 31, n. 2, p. 141-153, 2015.

BAHROLOLUMI, N. et al. Effect of water storage on ultimate tensile strength and mass changes od universal adhesives. **Journal of Clinical and Experimental Dentistry**, v. 9, n. 1, p. 78-83, 2017.

BRESCHI, L. et al. Dental adhesion review: aging and stability of the bonded interface. **Dental Materials**, v. 24, n. 1, p. 90-101, 2008.

BRESCHI, L. et al. Influence of chlorhexidine concentration on the durability of etch-and-rinse dentin bonds: a 12-month in vitro study. **The Journal of Adhesive Dentistry**, v. 11, n. 3, p. 191-198, 2009.

CARRILHO, M. R. O. et al. Effects of storage media on mechanical properties of adhesive systems. **American Journal of Dentistry,** 2004; v.17, n. 2, p. 104–108, 2004..

COURSON, F. et al. Bond strengths of nine current dentine adhesive systems to primary and permanent teeth. **Journal of Oral Rehabilitation**, v. 32, n. 4, p. 296-303, 2005.

CHEN, C. et al. Bonding of universal adhesives to dentin- old wine in new bottle? **Journal of Dentistry**, v. 43, n. 5, p. 525-536, 2015.

CUEVAS-SUÁREZ, C. E. et al. Impact of shelf-life simulation on bonding performance of universal adhesive systems. **Dental Materials**, v. 35, n. 9, p. 204-219, 2019.

DE MUNCK, J. et al. NaOCl degradation of a HEMA-free all-in-one adhesive bonded to enamel and dentin following two air-blowing techniques. **Journal of Dentistry**, v. 35, n. 1, p. 74-83, 2007.

DE MUNCK, J. et al. A critical review of the durability of adhesion to tooth tissue: methods and results. **Journal of Dental Research**, v. 84, n. 2, p. 118-132, 2005.

DE MUNCK, J. et al. Four-year water degradation of total-etch adhesives bonded to dentin. **Journal of Dental Reserch**, v. 82, n. 2, p. 136-140, 2003.

DENG, D. et al. Effects of different artificial ageing methods on the degradation of adhesive-dentine interfaces. **Journal of Dentistry**, v. 42, n. 12, p. 1577-1585, 2014.

FERRACANE, J. L. Hygroscopic and hydrolytic effects in dental polymer networks. **Dental Materials**, v. 22, n.3, p, 211-222, 2006.

FRANÇA, F. M. G.; SANTOS, A. J.; LOVADINO, J. R. A.Influence of air abrasion and long-term storage on the bond strength of self-etching adhesives to dentin. **Operative Dentistry**, v. 32, n. 3, p. 217– 224, 2007.

FRASSETTO, A. et al. Mechanisms of degradation of the hybrid layer in adhesive dentistry and therapeutic agents to improve bond durability - A literature review. **Dental Materials**, v. 32, n. 2, p. e41-e53, 2016.

FREEMAN, R. et al. Effect of air abrasion and thermocycling on resin adaptation and shear bond strength to dentin for an etch-and-rinse and self-etch resin adhesive. **Dental Materials Journal**, v. 31, n. 2, p. 180-188, 2012.

FUKOKA, A. et al. Hydrolytic stability of one-step self-etching adhesives bonded to dentin. **The Journal of Adhesive Dentistry**, v. 13, n. 3, p. 243–248, 2011.

GALE, M. S.; DARVELL, B. W. Thermal cycling procedures for laboratory testing of dental restorations. **Journal of Dentistry**, v. 27, n. 2, p. 89–99, 1999.

GUNAYDIN, Z.; YAZICI, A. R.; CEHRELI, Z. C. In Vivo and In Vitro Effects of Chlorhexidine Pretreatment on Immediate and Aged Dentin Bond Strengths. **Operative Dentistry**, v. 41, n. 3, p. 258-267, 2016.

HANABUSA, M. et al. Bonding effectiveness of a new ‘multi-mode’ adhesive to enamel and dentine. **Journal of Dentistry**, v. 40, n. 6, p. 475–484, 2012.

HARIRI, I. et al. The Effects of Aging on Shear Bond Strength and Nanoleakage Expression of an Etch-and- Rinse Adhesive on Human Enamel and Dentin. **The Journal of Adhesive Dentistry**, v. 14, n. 3, p. 235-243, 2012.

HASHIMOTO, M. et al. In vitro degradation of resin-dentin bonds analyzed by microtensile bond test, scanning and transmission electron microscopy. **Biomaterials**, v. 24, n. 21, p. 3795-3803, 2003.

HASHIMOTO, M. et al. In vitro degradation of resin–dentin bonds with one-bottle self-etching adhesives. **European Journal of Oral Sciences,** v. 117, n. 5, p. 611–617, 2009.

HEINTZE, S. D.; ROUSSON, V.; MAHN, E. Bond strength tests of dental adhesive systems and their correlation with clinical results - a meta-analysis. **Dental Materials**, v. 31, n. 4, p. 423–434, 2015.

HILGERT, L.A. et al. Adhesive procedures in daily practice: Essential aspects. **Compendium of Continuing Education in Dentistry**, v. 29, n. 4, p. 208-215, 2008.

HOSAKA, K. et al. Relationship between mechanical properties of one-step self-etch adhesives and water sorption . **Dental Materials,** v. 26, n. 4, p. 360-367, 2010.

ITO, S. et al. Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity. **Biomaterials**, v. 26, n.33, p.6449-6459, 2005.

KAMEL, M. et al. The effect of water storage on micro-shear bond strength of contemporary composite resins using different dentin adhesive systems. **Tanta Dental Journal,** v. 11, p. 47–55, 2014.

KITASAKO, Y. et al. The influence of storage solution on dentin bond durability of resin cement. **Dental Materials,** v. 16, n. 1, p. 1-6, 2000.

LAWSON, N. C. et al. Two-year clinical trial of a universal adhesive in total-etch and self-etch mode in non-carious cervical lesions. **Journal of Dentistry**, v. 43, n. 10, p. 1229–1234, 2015.

LELOUP, G. et al.Meta-analytical review of factors involved in dentin adherence. **Journal of Dental Research**, v. 80, n. 7, p. 1605-1614, 2001.

LOGUERCIO, A. D. et al. A comprehensive laboratory screening of three-step etch-and-rinse adhesives. **Operative Dentistry**, v. 39, n. 6, p. 652-662, 2014.

LOGUERCIO, A. D. et al. A new universal simplified adhesive: 36-month randomized double-blind clinical trial. **Journal of Dentistry**, v. 43, p. 9, p. 1083-1092, 2015.

MAKISHI, P. et al. Effect of storage time on bond strength and nanoleakage expression of universal adhesives bonded to dentin and etched enamel. **Operative Dentistry**, v. 41, n. 3, p. 305-317, 2016.

MALACARNE, J. et al. Water sorption/solubility of dental adhesive resins. **Dental Materials**, v. 22, n. 10, p. 973-980, 2006.

MARCHESI, G. et al. Adhesive performance of a multi-mode adhesive system: 1-year in vitro study. **Journal of Dentistry**, v. 42, n. 5, p. 603–612, 2014.

MASARWA, N. et al. Longevity of self-etch dentin bonding adhesives compared to etch-and-rinse dentin bonding adhesives: A systematic review. **The Journal of Evidence-Based Dental Practice,** v. 16 , n. 2, p. 96-106, 2016.

MONTICELLI, F. et al. Resistance to degradation of resin-dentin bonds using a one-step HEMA-free adhesive. **Journal of Dentistry,** v. 35, n. 2, p. 181-186, 2007.

MORRESI, A. L. et al. Thermal cycling for restorative materials: does a standardized protocol exist in laboratory testing? A literature review. **Journal of Mechanical Behavior Biomedical Materials**, v. 29, p. 295-308, 2014.

MORTIER, E. et al. Importance of water sorption and solubility studies for couple bonding agent—resin-based filling materials. **Operative Dentistry,** v. 29, n. 6, p. 669-676, 2004. 2004;29:669–76.

MUSANJE, L.; SHU, M.; DARVELL, B. W. Water sorption and mechanical behaviour of cosmetic direct restorative materials in artificial saliva. **Dental Materials,** v. 17, n. 5, p. 394–401, 2001.

NAKABAYASHI, N.; KOJIMA, K.; MASUHARA, E. The promotion of adhesion by the infiltartiomn of monomers into tooth substrates. **Journal of Biomedical Materials Research**, v. 16, n. 3, p. 265-273, 1982.

NAKABAYASHI, N.; SAIMI, Y. Bonding to intact dentin. **Journal of Dental Research,** v. 9, p. 1706-1715, 1996.

NICOLOSO, M. G. et al. Short-term aging and the dentin bond strength od adhesive systems. **General Dentistry**, v.67, n. 3, p. 71-76, 2019.

OKUDA, M. et al. Long-term durability of resin dentin interface: nanoleakage vs microtensile bond strength. **Operative Dentistry,** v. 27, n. 3, p. 289-296, 2002.

REIS, A. F.; GIANNINI, M. R.; PEREIRA, P. N. Influence of water-storage time on the sorption and solubility behavior of current adhesives and primer/adhesive mixtures. **Operative Dentistry,** v. 32, n. 1, p. 53-59, 2007.

REIS, A. F. et al. Effects of water-storage on the physical and ultramorphological features of adhesives and primer/adhesive mixtures. **Dental Materials Journal.** v. 29, n. 6, p. 697-705, 2010.

REIS, A. F. et al. Effects of Warm Air Drying on Water Sorption, Solubility, and Adhesive Strength of Simplified Etch-and-Rinse Adhesives. **Journal of Adhesive Dentistry,** v. 15, n. 1, p. 41–46, 2013.

RINASTITI, M. et al. Effects of surface conditioning on repair bond strengths of non-aged and aged microhybrid, nanohybrid, and nanofilled composite resins. **Clinical Oral Investigations**, v. 15, n. 5, p. 625–633, 2011.

SAI, K. et al. Influence of degradation conditions on dentin bonding durability of three universal adhesives. **Journal of Dentistry**, v. 54, p. 56-61, 2016.

SANO, H. Microtensile testing, nanoleakage, and biodegradation of resin-dentin bonds. **Journal of Dental Research**, v. 85, n. 1, p. 11-14, 2006.

SEZINANDO, A. et al. Influence of a hydrophobic resin coating on the immediate and 6-month dentin bonding of three universal adhesives. **Dental Materials**, v. 31. N. 10, p. e236-e246, 2015.

SHONO, Y. et al. Durability of resin-dentin bonds. **The Journal of Adhesive Dentistry**, v. 1, n. 3, p. 211-218, 1999.

TEIXEIRA, G. S.; PEREIRA G. K. R.; SUSIN, A. H. Aging methods- An evaluation of their influence on bond strength. **European Journal of Dentistry**, v. 28 (Online ahead of print).

TEZVERGIL-MUTLUAY, A.; D. PASHLEY, D.; M. M. MUTLUAY, M. M. Long-Term Durability of Dental Adhesives. **Current Oral Health Reports**, v. 2, p.174–181, 2015.

TOLEDANO, M. et al. Differential effect of in vitro degradation on resin–dentin bonds produced by self-etch versus total-etch adhesives. **Journal of Biomedical Materials Research Part A,** v. 77, n. 1, p. 128–135, 2006.

TSUJIMOTO, A. et al. Influence of duration of phosphoric acid pre-etching on bond durability of universal adhesives and surface free- energy characteristics of enamel. **European Journal of Oral Sciences**. V. 124, n. 4, p.377–386, 2016.

TSUJIMOTO, A. et al. Bonding performance and interfacial characteristics of short fiber-reinforced resin composite in comparison with other composite restoratives. **European Journal of Oral Sciences**, v. 124, n. 3, p. 301-308, 2016a.

TSUJIMOTO, A. et al. Interfacial characteristics and bond durability of universal adhesive to various substrates. **Operative Dentistry**, v. 42, n. 2, p. 59-70, 2017.

WAGNER. A. et al. Bonding performance of universal adhesives in different etching modes. **Journal of Dentistry**, v. 42, n. 7, p. 800-807, 2014.

WANG, R. et al. Adhesive interfacial characteristics and the related bonding performance of four self-etching adhesives with different functional monomers applied to dentin. **Journal of Dentistry**, v. 62, p.72-78, 2017.

YAMAUTI, M. et al. Degradation of resin-dentin bonds using NaOCl storage. **Dental Materials**, v. 19, n. 5, p. 399-405, 2003.

YIU, C.K. et al. Effect of resin hydrophilicity and water storage on resin strength. **Biomaterials**, v.25, n.26, p.5789-5796, 2004.

ZHANG, Z-Y. et al. Defying ageing: an expectation for dentine bonding with universal adhesives? **Journal of Dentistry**, v. 45, p. 43-52, 2016.

ANEXO A – NORMAS PARA PUBLICAÇO NO PERIÓDICO OPERATIVE DENTISTRY

Guidelines for Preparing Manuscripts for Operative Dentistry

- A Laboratory or Clinical Research Manuscript file must include:
  - a title
  - a running (short) title
  - a clinical relevance statement
  - a concise summary (abstract)
  - introduction, methods & materials, results, discussion and conclusion
  - references (see below)

  - The manuscript **MUST NOT** include any:
    - Identifying information such as:
    - Authors
    - Acknowledgements
    - Correspondence information
    - Figures
    - Graphs
    - Tables
- An acknowledgement, disclaimer and/or recognition of support (if applicable) must in a separate file and uploaded as supplemental material.
- All figures, illustrations, graphs and tables must also be provided as individual files. These should be high resolution images, which are used by the editor in the actual typesetting of your manuscript. Please refer to the instructions below for acceptable formats.
- All other manuscript types use this template, with the appropriate changes as listed below.

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

GENERAL INFORMATION

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

REQUIREMENTS

☐ FOR ALL MANUSCRIPTS

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

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

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

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

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

☐ FOR REFERENCES

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

EXAMPLES OF REFERENCE STYLE

- Journal article: two authors Evans DB & Neme AM (1999) Shear bond strength of composite resin and amalgam adhesive systems to dentin *American Journal of Dentistry* **12(1)** 19-25.
- Journal article: multiple authors Eick JD, Gwinnett AJ, Pashley DH & Robinson SJ (1997) Current concepts on adhesion to dentin *Critical Review of Oral and Biological Medicine* **8(3)**306-335.
- Journal article: special issue/supplement Van Meerbeek B, Vargas M, Inoue S, Yoshida Y, Peumans M, Lambrechts P &Vanherle G (2001) Adhesives and cements to promote preservation dentistry *Operative Dentistry* **(Supplement 6)** 119-144.
- Abstract: Yoshida Y, Van Meerbeek B, Okazaki M, Shintani H & Suzuki K (2003) Comparative study on adhesive performance of functional monomers *Journal of Dental Research* **82(Special Issue B)** Abstract #0051 p B-19.
- Corporate publication: ISO-Standards (1997) ISO 4287 Geometrical Product Specifications Surface texture: Profile method – Terms, definitions and surface texture parameters *Geneve: International Organization for Standardization* **1st edition** 1-25.
- Book: single author Mount GJ (1990) *An Atlas of Glass-ionomer Cements* Martin Duntz Ltd, London.
- Book: two authors Nakabayashi N &Pashley DH (1998) *Hybridization of Dental Hard Tissues* Quintessence Publishing, Tokyo.
- Book: chapter Hilton TJ (1996) Direct posterior composite restorations In: Schwarts RS, Summitt JB, Robbins JW (eds) *Fundamentals of Operative Dentistry* Quintessence, Chicago 207-228.
- Website: single author Carlson L (2003) Web site evolution; Retrieved online July 23, 2003 from: http://www.d.umn.edu/~lcarlson/cms/evolution.html
- Website: corporate publication National Association of Social Workers (2000) NASW Practice research survey 2000. NASW Practice Research Network, 1. 3. Retrieved online September 8, 2003 from: http://www.socialworkers.org/naswprn/default