UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

MARINA RODRIGUES SANTI

AVALIAÇÃO DE NANOPARTÍCULAS DE ZINCO NAS PROPRIEDADES MECÂNICAS E NA ESTABILIDADE DA INTERFACE ADESIVA

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Tese apresentada *à* Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dosrequisitos exigidos para a obtenção do título de Doutora em Clínica Odontológica, na Área de Dentística.

Thesis presented to the Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Dental Clinic, in Dentistry area.

Orientador: Prof. Dr. Luís Roberto Marcondes Martins

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RESUMO

Objetivo: Avaliar o efeito de nanopartículas de zinco como pré-tratamento dentinário na resistência de união resina-dentina, na nanodureza e no módulo de elasticidade da camada híbrida após 24 h e 12 meses; na atividade colagenolítica, namorfologia da interface adesiva, e o potencial antibacteriano sobre *S. mutans*. Materiais e Métodos: Terceiros molares hígidos foram divididos de acordo com a solução de pré-tratamento dentinário utilizada: Óxido de Zinco 5% (OZ5) e 10% (OZ10); Fluoreto de Zinco 1,5% (FZ1,5) e 3% (FZ3); Clorexidina 0,2% (CHX) como controle positivo; e o grupo sem pré-tratamento como controle negativo (CN). Após aaplicação do ácido fosfórico, as soluções foram aplicadas por 1 min, e o excesso foi removido com papel absorvente. Na sequência, realizou-se a aplicação do primer e do adesivo (Scotch Bond Multipurpose – 3M ESPE), seguido da restauração em resina composta. Os testes de resistência de união (RU) por microtração (n=8), padrão de falha, nanodureza (NA n=3), módulo de elasticidade (ME n=3), zimografia *in situ* (n=3) e morfologia da interface adesiva (n=3) foram avaliados após o armazenamento dos espécimes em solução de *body fluid*. O potencial antibacteriano foi avaliado através da mínima concentração inibitória (MCI) e bactericida (MCB). Para avaliar a RU, realizou-se ANOVA 2 fatores com post-hoc de Bonferroni e para NA e ME realizou-seANOVA 2 fatores com medidas repetidas (rANOVA) seguido de post-hoc de Bonferroni(α 5%). Os outros testes foram avaliados de forma descritiva. Resultados: Osresultados de RU mostraram que FZ3, OZ10, e CHX mantiveram os valores após 12 meses de armazenamento, e o FZ1.5 e o OZ5 aumentaram os valores. Falhas mistase coesivas foram as mais predominantes para todos os grupos. FZ3 apresentou maiores valores de NA e ME em ambos os tempos avaliados. CHX apresentou a menor atividade enzimática seguido do OZ5. FZ3 apresentou nanopartículas na baseda camada híbrida, enquanto OZ5, OZ10 e FZ1.5 apresentaram nanopartículasincorporadas no adesivo. OZ apresentou MCI e MCB de 62,5 µg/mL e FZ apresentou 31,25 µg/mL respetivamente. Conclusão: Pré-tratamentos com nanopartículas de zinco, especialmente com fluoreto de zinco, apresentaram uma abordagem promissora para aumentar a longevidade da restauração, mantendo os valores de resistência de união e melhorando as propriedades mecânicas da camada

híbrida.

Palavras-Chave: Nanopartículas. Zinco. Adesivos Dentinários. Dentina.

ABSTRACT

Objective: To evaluate the effect of zinc nanoparticle solution as pretreatment on the microtensile bond strength, nanohardness and young's modulus of hybrid layer after 24 h and 12 months of storage. As well as the collagenolytic activity, interface morphology and antibacterial potential against *S. mutans*. Materials and Methods: Human third molars were divided into 6 groups according to pretreatment solution: 5% Zinc Oxide (ZO5); 10% Zinc Oxide (ZO10); 1.5% Zinc Fluoride (ZF1.5); 3% Zinc Fluoride (ZF3); 0.2% Chlorhexidine was used as positive control (CHX); and no pretreatment was considered as negative control (NC). After etching with phosphoric acid, the solutions were applied for 1 min and the excess was dried. Next,primer and adhesive (Scotch Bond Multipurpose – 3M ESPE) were applied, followed by the restauration in resin composite. Microtensile bond strength (μTBS n=8), failuremode, nanohardness (NH n=3), young's modulus (YM n=3), in situ zymography (n=3)and interface morphology (n=3) were evaluated after body fluid solution immersion. The antibacterial activity was determined by minimum inhibitory (MIC) and bacterial (MBC) concentration. The μTBS was evaluated by two-way ANOVA followed by Bonferroni post-hoc; and NH and YM data were analyzed by two-way repeated-measures analyses of variance (rANOVA) followed by Bonferroni post-hoc (α 5%). *In situ* zymography, interface morphology, MIC and MBC were descriptively analyzed. Results: ZF3, ZO10 and CHX maintained the μTBS values over time, while ZF1.5 and Z05 increased values. Mixed and cohesive failures were the most prevalent typesfor all groups. ZF3 presented higher values of NH and YM on both times evaluated. CHX presented the lowest enzymatic activity followed by ZO5. ZF3 presented nanoparticles on the bottom of the hybrid layer, while ZO5 and ZO10 presented nanoparticles within adhesive layer on the interface morphology images. MIC and MBCfor ZO was 62,5 μg/mL and for ZF was 31,25 μg/mL respectively. Conclusion: Pretreatment with zinc nanoparticles, specially zinc fluoride, may be a promising method to increase the longevity of restorations, maintaining the bond strenght valuesand improving the mechanical properties of the hybrid layer.

Key Words: Nanoparticles. Zinc. Dentin-Bonding Agents. Dentin.

SUMÁRIO

1 INTRODUÇÃO

As restaurações adesivas têm sido muito utilizadas por apresentarem diversas vantagens, como por exemplo, o fato de serem estéticas e não necessitarem de preparos cavitários amplos, sendo considerado um procedimento conservador (Askar *et al.,* 2021). Entretanto, os insucessos relacionados a resina compostacorrespondem de 80 a 90% em falhas adesivas (Spencer *et al*., 2014; Ferracane *et al*., 2017; Breschi *et al*., 2008). As falhas adesivas podem estar relacionadas a diversos fatores, como por exemplo, cárie recorrente (Ferracane, 2017; Tao *et al.,* 2019; Askar *et al.,* 2020). Nesse caso, o principal microrganismo encontrado é o *Streptococcus mutans,* devido a sua alta capacidade em aderir a diferentes substratos e glicoproteínas presentes na saliva (Spencer *et al*., 2014; Tao *et al.,* 2019).

Além do biofilme, a bactéria S. *mutans* também está presente nos túbulos dentinários, e que através do metabolismo, produzem ácido lático e ocasionam constantes quedas de pH, tornando-o crítico abaixo de 5.5 para esmalte e 6.5 para dentina, dissolvendo minerais e expondo as fibrilas de colágeno que são suscetíveisa degradação (Osorio *et al.,* 2014; Gutierrez *et al.,* 2015). Portanto, para que a incidência de falhas adesivas diminua, também deve-se levar em consideração a formação da camada híbrida (Pashley *et al.,* 2011). Para que a camada híbrida seja formada, é necessário que a superfície dentinária seja desmineralizada, e que a redede fibrilas colágenas seja exposta, permitindo que o sistema adesivo infiltre por entreestas fibrilas, criando um entrelaçamento micromecânico (Breschi e*t.,* 2008; Pashley *et al.,* 2011).

O processo de desmineralização dentinária irá depender da forma em queo sistema adesivo interage com o substrato (Breschi e*t.,* 2008; Pashley *et al.,* 2011). Os sistemas adesivos convencionais requerem o uso do ácido fosfórico (técnica *etchand rinse*) antes de sua aplicação para remover a *smear layer*, já os adesivos autocondicionantes dispensam o uso do ácido fosfórico (técnica *self-etch*), visto que os mesmos contêm monômeros ácidos capazes de condicionar a *smear layer* e infiltrarno substrato dentinário simultaneamente (Pashley *et al.,* 2011; Wang *et al.,* 2019). Entretanto, ao reduzir a quantidade de passos clínicos na técnica *self-etch*, resultados inconsistentes têm sido relatados em estudos laboratoriais e revisões sistemáticas devido à dificuldade

em criar microembricamento mecânico e por monômeros ácidosserem mais hidrofílicos quando comparado a monômeros convencionais (Van Landuyt*et al.,* 2007; Giannini *et al*., 2015; Chen *et al.,* 2015; Breschi e*t.,* 2008; Pashley *et al.,* 2011; Loguércio *et al.,* 2014; Wang *et al.,* 2019).

Por outro lado, os maiores valores de resistência de união dos sistemas adesivos convencionais estão relacionados a remoção da smear layer, facilitando a infiltração do *primer* e do *bond.* (Pashley *et al.,* 2011; Loguércio *et al.,* 2014; Schwendicke *et al*., 2019). Ao aplicar o ácido fosfórico, 50% do volume dos minerais é substituindo por água, e as fibrilas colágenas são expostas ao meio. (Sauro *et al.,* 2015; Yu *et al*., 2021) O seguinte passo é a aplicação do *primer*, que por sua vez, contêm monômeros hidrofílicos associado a solventes que removem a água interfibrilar permitindo com que os monômeros resinosos presentes no adesivo (*bond*)infiltrem entre as fibras de colágeno e nos túbulos dentinários, promovendo o imbricamento mecânico após a polimerização, formando então a camada híbrida. (Pashley *et al.,* 2011)

Entretanto, a técnica convencional *etch-and-rinse* envolve uma série de problemas que podem ocorrer ao longo do tempo, como por exemplo, a degradação hidrolítica. Após a lavagem do ácido fosfórico, a água residual presente na dentina pode prejudicar a difusão do *bond* na parte inferior da camada híbrida e, consequentemente, deixar as fibrilas de colágeno desprotegidas (Yu *et al.,* 2021). A presença da água também pode ocasionar a hidrólise de ligações éster em metacrilatos, presente na composição química do adesivo. Além disso, em caso de cavidades profundas, as bactérias residuais resultantes do processo de remoção parcial do tecido cariado, podem acelerar o processo de degradação pois também sãocapazes de hidrolisar esse tipo de ligação (Bourbia *et al*., 2013; Spencer *et al*., 2014).

Porém, se a água residual for totalmente removida e o substrato for seco, ocorrerá o colapso das fibras de colágeno, dificultando ainfiltração dos monômeros resinosos (De Munck *et al.,* 2009). Sendo assim, o substrato deve ser mantido úmido para que o sistema adesivo consiga infiltrar por completo entre as fibrilas de colágeno e evitar que ocorra a degradação enzimática pelas enzimas metaloproteinases (MMPs) e catepsinas (CTs) (De Munck *et al.,* 2009; Gutiérrez *et al.,* 2019; Yu *et al.,* 2021). As enzimas endógenas são zinco e cálcio

dependentes que participam do processo de odontogênese, mas permanecem inativas no dente por estarem mineralizadas. Entretanto, através das mudanças de pH resultante do condicionamento dentinário e/ou da liberação de lactato de bactériascariogênicas, essas enzimas são ativadas e degradam o colágeno não infiltrado peloadesivo (Mazzoni *et al.,* 2006; Spencer *et al*., 2014; Tjäderhane *et al.,* 2015; Gutiérrez*et al.,* 2019).

O digluconato de clorexidina (CHX) é o agente sintético mais estudado capaz de inativar as proteases endógenas da área de união dentina-adesivo. (Mazzoniet al., 2019) O CHX, em concentração de até 0,2%, pode se ligar a várias proteínas através do mecanismo de quelação, ou seja, evitando a ligações de íons como Zn^{2+} eCa^{2+} às estruturas das MMPs, inibindo a sua atividade catalítica. (Mazzoni et al., 2019) Entretanto, apesar do CHX ser considerado por vários autores um material que aumenta a longevidade do procedimento restaurador (Mazzoni et al., 2019; Toledano*et al.,* 2013), existem controvérsias na literatura devido ao fato desse composto ser lixiviado ao longo do tempo (Mazzoni et al., 2019).

Atualmente, sabe-se que a degradação da camada híbrida ocasionada pelas MMPs deve ser levada em consideração, mas que contribui pouco para as falhas das restaurações de compósito, e que o principal fator é o processo de cárie recorrente e acúmulo de biofilme bacteriano (Spencer *et al*., 2014; Tjaderhane *et al*, 2015). Dessa forma, nanopartículas bioativas tem sido estudas como forma de solucionar os problemas relacionados a diversos cenários clínicos. As nanopartículasbioativas apresentam como vantagem a reatividade química e biológica através da liberação de íons. Sendo assim, quando em contato com fluidos fisiológicos como por exemplos fluidos dentinários, que por sua vez são ricos em cálcio e fosfato, ocorre a precipitação de minerais. (Osorio *et al.,* 2014;). Além disso, nanopartículas possuem alta capacidade de infiltração em túbulos dentinários peri- e intertubular devido a pequena dimensão (menor que 100 nm), podendo melhorar as propriedades mecânicas da camada híbrida (Padovani *et al.,* 2015; Gutiérrez *et al*., 2019).

Sendo assim, compostos de zinco e suas derivações têm sido incorporadosem materiais adesivos com o intuito de estimular o processo de deposição de minerais(Wang *et al*., 2019; Nishida *et al*., 2021). O óxido de zinco,

por exemplo, libera íons Zn^{2+}, facilitando a deposição de cálcio e fosfato na superfície durante o processo de remineralização, onde a água intra- e interfibrilar é substituída por cristais de apatita, melhorando a integridade estrutural da dentina e da ligação dentina/adesivo (Osorio *et al*., 2014). Além disso, acredita-se que o óxido de zinco é capaz de inibir a ação proteolítica, uma vez que a presença desses cristais de apatita intrafibrilares bloqueiam a movimentação e acesso das MMPs e CTs aos sítios catalíticos no colágeno (Moreira *et al.,* 2021).

Outra vantagem desse composto, é o efeito bactericida (Gutiérrez et al., 2019; Nishida *et al*., 2021). O zinco se liga fortemente a lipídios e proteínas, alterandoo equilíbrio osmótico e aumentando a permeabilidade da membrana celular das bactérias orais gram-positivas e gram-negativas (Padovani *et al*., 2015; Garcia *et al*., 2021). Devido a essas vantagens, alguns autores têm relatado resultados promissoresem relação a resistência de união ao incorporar esse composto em adesivos (Toledano *et al*., 2013; Osorio *et al*. 2014; Gutiérrez *et al.,* 2019; Mahamuni-Badiger *etal.,* 2019; Garcia *et al*., 2020). Porém, não existe um consenso entre a porcentagem de peso por volume adequada para a utilização desse composto, além de não ter sidoutilizado como forma de pré-tratamento dentinário.

Outro composto derivado do zinco que vem sendo utilizado, é o fluoreto dezinco. Recentemente, a empresa GC (GC - Tokyo, Japan) lançou o produto Caredyne™ Shield, composto por uma solução aquosa de nanopartículas de vidro de fluoreto de zinco, que é utilizada como agente descontaminante em canais radicularesdevido a ação antibacteriana (Saad *et al*., 2019). Além do potencial antibacteriano, devido à capacidade remineralizante do flúor, a mesma empresa incorporou esse composto em um cimento de ionômero de vidro (Caredyne Restore, GC - Tokyo, Japan), dessa forma, o fluoreto pode se ligar aos íons de cálcio presente no substrato desmineralizado, e por ser uma ligação fraca, pode precipitar formando cristais de fluorapatita, assim, todo o mineral perdido é reposto e a probabilidade de cárie recorrente ou as margens da restauração é reduzido. (Nishida *et al*., 2019; Hasegawa*et al*., 2020).

Autores como Saad et al., (2019) Hasegawa et al., (2020) e Nishida

et al., (2021) em seus estudos, concluíram que o uso de Caredyne™ Shield e Caredyne Restore™ respectivamente, foram capazes de inibir a formação de biofilme e evitou adesmineralização dentinária. Contudo, baseado em nossos conhecimentos, o único estudo relacionado à adesão dentina-adesivo e fluoreto de zinco é de Wang et al., (2019), onde uma solução aquosa composta por nanopartículas de vidro e fluoreto de zinco foi aplicada previamente ao procedimento adesivo, e concluiu-se que a resistência de união foi aumentada.

Portanto, de acordo com o exposto, a incorporação de óxido de zinco e fluoreto de zinco como pré-tratamento dentinário, poderiam trazer benefícios na integridade e na estabilidade da camada híbrida. Dessa forma, o objetivo desse estudo foi avaliar o efeito de nanopartículas de zinco como pré-tratamento dentinário na adesão, na degradação de colágeno e no potencial antibacteriano sobre S. mutans. As hipóteses de pesquisa são: (1) Pré-tratamentos a base de nanopartículas de zinco associados a um adesivo convencional de três passos, são capazes de manter ou aumentar os valores de resistência de união após 12 meses de armazenamento; (2) Nanopartículas de zinco são capazes de aumentar a nanodureza e (3) módulo de elasticidade na camada híbrida.

2 ARTIGO

Effect of zinc nanoparticles on the mechanical properties and stability of resin-dentin bonded interface

ABSTRACT

Objective: To evaluate the effect of zinc nanoparticle solution as pretreatment on the microtensile bond strength (μTBS), nanohardness (NH) and young's modulus (YM) of hybrid layer. As well as the metalloproteinases inhibition and antibacterial potential against *S. Mutans*. **Materials and Methods:** Pretreatment solutions were applied after phosphoric acid: 5% Zinc Oxide (ZO5); 10% Zinc Oxide (ZO10); 1.5% Zinc Fluoride (ZF1.5); 3% Zinc Fluoride (ZF3); 0.2% chlorhexidine (CHX-positive control) and no pretreatment (NC-negative control). Teeth were restored and stored in body fluid solution for 24h and 12 months for μTBS (n=8), failure mode, NH and YM (n=3). *In situ* zymography (n=3) and interface morphology (n=3) were evaluated after 24h. The antibacterial activity was determined by minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC). The μTBS was evaluated by two-way mixed ANOVA and NH and YM data were analyzed by two-way repeated-measures analyses of variance (rANOVA) (α= 0.05). *In situ* zymography, interface morphology, MIC and MBC were descriptively analyzed. **Results:** The μTBS of ZF3, ZO10 and CHX was maintained over time, while for ZF1.5 and Z05 it was increased. Mixed failures were the most prevalent for all groups. ZF3 presented higher values of NA and YM with incorporation of nanoparticles being observed within the hybrid layer on the interface morphology image. CHX presented the lowest enzymatic activity followed by ZO5. MIC and MBC for ZO was 62,5 μg/mL and ZF 31,25 μg/mL. **Conclusion: P**retreatment with zinc nanoparticles, specially zinc fluoride, may be a promising method to increase the longevity of restorations.

Key words: Zinc; Fluoride; Adhesion; Bond Strength; Remineralization

Introduction

The use of resin composite restorations has increased because the material provides superior esthetics,(1) the possibility to perform a minimally invasive approach(2) and low cytotoxicity (1,2). Nonetheless, it is common for failures to occur and require replacement of the restorations, in less than 10 years. (1-4) It has been suggested that the longevity of resin composite restoration depends highly on the clinical performance of dental adhesives(4,5); and the dentin-composite bonded interface has been proposed as the weakest link in restorations (5) because regardless of the adhesive system, bonding relies mostly on the formation of a hybrid layer, produced by demineralized dentin collagen fibrils infiltrated with resin monomers. (1-5)

Resin monomers in adhesives can create microporosities below and inside the hybrid layer, as a result of a limited diffusion, an incomplete displacement of water and infiltration of the interfibrillar spaces, thus resulting in water-filled gaps on the unprotected collagen fibrils that cause hydrolytic degradation over time. (1,4) Furthermore, host-derived matrix metalloproteinase (MMPs) and cysteine cathepsins (CTs) that are activated by the acid etching process and/or by the acidic pH produced by cariogenic bacteria, also play a relevant role on degradation of the hybrid layer. (3,4,6,7,8,9) Thus, in order to increase the durability of adhesive/dentin interface without compromising the mechanical properties of the adhesive layer, bioactive nanoparticles have been highlighted as a promising approach due to their small sizes that can promote better infiltration and high surface areas to interact or release ions. (10)

Zinc is commonly used in dental materials due to its properties, (11,12) and it has been reported to inhibit the activity of matrix metalloproteinases, reduce collagen degradation, inhibit demineralization, and promote remineralization. (12-15) Recently, a study demonstrated that the quality and the longevity of the resin–dentin interface may be increased by using innovative dental adhesives containing zinc oxide within their composition. (16) Also, another study (12) reported that when zinc is incorporated in an adhesive, the compound was able to inhibit and stabilize collagen degradation at the hybrid layer and did not affect mechanical properties. Nonetheless, there is no consensus regarding the recommended concentration nor have zinc compounds been evaluated as dentinal pretreatments. (12,14,15,16) This approach may be useful

because zinc-related substances contribute to antibacterial activity on *S. mutans* biofilms. (6)

On the other hand, fluoride ions typically show tooth remineralizing and antibacterial effects. (15,16) Clinically, fluoride toothpaste, varnish and mouthwash demonstrated a caries-preventive effect and suppressed dentin demineralization by replacing the hydroxyl groups of hydroxy apatite in fluorapatite, which is less soluble and thus more resistant to low pH. (18,19,20) Therefore, considering the mechanism underlying the inhibitory activity of zinc and the capacity of fluoride to stimulate hard tissue mineralization (21,22,23), the association of both compounds may provide beneficial effects as a pretreatment to improve the longevity of the hybrid layer.

Thus, considering the importance of innovative bioactive molecules for restorative materials, analysis of the effects of zinc and fluoride ions becomes relevant. Because the study of the therapeutic effects on the mineral depleted sites within the bonded-dentin interface remains one of the main targets of dental biomaterial research, the purpose of this study was to evaluate the effect of Zn nanoparticles on the adhesive layer of resin composite restorations. The research hypotheses were: (1) Pretreatment with zinc nanoparticles using an etch-and-rinse adhesive system would improve the microtensile bond strength after 12 months of aging; (2) Zinc nanoparticles would be able to improve the nanohardness and (3) the Yong's modulus of the hybrid layer.

Materials and Methods

Teeth Selection and Solution Preparation

Eighty-four sound human third molars were collected according to the Ethics Committee in Research of the Piracicaba Dental School – University of Campinas (CAAE: 61739622.7.0000.5418) and stored in thymol solution (Labsynth; Diadema,SP, Brazil) at 4°C for no longer than three months. (3)

To obtain the pretreatment solutions, zinc oxide and zinc fluoride powder were diluted in 0.5 ml of distilled water and 0.5 ml of absolute ethanol at 24°C (room temperature). Then, solutions were transferred to a magnetic stirrer (Marconi-MA 085, Piracicaba/São Paulo, Brazil) for 60 s. The evaluated concentrations of zinc oxide were 5 wt% (ZO5) and 10 wt% (ZO10). (6,12,14,15,17) Zinc fluoride was evaluated in 1.5

wt% (ZF1.5) and 3 wt% (ZF3). (16) Chlorhexidine (CHX) at 0.2 wt% was diluted in distilled water and used as a positive control. (3,8) The negative control (NC) was considered the group without pretreatment. Summarized information about the commercial name, manufacturer lot number and composition of the materials used are present in Table 1.

Table 1. Specification of the products.

Commercial name, (#) lot number and Manufacturer	**Composition**
Zinc Oxide nanopowder (#MKCQ2445) Sigma Aldrich (Saint Louis, MO, USA)	99% zinc oxide; water; alcohol Particles size: <100 nm
Zinc Fluoride nanopowder (#MKCPS5120) Sigma Aldrich (Saint Louis, MO, USA)	99% zinc fluoride; water; alcohol Particles size: <100 nm
Chlorhexidine (#MFCD009673) Sigma Aldrich (Saint Louis, MO, USA)	99% chlorhexidine; water
Adper Scotchbond Multipurpose Primer (#NE23064) Adhesive (#NE94470) (3M ESPE, St Paul, MN, USA)	Primer: water, 2-hydroxyethyl methacrylate, copolymer of acrylic and itaconic acids Adhesive: bisphenol A diglycidyl ether dimethacrylate, 2-hydroxyethyl methacrylate

Bonding Protocol

The roots and occlusal enamel of the teeth were removed with a diamond saw, under water-cooling, using a low-speed wafering blade (Buehler Ltd., Lake Bluff, IL, USA) to expose middle-depth dentin. (3) Dentin surfaces were abraded with 600-grit silicon carbide paper for 5 s, under water-cooling, to standardize the smear layer and flatten the substrate. (3) Next, a 37% phosphoric acid gel (Ultradent Products Inc – South Jordan, UT, USA) was applied for 15 s and water rinsed for 30 s. In sequence, dentin surfaces were treated with the corresponding pretreatments of each group, passively with a microbrush, for 1 minute. Excess solution was removed with absorbent paper and the primer and adhesive (Adper Scotchbond Multipurpose – 3M ESPE, St

Paul, MN, USA) were applied and light cured according to the manufacture instructions. Finally, the teeth were restored by incremental placing a 4 mm height block of resin composite (Z250 – 3M ESPE). All light curing procedures were performed using a multiple peak LED light curing unit (1000 mW/ cm^2 radiant emittance; Valo - Ultradent Products Inc) for 20 s. (3)

Microtensile Bond Strength (μTBS)

For each group, the teeth (n=8) were restored using the previously described procedure and stored at relative humidity at 37°C for 24 h. Then, the teeth were sectioned into beam-shaped specimens with a cross-section area around 1.0 $mm^{2.}$ (24) For each tooth, the number of obtained sticks ranged between 8 and 16. Half of the specimens was tested after 24 h of water storage, while the other half was tested after 12 months of storage in body fluid solution (SBF).

The SBF was prepared by dissolving reagent grade NaCl (16.070 g), $NaHCO_3$ (0.710 g), KCl (0.450 g), $K_2HPO_4 \cdot 3H_2O$ (0.462 g), $MgCl_2 \cdot 6H_2O$ (0.622 g), $CaCl_2$ (0.584 g) and Na_2SO_4 (0.144 g) into double-distilled water. The solution was buffered to a pH of 7.4 using tris-hydroxymethylaminomethane [$(CH_2OH)3CNH_2$] (12.236 g) and 1 M hydrochloric acid (HCl) (0–10 mL). The storage solution was replaced every week during the 12 months of aging. (12)

For μTBS evaluation, the specimens were attached and tested to a universal testing machine (EZ Test, Shimadzu, Kyoto, Japan) at a crosshead speed of 1 mm/min until failure of the bonded specimen. The specific area of each stick was measured with a digital caliper (Mitutoyo Co., Kanagawa, Japan). The microtensile bond strength result in MPa of each specimen was calculated by dividing the obtained result by the area of the stick (mm^2). (3)

Failure mode

The fractured surfaces of each specimen were analyzed by scanning electron microscopy (SEM - JSM 5600LV, Jeol, Tokyo, Japan) at magnifications of 100x and 400x. Failure modes were descriptively evaluated in seven modes: Type-I: cohesive failure of composite; Type-II: adhesive failure between composite and adhesive resin; Type-III: adhesive failure between dentin and adhesive resin; Type IV: mixed failure showing the different components of the bonding interface; Type-V: cohesive failure within the adhesive layer; Type-VI: cohesive failure within the hybrid

layer; and Type- VII: and cohesive failure of dentin. The type of fracture was classified as mixed unless a single specific failure mode covered 70% or more of the evaluated surface area.(3)

Nanohardness (NH) and Young's Modulus (YM)

Eighteen sound human third molar teeth were prepared and restored (n=3) as described. Restored teeth were longitudinally sectioned mesio-distally to obtain six 1 mm thick slices. The slices were subdivided and kept in body fluid solution to be evaluated after 24 h and 12 months aging. The solution was replaced every week. Afterwards, samples were individually embedded in epoxy resin (Buehler – Chicago, IL, USA) and polished with grit SIC papers (#600; #1200; #2000) followed by diamond pastes (9; 6; 3; 1; 0.5 μm), being ultrasonically cleaned after each polishing step. (25)

To measure the NH and YM, a computer controlled triboindenter (Custom Triboindenter, Hysitron: Minneapolis, MN, USA) with a cell Berkovich point was used. In all samples, ten equally spaced (200 μm) nanoindentations were performed at the hybrid layer. The nanoindentations were performed with a load of 1000 μN and a standard trapezoidal load function of 5–2–5 s. (25) The nanohardness and young's modulus of each area were computed according to Oliver and Pharr's method. (26)

***In situ* Zymography**

Eighteen molars (n=3) were prepared and restored as described. After 24 h of storage, the teeth were longitudinally sectioned in a cutting machine to obtain six slices of 1 mm thickness from the middle portion. The six slices of each tooth were further subdivided in two groups where one set of three dentin slices was used for *in situ* zymography and the other set for interface morphology characterization.

For the in situ zymography, a modified protocol described by Giacomini et al. (7) was used. The slices were immersed in 1% phosphoric acid solution (Sigma-Aldrich) for 30 s to remove the smear layer. Next, self-quenched fluorescein-conjugated gelatin (EnzChek gelatinase / collagenase assay kit, Molecular Probes, Eugene, OR, USA) was prepared as MMP substrate. Gelatin was diluted 1:8 in the dilution buffer (NaCl 150 mM, CaCl2 5 mM, Tris-HCl 50 mM, pH 8.0) and an anti-fading agent (Mounting Medium with Dapi H-1200, Vectashield, Vector Laboratories LTD, Cambridgeshire, UK) was added. In sequence, specimens were covered by gelatin mixture solution on a culture plate of 150 μL and incubated in a dark humid chamber

at 37°C for 24 h. (7) Afterwards, the specimens were analyzed by confocal laser scanning microscopy (Microscope Leica TCS SPE, Leica Microsystems, Mannheim, BW, GER) with excitation and emission peaks at 460 and 520 nm respectively. Each resin-dentin interface was entirely characterized, and images representing the MMP-activity were obtained along the bonded interfaces. (3,7,25)

Interface Morphology Characterization

The remaining dentin slab (n=3) obtained from the preparation of *in situ* zymography were used for interface morphology characterization. The samples were embedded in epoxy resin, polished with grit SIC papers (#600; #1200; #2000 and #4000) and immersed in 37% phosphoric acid solution for 10 s and 5% NaClO for 5 min to expose the resin tags. (3,13) Then, the samples were dehydrated by an increasing concentration of ethanol (25%, 50%, 70%, 90% and absolute ethanol) for 10 min each and dried overnight. Afterwards, specimens were sputter-coated with gold (SDC 050 Sputtercoater, Baltec, Balzers, Liechtenstein) and analyzed by SEM at x1000 magnification. (3,13)

Antibacterial activity: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The experiments were performed using the *S. mutans* (reference strain UA159). The bacterial culture containing 20% (*v*/V) glycerol was stored at −80◦C in brain heart infusion (BHI) medium (Difco Laboratories, Detroit, MI, USA) until needed. The antimicrobial activity of ZO and ZF was evaluated by determining their MIC and MBC. Cultures of *S. mutans* were grown in BHI-broth culture medium supplemented with 0.1% sucrose for 18 h at 37◦C and 5% CO_2 were used. The cultures were measured for optical density at (OD600) ≥ 0.900 and adjusted between 0.08 to 0.1 with fresh BHI broth. To prepare the solutions, absolute ethanol was used as a diluent and it was diluted from 8% to 0.015%. Afterwards, ZO and ZF were diluted from 4000 µg/mL to 31,25 µg/mL on the diluent. Absolute ethanol in a concentration of 8% was used as a negative control group.

One hundred microliters of the adjusted inoculum were mixed with 100 µL of each compound with BHI broth dilutions. (27) Plates were incubated at 37◦C and 5% CO_2 for 24 h. After incubation, the row of wells with the lowest dilution showing no turbidity was defined as the MIC, to confirm the results a resazurin method was applied.

(27) A dilution of resazurin sodium salt (0.1% - Sigma-Aldrich, S. Louis, MO, USA) was added to all wells (20 µL per well), and further incubated for 4 h at 37◦C and 5% CO_2 in dark conditions for the observation of color change.

The MBC was determined by plating in triplicate 10 µL the content of the wells with concentrations 5 times higher than the MIC, before to the staining with resazurin. The MBC value was determined by plating on agar the lowest concentration of the extracts required to kill 99.9% of bacteria from the initial inoculum. Three independent experiments were performed for MIC and MBC. (27)

Statistical Analyses

Data from the µTBS and NH passed the Shapiro-Wilk normality test, as well as Levene's test for equality of variances. The µTBS results were analyzed by two-way mixed ANOVA (between-subjects factors: 1- pretreatments and 2- evaluation time), followed by Bonferroni's test. For NH and YM, two-way repeated-measures analyses of variance (rANOVA) followed by Bonferroni multiple comparison were applied. A level of significance of 5% was adopted. The *in situ* zymography, interface morphology characterization, MIC and MBC were descriptively analyzed.

Results

Microtensile Bond Strength

Two-way ANOVA showed that "storage time", "groups" and the factor interaction significantly influenced the µTBS ($p=0.001$, $p<0.001$ and $p=0.015$, respectively). Table 2 presents the µTBS results of the tested groups, according to the pretreatment applied at both times.

At 24h of evaluation, NC presented higher µTBS than all other groups ($p<0.005$), and there was no difference between the experimental groups and the positive control group (CHX). On the other hand, after 12 months storage, ZF3 and ZO10 presented the lowest µTBS values while the other groups did not differ between them. For most groups, the microtensile bond strength did not differ over time, with the exception of ZO5 and ZF1.5, which showed an increase after 12 months ($p<0.001$ and $p=0.002$, respectively).

Table 2. Means (standard deviation) of microtensile bond strength (MPa), comparing the groups at different evaluation time.

Treatment	24 hours	12 months
Negative Control (NC)	53.25 (13.0) Aa	47.72 (12.3) Aa
0.2% Chlorhexidine (CHX)	38.64 (9.2) Ab	48.20 (9.1) Aa
5% Zinc Oxide (ZO5)	31.18 (4.8) Bb	46.17 (4.3) Aa
10% Zinc Oxide (Z010)	36.53 (7.1) Ab	42.94 (13.3) Aab
1.5% Zinc Fluoride (ZF1.5)	35.60 (9.4) Bb	51.61 (6.7) Aa
3% Zinc Fluoride (ZF3)	33.83 (6.0) Ab	34.01 (8.8) Ab

Means followed by different letters indicate significant difference ($p<0.05$). Lowercase letters compare pretreatments within the same evaluation time (column). Uppercase letters compare the same pretreatment in different times (row).

Failure Mode

The frequencies of occurrence of the different failure modes are present in Figure 1. For the 24 hours evaluation, Type-I was the most predominant failure mode for NC, CHX, ZF1.5 and ZF3 group. The most predominant failure mode for ZO5 and ZO10 was type IV. For the 12 months evaluation, the groups CHX; ZO5; ZF1.5 and ZF3 maintained the same results. Type-VI was the most predominant failure for NC.

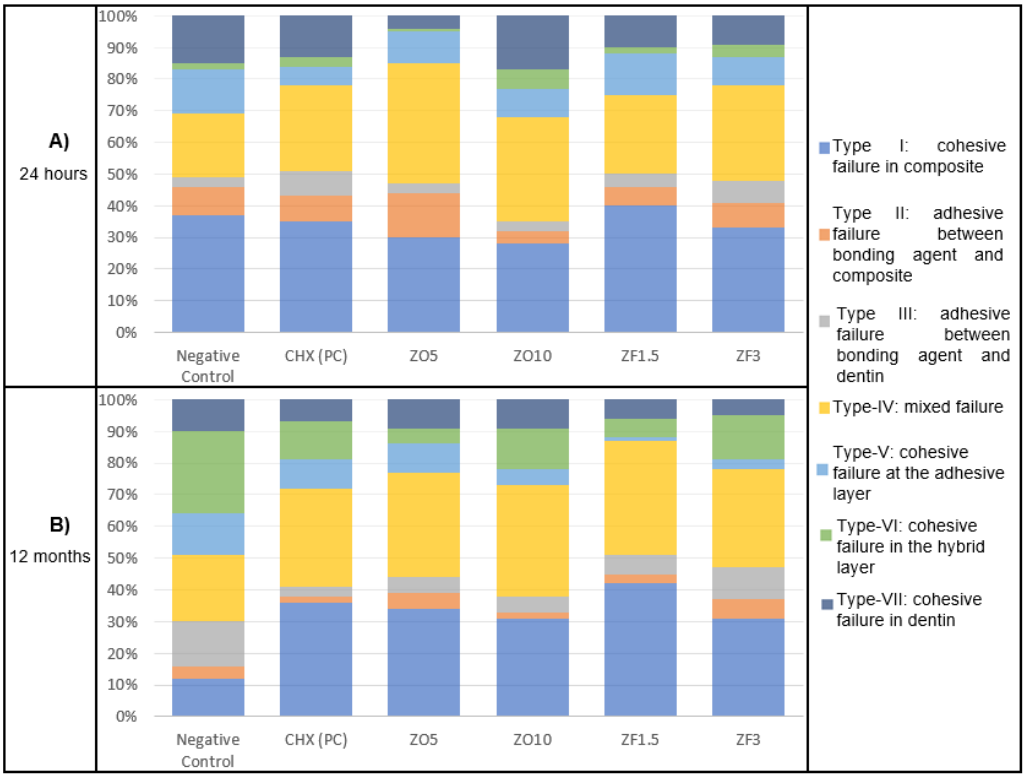

Figure 1: Distribution of failure modes at 24 h (A) and 12 months (B) according to the tested groups.

Nanohardness and Young's modulus

The mean (± SD) NH and YM are presented in Table 3. Regarding NH, The "storage time" and "groups" factors significantly influenced the nanohardness ($p<0.001$). At 24 hours of evaluation, ZF3 presented the highest NH of all groups ($p<0.001$). On the other hand, CHX and NC presented the lowest values ($p<0.002$). ZO5, ZO10, and ZF1.5 did not differ from each other ($p>0.05$). At 12 months, ZF3 presented the highest value compared to other groups ($p<0.001$), followed by ZF1.5, ZO5 and ZO10. CHX and NC presented the lowest values compared to other groups ($p<0.001$). Also, when comparing the same pretreatment in different evaluation time, the NH decreased for all groups after 12 months ($p<0.001$).

Regarding YM, the "storage time" and "groups" factors significantly influenced the results ($p<0.050$). However, the factor interaction was not significant ($p=0.944$). At 24 hours of evaluation, ZF3 was statistically different from NC and ZO10 ($p=0.006$ and $p=0.019$, respectively). Besides, NC presented lower values compared to ZF1.5 and ZF3 ($p<0.017$). CHX, ZO5, ZO10, AND ZF1.5 did not differ from each other ($p>0.05$). At 12 months, ZF3 was statistically different from NC, Z05, and ZO10 ($p<0.033$). NC presented the lowest value compared to the other groups of evaluation ($p<0.007$). CHX, ZO5, ZF1.5 did not differ from each other ($p>0.05$). when comparing the same pretreatment in different evaluation time, all groups decreased the YM after 12 months ($p<0.001$).

Table 3. Nanohardness (GPa) and Young's modulus (GPa) of hybrid layer

	Nanohardness		Young's modulus	
Treatment	24 hours	12 months	24 hours	12 months
Negative Control (NC)	0.33 (0.05) Ac	0.18 (0.05) Bd	4.65 (1.5) Ac	2.32 (0.8) Bd
0.2% Chlorhexidine (CHX)	0.34 (0.06) Ac	0.20 (0.05) Bd	5.29 (1.5) Aabc	3.20 (1.2) Babc
5% Zinc Oxide (ZO5)	0.38 (0.04) Ab	0.32 (0.03) Bbc	5.29 (1.4) Aabc	3.27 (1.0) Bbc
10% Zinc Oxide (ZO10)	0.39 (0.03) Ab	0.30 (0.06) Bc	4.82 (1.4) Abc	3.09 (0.9) Bc
1.5% Zinc Fluoride (ZF1.5)	0.39 (0.03) Ab	0.33 (0.03) Bb	5.39 (1.0) Aab	3.67 (1.1) Bab

3%Zinc Fluoride (ZF3)	0.43 (0.03) Aa	0.40 (0.03) Ba	5.77 (1.3) Aa	4.02 (1.4) Ba

Means followed by different letters indicate significant difference ($p<0.05$). Lowercase letters compare treatments within the same evaluation time (column). Uppercase letters compare the same treatment in different times (row).

In situ Zymography

Figure 2 shows representative images of the dentin enzymatic activity performed by CLSM. The *in situ* zymography revealed an intense MMP activity at the hybrid layer and dentinal tubules for NC. All the pretreatments groups presented a decrease in fluorescence intensity values. The CHX group presented higher fluoresce in the hybrid layer while ZO5, ZO10, ZF1.5 and ZF3 presented higher fluoresce in dentinal tubules. ZO5 presented the lowest enzymatic activity.

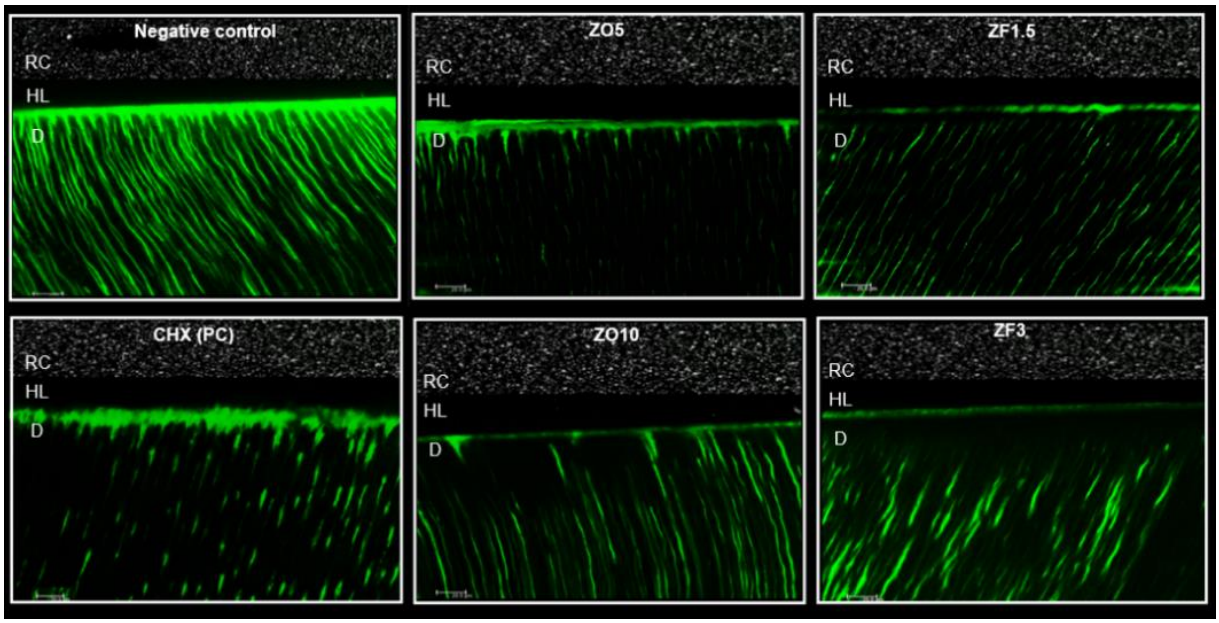

Figure 2: Gelatinolytic activity at the resin-dentin interface indicated by the green fluorescence. RC: resin composite; HL: hybrid layer; D: dentin.

Interface Morphology Characterization

Figure 3 shows representative images of the interface morphology analysis performed by SEM. For all the groups, thick hybridization zones (over 6 µm) were observed. The NC, CHX, ZO10 and ZF1.5 presented multiple deep resin tags while ZO5 and ZF3 presented shorter resin tags. All the pretreatments reveled nanoparticles on the adhesive layer, and ZF3 presented nanoparticles also on the hybrid layer.

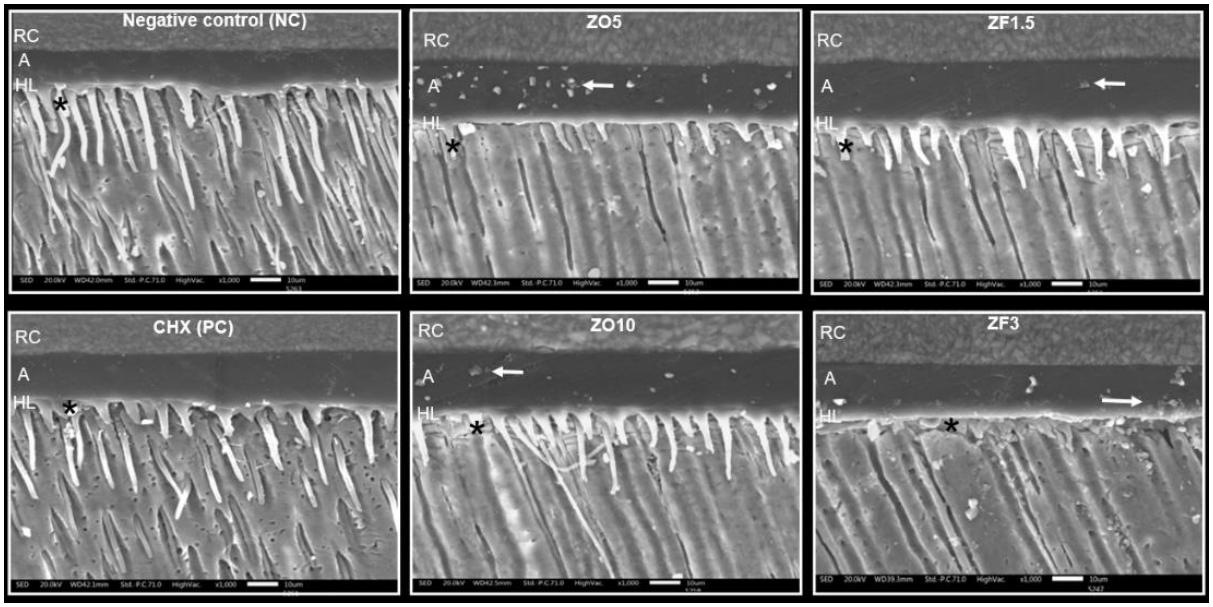

Figure 3: SEM images (x1000) of the bonding interface. RC: resin composite; A: adhesive layer; HL: hybrid Layer; (*): resin tags; Arrow: particles.

Antibacterial activity: MIC and MBC

The MIC for ZO was identified at the concentration of 62,5 µg/mL and the MIC of ZF at 31,25 µg/mL. The same values were obtained for the MBC in both compounds. Table 4. represents how many times the percentage of the pretreatments represents the MIC and MBC of each compound. The percentage of 5% and 10% of ZO represented 8x and 16x the concentration of MIC and MBC respectively. On the other hand, the percentage of 1.5% and 3% of ZF represents 4.8x and 9.6x the concentration of MIC and MBC.

Table 4. Effectiveness of the concentration used in the study in relation to MIC and MBC

Pretreatment	MIC and MBC	Correlation between pretreatments and MIC / MBC
5% ZO	62,5 µg/mL	8x
10% ZO	62,5 µg/mL	16x
1.5% ZF	31,25 µg/mL.	4.8x
3% ZF	31,25 µg/mL.	9.6x

Discussion

Dental adhesives have constantly improved in relation to bond strength, however, the issue degradation of the hybrid layer over time remains unresolved.(8) Yet the results of this study show that bond strength to dentin was influenced by

pretreatments, because when the pretreatments were applied, the μTBS increased or remained stable overtime, thus, based on that, the first hypothesis can be accepted. Even though the negative control group presented the higher μTBS on the 24 h evaluation and maintained the values over time, it must be highlighted that the hybrid layer created by total etch and rinse adhesives undergo degradation within 6 months to 3-5 years. (28,29). Therefore, in a longer time evaluation, it can be assumed that despites this adhesive system presents a more hydrophobic, dense adhesive that seal primed dentin, as a consequence, the collagen fibrils are exposed and latent MMP are activated by the application of the phosphoric acid, increasing dentin permeability due to water diffusion from the dentin tubules, creating a permeable hybrid and adhesive layer, leading to degradation. (4,28,29)

Conversely, all the zinc-based pretreatments reached the minimum μTBS values considered for an accepted adhesion (18–20 MPa) (29) and did not differ from the positive control group (CHX) on the 24h evaluation. Also, CHX, ZO10 and ZF3 maintained the values while ZO5 and ZF1.5 had an increase on the results after aging. Thus, it can be hypothesized that lower concentrations of zinc can improve the results over the time, however, higher concentration of the compound can saturate and only maintain the values obtained. Furthermore, it should be considered that there are controversial studies regarding the beneficial effect of CHX related to bond strength. (8, 9) On the other hand, several studies confirm that zinc compounds do not show negative effects on the bond strenght. (11,13,14) Thus, it can be hypothesized that the incorporation of zinc nanoparticles may increase the collagen resistance and indirectly decrease the immediate nanoleakage, promoting adhesion and an integrity hybrid layer. (12,14,15,30)

Likewise, the improvement of the collagen resistance can reflect on the failure mode results. Cohesive in resin composite and mixed failures were the most common failure types for both evaluation times for all the groups. Cohesive failures in resin composite suggests that the cohesive strenght of the composite was lower than the resin-dentin bond strength (8). However, in the presence of a strong bond, the fracture path starts in the composite and propagates through the bonding interface, reaching the dentin substrate. (3,8,28) Thus, mixed failures can be considered as preferable, as they indicate that the structures involved in dentin bonding acted as a single unit rather than separate layers. (3,28) Yet, it can be noticed that NC group had

an increase in failure type VI after 12 months of storage. The cohesive failure in the hybrid layer imply that a defective hybridization zone was formed, leading to water diffusion and degradation over time. (28)

The longevity of hybrid layer can also be correlated with enzymatic activity. Human dentin contains many MMPs, like -2, -8, and -9, which are capable of degrading type I collagen fibrils. (6,7,8,25). The results of *in situ* zymography (Fig. 2) showed green fluorescence within the hybrid layer and dentinal tubules for the NC and this fact may be attributed to water degradation of exposed collagen fibrils at the deepest area of demineralized dentine that was not resin encapsulated by the adhesive. (25) Yet, when the pretreatments were applied, lower or weaker fluorescence signals were detected, and zinc-based nanoparticles presented slightly less enzymatic activity than CHX. Despite MMPs being part of the zinc-activated and calcium-dependent endopeptidases, (2,3,7,8), it is believed that when zinc nanoparticles are applied, Zn^{2+} ions in the active site will bind to a water molecule, by which the enzyme is activated, and will cleave peptide bonds within the proteins inhibiting the enzymatic activity. (22)

Also, when incorporating bioactive nanoparticles on the dentin-resin interface, the mechanical properties of the hybrid layer and the longevity of the restoration can be improved. (12,15) According to this study, even though the NH of all the groups decreased after 12 months of storage, ZF3 present the higher value in both evaluation times, followed by ZF1.5, ZO5, and ZO10. Therefore, the second hypothesis that zinc nanoparticles would be able to improve the nanohardness on the hybrid layer can be accepted. This might be a result of the higher solubility of zinc when the pH is lowered by the phosphoric acid, which favors the release of ions at the resin-dentin-interface (17) and interact with the acid primer of the adhesive (15). However, after pH is gradually raised by the adhesive, precipitations of crystal salts and undissolved powder nanoparticles can be incorporated into the adhesive layer (14,16) and improve the integrity of the resin-dentin-interface.

Although all the groups in this study formed thick hybridization zones, ZF3 presented shorter resin tags than the other groups. However, the main factor that contribute to adhesion is the infiltration of the adhesive in the intertubular collagen fibrils, thus, the presence of deep resin tags does not contribute much to bond strength. (31) Also, it can be noticed ZF3 present a higher quantity of inorganic particles on the bottom of the hybrid layer, explaining the higher values of NH, while ZF1.5, ZO5, and

ZO10 revealed nanoparticles on the adhesive layer. Yet, as an advantage of ZF3, sealing the dentinal tubules with nanoparticles may reduce the intertubular fluid and dentin permeability. (15, 16) Despites that, it can be hypothesized that the deposition of crystals salts and undissolved particles could “fossilize” MMPs, blocking their movement and access to catalytic sites on collagen, and consequently, improving the NH and maintaining the μTBS after aging. (17,30)

Furthermore, another fact that may have influenced the NH results is that precipitated mineral might work as a constant site for further nucleation. As reported by Osório et al., (17), under body fluid solution, Zn-OH groups can be formed and will induce phosphate and calcium ions deposition, and once apatite nuclei are formed on the surface, they can easily grow spontaneously by consuming calcium and phosphate ions from SBF. Previous studies have shown that mineralization process can take 21 days to 3 months, because the mechanics of action occurs by replacing free and loosely bound water by apatite crystallites into the collagen matrix. (10, 17, 23). Therefore, considering that nanohardness can be an indirect method to evaluate the remineralization (12) and the experimental groups presented higher values on the 24 h and after 12 months of storage when comparing to NC and CHX, hypothetically, zinc nanoparticles might have an effect in hard tissue mineralization, protecting the seed crystallite sparse collagen fibrils of the scaffold from degradation and permitting they could be remineralized. (14, 15, 21, 22)

However, when comparing zinc oxide and zinc fluoride, it can be noticed that ZF3 and ZF1.5 presented less reduction of values of YM of the hybrid layer over time. Therefore, the third hypothesis that zinc nanoparticles would be able to improve the YM of the hybrid layer can be partially accepted. it can be assumed that zinc fluoride might present a better collagen stabilization by increasing the collagen resistance. (17) Besides, considering the ability of zinc to induce remineralization (14,15,21,22) and fluoride to replace the hydroxyl groups of the new formed hydroxyapatite (19), when zinc fluoride is used, fluorapatite can be formed. (19) Thus, the fact that fluorapatite is less soluble than hydroxyapatite, the results of YM can be associated with the properties of each ion that are deposited over time.(16,19)

Moreover, it must be highlighted that restorations with resin composites can increase the outgrowth of the biofilm (22, 32,34,36) where *S. mutans* is the major cariogenic oral microorganism. (33,34). Thus, considering that marginal gap formation

can occur due to the shrinkage stress of the adhesive and the composite, and during secondary caries development, bacterial acid production causes demineralization that cannot be reversed merely via saliva (37), compounds that can release ions and has antibacterial potential are highly wanted to strengthen bonded interfaces in the dental biofilm environment. (15,16, 18, 22,) As an alternative, in a previous study, chlorhexidine was added as releasing antibacterial agent, however, it resulted in a short-term effect. (37,38)

On the other hand, according to this study, zinc oxide and zinc fluoride where able to inhibit and kill *S. mutans* in a very low concentration, and previous studies reported that this antibacterial potential is correlated to the balance during the demineralization and remineralization process, where Zn it is slowly liberated when the pH is lowered. (32,34,35) Thus, the lower MIC for ZF can be associated with fluoride that can also be released and potentialize bacterial inhibition. (11,15,18,19,) Also, as another advantage, fluoride can be rechargeable over time.(13,18) Likewise, the pretreatments solution presented MBC. Hence, when considering a deep cavity where the caries lesion is not completely removed, zinc-based pretreatments can be used as a disinfectant solution (15) as well as it can reduce the biofilm formation in marginal gaps and induce deposition of calcio and phosphate.(17,36,37) Therefore, future studies should investigate the incorporation of the compounds on adhesive or resin composite to develop bioactive materials.

Yet, when developing new materials, the cytotoxicity must be evaluated. Despites the concentration used for the pretreatments in this study can correspond up to 10x the MIC and MBC for ZO and ZF, a recent study evaluated the cytotoxicity of 5% zinc oxide incorporated into two different adhesives and no statical differences were observed compared to the commercial material. (15) Regarding ZF, a study evaluated 2% ZF eluting solution and no cytotoxicity were observed. (18) Thus, despite the limitation of this compound that cannot interact with adhesives that contain MDP monomer (39) and further investigations about the potential antimicrobial activity in biofilms are needed (36), the present study was capable to demonstrate that the addition of a step that only takes one minute during the restoration procedure, it can maintain the μTBS results over time, reduce MMP activity, improve the mechanical properties of the hybrid layer and it has a potential to inhibit and kill the major cariogenic oral microorganism.

Conclusion

Pretreatments based on zinc nanoparticles proved to be able to maintain a stable μTBS after 12 months evaluation. Also, the use of the evaluated solutions resulted in a slightly lower enzymatic activity than the observed with CHX. The application of the ZF3 solution produced higher NH and YM values, as well as the incorporation of nanoparticles within the hybrid layer on the interface morphology image. The compounds were able to inhibit and kill *S. mutans* in a very low concentration.

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3 CONCLUSÃO

A adição de mais um passo no procedimento restaurador através de materiais bioativos, pode trazer inúmeros benefícios promovendo longevidade ao tratamento. Os pré-tratamentos com nanopartículas de zinco utilizados neste estudo, especialmente o fluoreto de zinco, apresentaram uma abordagem promissora, mantendo os valores de resistência de união e melhorando as propriedades mecânicas e a integridade da camada híbrida, além de possuir potencial antibacteriano sobre o microrganismo mais incidente presente no biofilme da cavidadeoral.

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ANEXOS

Anexo 1 – Comitê de Ética

UNICAMP - FACULDADE DE ODONTOLOGIA DE PIRACICABA DA UNIVERSIDADE DE CAMPINAS - FOP/UNICAMP

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Situação do Parecer:
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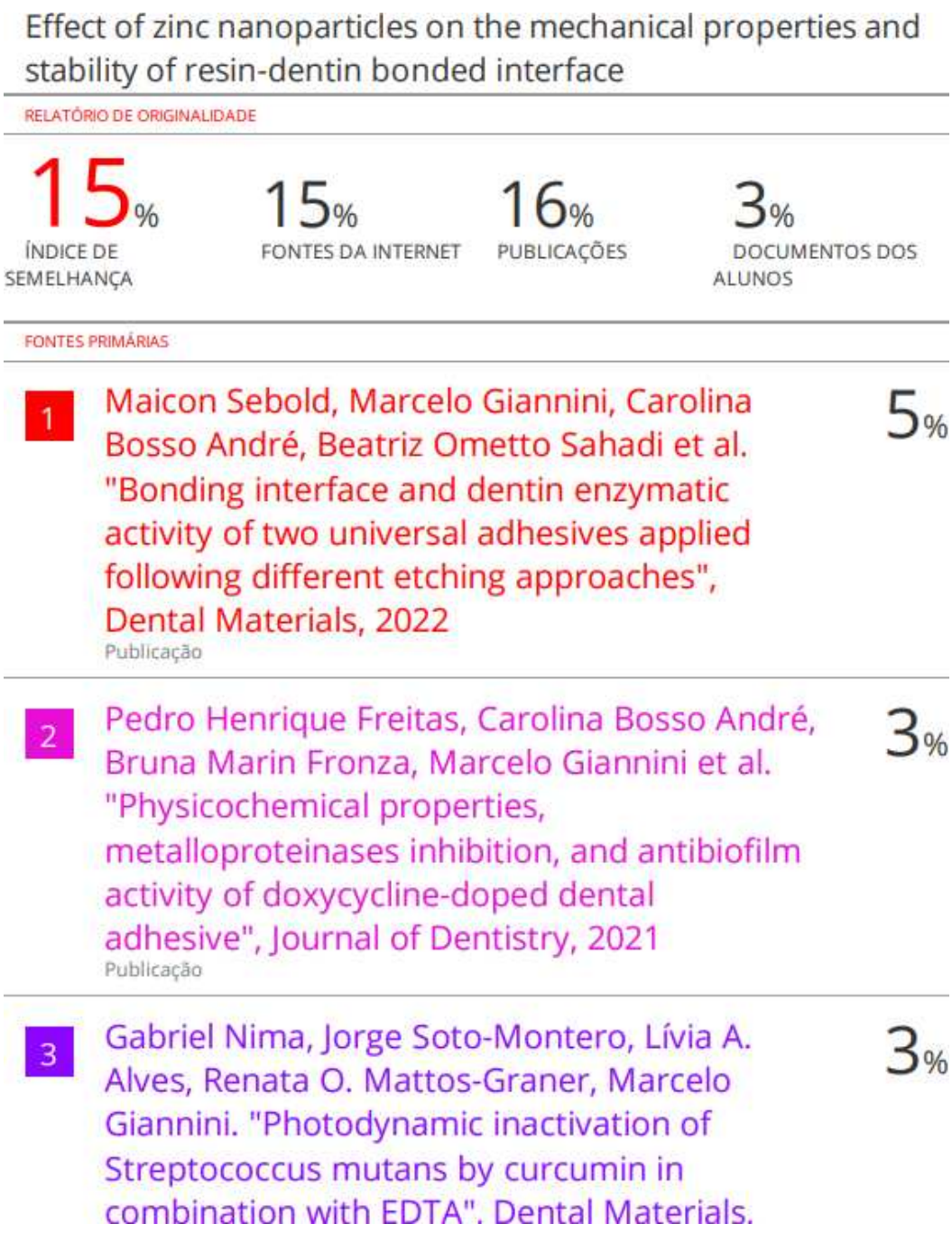
Necessita Apreciação da CONEP:
Não

PIRACICABA, 30 de Setembro de 2022

Assinado por:
jacks jorge junior
(Coordenador(a))

Endereço: Av.Limeira 901 Caixa Postal 52
Bairro: Areião **CEP:** 13,414-903
UF: SP **Município:** PIRACICABA
Telefone: (19)2106-5349 **Fax:** (19)2106-5349 **E-mail:** cep@fop.unicamp.br

Anexo 2 – Relatório de Similaridade



Effect of zinc nanoparticles on the mechanical properties and stability of resin-dentin bonded interface

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ÍNDICE DE SEMELHANÇA	FONTES DA INTERNET	PUBLICAÇÕES	DOCUMENTOS DOS ALUNOS

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1	Maicon Sebold, Marcelo Giannini, Carolina Bosso André, Beatriz Ometto Sahadi et al. "Bonding interface and dentin enzymatic activity of two universal adhesives applied following different etching approaches", Dental Materials, 2022 Publicação	5%
2	Pedro Henrique Freitas, Carolina Bosso André, Bruna Marin Fronza, Marcelo Giannini et al. "Physicochemical properties, metalloproteinases inhibition, and antibiofilm activity of doxycycline-doped dental adhesive", Journal of Dentistry, 2021 Publicação	3%
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Anexo 3 – Comprovante de submissão

Dear Marina Santi,

Thank you for your submission.

Submission ID	**233467753**
Manuscript Title	**Effect of zinc nanoparticles on the mechanical properties and stability of resin-dentin bonded interface**
Journal	**Journal of Adhesion Science and Technology**

If you made the submission, you can check its progress and make any requested revisions on the Author Portal

Thank you for submitting your work to our journal.
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