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CAPACIDADE ANTIMICROBIANA E DE BIOMODIFICAÇÃO DO RESVERATROL EM DENTINA AFETADA POR CÁRIE

CAROLINE NÁGILA DO NASCIMENTO TERTO

FORTALEZA

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

Área de Concentração: Clínica Odontológica.

Orientador: Profa. Dra. Vanara Florêncio Passos

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RESUMO

Para preservar a estrutura dental, a remoção seletiva do tecido cariado tem sido indicada. Entretanto, por ser um substrato que apresenta uma maior variabilidade ao longo da interface, são relatados prejuizos na união. Estratégias são desenvolvidas com o intuito de minimizar as limitações desse substrato. O Resveratrol (RVT) é um composto fenólico natural com ação antioxidante que apresenta biocompatibilidade em dentina. O objetivo desse estudo foi avaliar *in vitro*, o potencial antimicrobiano e a influência na adesão à dentina afetada por cárie do pré-tratamento dentinário com uma solução de resveratrol/etanol nas concentrações de 0,001%, 0,002% e 0,003% p/v comparados aos grupos controle (água destilada ou etanol 100%) e solução de clorexidina 2%. Foram utilizados 60 terceiros molares hígidos submetidos a indução de dentina afetada pela ciclagem de pH. Para a microtração (n=6), os dentes foram tratados com as soluções de tratamento durante 1 minuto, restaurados e analisados em uma maquina de ensaios universais imediatamente e após 6 meses de armazenamento em água destiliada. Micropermeabilidade (n=2) foi utilizada para a análise da morfologia da camada híbrida por meio da microscopia confocal a laser. Para o teste microbiológico (n=3) foram confeccionados espécimes de dentina de 4x4x2 mm^3 e divididos em seis grupos. Os espécimes foram submetidos a um desafio cariogênico, durante 5 dias, *in vitro*, imersos em TSB contendo extrato de levedura com sacarose a 10% e inoculados com *S. mutans* ATCC 25175. Após o período experimental, o biofilme formado foi coletado e a relação de unidades formadoras de colônia foi estabelecida. Para o teste de microtração realizou-se o teste de ANOVA two-way, seguido de teste de Tukey. Para a microbiologia, os dados foram tranformados em logarítmo e analisados por ANOVA, seguido de teste de Tukey. O nível de significância foi estabelecido em 5%. O grupo tratado com etanol obteve maior resistência de união ($p=0,028$) quando comparado ao grupo apenas adesivo, mas não apresentou diferença significativa entre os grupos RVT e CHX ($p>0,05$). Todos os grupos apresentaram resistência de união inferior após 6 meses em comparação com o teste imediato ($p<0,001$).Observou-se diferença estatística em relação ao potencial antimicrobiano entre a clorexidina e os demais grupos ($p<0,001$). O pré-tratamento com resveratrol não prejudicou a resistência de união à dentina desmineralizada artificialmente e não demonstrou potencial antimicrobiano significativo contra o *S. mutans*.

Palavras-chave: Adesivos dentinários; Antibacterianos; Polifenóis; Colágeno

ABSTRACT

To preserve tooth structure, selective removal of carious tissue has been indicated. However, as it is a substrate that presents greater variability along the interface, damages in the union are reported. Strategies are developed in order to minimize the limitations of this substrate. Resveratrol (RVT) is a natural phenolic compound with antioxidant action that presents biocompatibility in dentin. The objective of this study was to evaluate in vitro the antimicrobial potential and the influence on adhesion to caries-affected dentin of dentin pretreatment with a resveratrol/ethanol solution at concentrations of 0.001%, 0.002% and 0.003% w/v compared to control groups (distilled water or 100% ethanol) and 2% chlorhexidine solution. Sixty sound third molars submitted to induction of dentin affected by pH cycling were used. For microtensile (n=6), teeth were treated with the treatment solutions for 1 minute, restored and analyzed in a universal testing machine immediately and after 6 months of storage in distilled water. Micropermeability (n=2) was used to analyze the morphology of the hybrid layer using confocal laser microscopy. For the microbiological test (n=3) 4x4x2 mm^3 dentin specimens were made and divided into six groups. The specimens were submitted to an in vitro cariogenic challenge for 5 days, immersed in TSB containing yeast extract with 10% sucrose and inoculated with S. mutans ATCC 25175. After the experimental period, the biofilm formed was collected and the ratio of colony forming units has been established. For the microtensile test, the two-way ANOVA test was performed, followed by the Tukey test. For microbiology, data were log-transformed and analyzed by ANOVA, followed by Tukey's test. The significance level was set at 5%. The ethanol-treated group achieved higher bond strength ($p=0.028$) when compared to the adhesive-only group, but it did not show a significant difference among the RVT groups and CHX ($p>0.05$). All groups showed lower bond strengths after 6 months compared with immediate test ($p<0.001$).There was a statistical difference in relation to the antimicrobial potential between chlorhexidine and the other groups ($p<0.001$). Pretreatment with resveratrol did not impair bond strength to dentin artificially demineralized and did not demonstrate significant antimicrobial potential against *S. mutans*.

Keywords: Dentin-bonding agents; Anti-bacterial agents; Polyphenols; Collagen

SUMÁRIO

Introdução Geral

1 INTRODUÇÃO GERAL

Os sistemas adesivos são responsáveis pela união entre o substrato dentário e os compósitos restauradores estéticos; tais sistemas agem promovendo uma união em dentina, por meio da infiltração de seus monômeros por entre as fibrilas colágenas expostas durante o condicionamento ácido. Essa interface obtida pela infiltração dos monômeros adesivos por entre as fibrilas colágenas origina uma zona denominada camada híbrida (NAKABAYASH et al., 1982).

Sabe-se que, em longo prazo, a camada híbrida está sujeita a degradação. Essa degradação é decorrente de vários fatores, entre eles: as propriedades hidrófilas dos monômeros resinosos e a permanência de solventes residuais devido a evaporação incompleta durante a técnica adesiva. Além disso, a interface adesiva estar sujeita a degradação devido à ativação das MMP's (Metaloproteinases de Matriz), enzimas proteolíticas capazes de hidrolisar a matriz orgânica da dentina desmineralizada (KHAMVERDI; REZAEI-SOUFI; ROSTAMZADEH, 2015).

Durante a progressão do processo carioso, há a formação de dois tipos distintos de dentina, classificadas de acordo com a sua consistência clínica em: mole ou macia e a dura. A dentina mole ou macia se configura como uma zona ne crótica superficial, de substrato vastamente desmineralizado, apresentando fibrilas de colágeno degeneradas, que perderam suas ligações cruzadas e uma grande biomassa bacteriana. Já a dentina dura, é considerada uma variação da dentina reacional, apresentando pequenas alterações na reticulação das suas fibrilas colágenas e precipitados mineralizados dentro dos túbulos dentinários (RODRIGUES et al., 2021; FATTAH et al., 2021 INNES et al., 2016). Entretanto, estudos mostram que a dentina dura pode ser remineralizada, tornando-se um possível substrato para a adesão em procedimentos restauradores. No entanto, por ser um substrato que apresenta uma maior variabilidade e uma composição irregular ao longo da interface, em comparação com a dentina hígida, pode desencadear prejuízos relacionados a longevidade e efetividade da interface adesiva restauradora. Assim, alguns estudos mostram uma menor resistência de união, uma menor infiltração adesiva e uma maior propensão a degradação hidrolítica em dentinas afetadas por cárie, quando comparadas a dentinas sadias (COSTA et al., 2017; ISOLAN et al., 2018; JOWKAR et al., 2021).

Durante muito tempo, o tratamento para a cárie preconizou a total remoção do tecido cariado, com o intuito de evitar futuras atividades cariogênicas, fornecendo uma base bem mineralizada de dentina para a confecção da restauração (ARAUJO et al., 2017).

Porém, em lesões de cárie profundas, a remoção total do tecido cariado apresenta risco significativo de exposição pulpar. Assim, a remoção seletiva de tecido cariado é recomendada. Durante essa remoção seletiva, a dentina afetada por cárie permanece na parede pulpar e é vedada sob a restauração (SCHWENDICKE et al., 2019). Nesse contexto, surgiu a discussão sobre a real necessidade da remoção completa dos tecidos cariados, durante o preparo cavitário, para o tratamento restaurador de dentes com presença de cáries profundas (BARROS et al., 2020; COSTA et al., 2017).

Estudos mostram que, há uma paralização da atividade cariogênica de lesões parcialmente escavadas, se forem perfeitamente isoladas da cavidade oral. Essa paralização ocorre, pois, a microbiota residual fica exposta a uma maior homogeneidade de nutrientes, principalmente proteínas séricas ao invés de carboidratos, gerando um estresse de inanição que afeta significativamente os microrganismos, diminuindo a carga microbiana (FIRMINO et al., 2018; MALTZ et al., 2002; PADDICK et al., 2005).

Em regiões desmineralizadas por cárie, uma camada híbrida adequada dificilmente é formada (HAJ-ALI et al., 2006). Esse substrato apresenta-se mais poroso, devido a hipomineralização. Assim, ocorre uma desmineralização mais profunda, após o condicionamento ácido, dando origem a uma zona mais profunda de colágeno parcialmente exposto, tornando mais difícil a infiltração dos monômeros resinosos. Assim, há a presença de um maior número fibrilas colágenas não infiltradas e expostas a degradação (HAJ-ALI et al., 2006; LENZI et al., 2014)

Dessa forma, procedimentos e materiais que melhorem a qualidade da união, reduzindo a infiltração da água e a degradação do colágeno da camada híbrida, são benéficos para tornar a interface resina-dentina mais estável, mesmo em substratos alterados (COSTA et al., 2017). Além disso, materiais que exibam atividade antimicrobiana, também podem ser favoráveis, tendo em vista, um substrato com bactérias remanescentes. Adicionalmente, o aumento da longevidade de restaurações de resina composta proporcionará uma redução significativa nos custos dos serviços odontológicos públicos e privados, uma vez que, as trocas de restaurações serão minimizadas.

O resveratrol (RVT) é um antioxidante polifenílico, de ocorrência natural, presente em diversas espécies de vegetais, como uva, amora, amendoim e eucalipto. Seu nome químico é trans-33,5,4´-trihidroxi estilbeno. O resveratrol ocorre em duas isoformas *cis* e *trans*-resveratrol, sendo sua isoforma *trans* a mais biologicamente ativa. Nas últimas décadas, tem ganho a atenção dos cientistas devido a seus efeitos anticancerígenos, anti-inflamatórios, redutores de açúcar no sangue e outros efeitos cardiovasculares benéficos. Na terapia anticâncer

é considerado, por alguns, como um agente promissor, pois é capaz de afetar as 3 fases distintas da carcinogênese: iniciação, promoção e progressão (KRAFT et al., 2009). Além disso, também possui a capacidade de inibir o crescimento de alguns microrganismos, como bactérias gram positivas, gram negativas e fungos (CHAN et al., 2002; PAULO et al., 2010).

Tal substância vem sendo analisada em distintas estratégias na Odontologia restauradora, seja em pré-tratamento dentinário ou em incorporações nos sistemas adesivos. Estudos mostram que a adição de reservatrol pode melhorar a biocompatibilidade sem causar influência negativa na resistência de união de adesivos autocondicionantes (ATALAYIN et al., 2019; ATALAYIN et al., 2015; GUO et al., 2021). Quando usado como solução de pré tratamento, o reservatrol pode proporcionar estabilidade de união desejável entre os adesivos e a dentina, reduzindo a frequência de substituição de restaurações de resina composta (PORTO et al., 2018). Peng e colaboradores, em 2020, mostraram em seu estudo que a solução de resveratrol/etanol pode ser usada como um primer de dentina versatil, pois pode biomodificar as fibras de coláeno, aumentar a força da interface de união, reduzir a ocorrência de nanoinfiltração, inibir a atividade de MMP’s e atuar como agente bacteriostático.

Assim, o resveratrol vem apresentando resultados promissores, os quais, podem também apresentar perspectivas positivas, quando aplicadas sobre a dentina afetada, visando minimizar as adversidades adesivas relacionadas a esse substrato.

Dessa forma, o objetivo dessa pesquisa foi avaliar, *in vitro*, o potencial antimicrobiano e a influência na adesão à dentina afetada por cárie do pré-tratamento dentinário com uma solução de resveratrol nas concentrações de 0,001%, 0,002% e 0,003% p/v comparados ao grupo controle (água destilada ou etanol) e solução de clorexidina 2%.

Proposição

2. PROPOSIÇÃO

O presente trabalho teve como objetivos:

2.1 Objetivo geral

Avaliar, *in vitro*, o potencial antimicrobiano e a influência na adesão à dentina afetada por cárie do pré-tratamento dentinário com uma solução de resveratrol/etanol nas concentrações de 0,001%, 0,002% e 0,003% p/v comparados ao grupo controle (água destilada ou etanol 100%) e solução de clorexidina 2%.

2.2 Objetivos específicos

- Analisar *in vitro* o potencial antimicrobiano do pré-tratamento de resveratrol/etanol nas concentrações de 0,001%, 0,002% e 0,003% p/v.
- Analisar, *in vitro*, a influência do pré-tratamento de resveratrol/etanol nas concentrações de 0,001%, 0,002% e 0,003% p/v sobre a resistência de união imediata e, após 6 meses de armazenamento, de um sistema adesivo universal à dentina afetada por cárie.
- Analisar a morfologia da camada híbrida promovida por um sistema adesivo universal, após aplicação do pré-tratamento de resveratrol/etanol, nas concentrações 0,001%, 0,002% e 0,003% p/v, sobre à dentina afetada por cárie, por meio de microscopia confocal a laser.

Capítulo

3. CAPÍTULO

Esta dissertação está baseada no Artigo 46 do Regimento Interno do Programa de Pós-Graduação em Odontologia da Universidade Federal do Ceará que regulamenta o formato alternativo para dissertações de Mestrado e teses de Doutorado, e permite a inserção de artigos científicos de autoria ou coautoria do candidato. Por se tratar de estudos envolvendo seres humanos, ou parte deles, o projeto de pesquisa foi submetido à apreciação do Comitê de Ética em Pesquisa da Universidade Federal do Ceará, tendo sido aprovado (Parecer n° 4.651.854 15/04/2021). Assim sendo, esta dissertação é composta de um artigo científico que será submetido à publicação conforme descrito abaixo:

CAPÍTULO 1: Antimicrobial and biomodification capacity of resveratrol in caries-affected dentin. Este artigo será submetido à publicação no periódico Archives Oral Biology (ANEXO B)

3.1 Capítulo 1

TÍTULO: Antimicrobial and biomodification capacity of resveratrol in caries-affected dentin.

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ANTIMICROBIAL AND BIOMODIFICATION CAPACITY OF RESVERATROL IN CARIES-AFFECTED DENTIN

ABSTRACT

Objective: To evaluate the antimicrobial potential and influence on adhesion to caries-affected dentin of dentin pretreatment with a resveratrol/ethanol solution at 0.001%, 0.002% and 0.003% w/v.

Design: Sixty sound third molars submitted to induction of dentin affected by pH cycling were used. For microtensile (n=6) teeth were divided into 6 groups: RVT1 (resveratrol/ethanol 0.001%), RVT2 (resveratrol/ethanol 0.002%), RVT3 (resveratrol/ethanol 0.003%), ETN (ethanol), CLX (2% chlorhexidine) and BOND (adhesive) and analyzed in a universal testing machine. Micropermeability (n=2) was analyzed using confocal laser microscopy. For the microbial test (n=3) the 4x4x2 mm^3 specimens were divided into the same 6 groups, but with the replacement of the (BOND) group by the H2O group (distilled water). The specimens were submitted to an in vitro cariogenic challenge for 5 days, immersed in TSB containing yeast extract with 10% sucrose and inoculated with *S. mutans* ATCC 25175. The biofilm formed was collected and the ratio of colony forming units was established. For the microtensile test, the two-way ANOVA test was performed, followed by the Tukey test. For microbiology, data were log-transformed and analyzed by ANOVA, followed by Tukey's test. The significance level was set at 5%.

Results: The ethanol-treated group achieved higher bond strength ($p=0.028$) when compared to the adhesive-only group, but it did not show a significant difference among the RVT groups and CHX ($p>0.05$). All groups showed lower bond strengths after 6 months compared with immediate test ($p<0.001$).There was a statistical difference in relation to the antimicrobial potential between chlorhexidine and the other groups ($p<0.001$).

Conclusion: Pretreatment with resveratrol did not impair bond strength to dentin artificially demineralized and did not demonstrate significant antimicrobial potential against *S. mutans*.

Keywords: Dentin-bonding agents; Anti-bacterial agents; Polyphenols; Collagen

1. INTRODUCTION

One of the main causes of restoration unsuccessful is the failure of the adhesive/dentin interface (Guo et al.,2021). This interface is called the hybrid layer and is formed by the infiltration of adhesive monomers between the collagen fibrils exposed during acid etching (De Munck et al., 2005, Van Meerbeeck et al., 2011).

It is known that, in the long term, the hybrid layer is subject to degradation. This degradation is due to several factors, including: the hydrophilic properties of the resin monomers and the permanence of residual solvents due to incomplete evaporation during the adhesive technique. In addition to the adhesive interface, it is subject to degradation due to the activation of matrix metalloproteinases (MMP's) and cysteine cathepsins (CTP's), proteolytic enzymes capable of hydrolyzing the organic matrix of demineralized dentin (Silva et al., 2020).

In the context of minimally invasive dentistry, conservative treatments have been advocated, such as selective removal of carious tissue (Rodrigues et al., 2021). However, adhesion in affected dentin is even more critical, due to the morphological changes found in the substrate, which makes a perfect hybrid layer difficult to form. The affected dentin is hypomineralized and after acid conditioning a deeper layer of partially exposed collagen is created, which makes infiltration of adhesive monomers difficult and culminates in a zone rich in non-infiltrated collagen fibrils and exposed to degradation (Rodrigues et al., 2021, Neves et al., 2011, Schwendicke et al., 2016).

Dentin biomodification has been proposed as a strategy to improve the mechanical and biochemical properties of dentin through the formation of collagen cross-links. During the adhesive procedure, the prior application of bioactive agents on demineralized dentin increased the bond strength of the adhesive to the dentin. This is possible due to the reduction of the hydrophilicity of the hybrid layer, increase in the number of collagen cross-links and nonspecific inhibition of the proteolytic activity of MMP's and CTP's, thus, the collagen network becomes more stable and resistant to biodegradation (Seseogullari-dirihan et al., 2016).

Resveratrol (RVT) is a naturally occurring polyphenolic antioxidant present in several plant species, such as grapes, blackberries, peanuts and eucalyptus (Vestergaard et al., 2016). This substance has been analyzed in different strategies in restorative dentistry, whether in dentin pretreatment (Porto et al., 2018, Peng et al., 2020) or incorporation into adhesive systems (Guo et al., 2021, Porto et al., 2021). Studies show that when used as a pretreatment solution, resveratrol can provide desirable bond stability between adhesives and dentin (Guo et al., 2021, Porto et al., 2018, Peng et al., 2020). Thus, the resveratrol has shown promising results related

to the improvement of dentin adhesion, however, there are no studies evaluating its performance in caries-affected dentin, in addition, the results about its antimicrobial capacity against are still conflicting *S. mutans*. Thus, the objective of this study was to evaluate, in vitro, the antimicrobial potential and the influence on adhesion to caries-affected dentin of dentin pretreatment with a resveratrol/ethanol solution at concentrations of 0.001%, 0.002% and 0.003% w/v compared to control groups (distilled water or 100% ethanol) and 2% chlorhexidine solution. The null hypotheses were (1) there is no difference between the tested groups in terms of adhesive efficacy and (2) there is no difference between the tested groups in terms of antimicrobial potential.

2. MATERIALS AND METHODS

2.1. Formulation of resveratrol/ethanol solutions

Absolute ethyl alcohol was chosen as the solvent in our study because of the poor water solubility of resveratrol (0.03 g/L at 25 °C). Resveratrol (RVT) (SM Empreendimentos Farmeceuticos Ltda, SP, Brazil) will be dissolved in ethyl alcohol (100%) (Peng et al., 2020) to produce three experimental primer solutions at 0.001%, 0.002% and 0.003% w/v.

2.2 Induction of caries-affected artificial dentin

Sixty sound human third molars extracted in private clinics were used, with the consent of the patients, through the Tooth Donation Term, under the approval of the institutional Ethics Committee (Committee opinion nº 4.651.854) and stored in 0.1% thymol solution. at 4 °C, for a maximum period of three months (Humel et al., 2007). The teeth will be randomly distributed among the groups (Table 1), through an Excel software (Microsoft Excel®, Random command). Each tooth was sectioned to expose a flat dentin surface using a water-cooled, low-speed diamond disc (Isomet 4000; Buehler, Lake Bluff, USA), thereby removing the occlusal enamel and roots.

The exposed occlusal dentin surface was polished with #600 silicon carbide sandpaper (Carbimet, Buehler, Lake Bluff, IL, USA) to create a smooth, patterned surface. All other surfaces were coated with acid resistant nail varnish (Colorama Maybelline Ltda, São Paulo, Brazil). A layer of partially demineralized dentin was created on the uncoated surface through pH cycling.

The demineralizing solution was composed of 1.5 mM of calcium chloride ($CaCl_2$); 0.9 mM monopotassium phosphate (KH_2PO_4); 50 mM acetic acid and 5 mM sodium sorrel (NaN_3) adjusted to pH 4.8. The remineralizing solution was composed of 1.5 mM of calcium chloride ($CaCl_2$); 0.9 mM monosodium phosphate (NaH_2PO_4); 0.13 M sodium chloride (KCl) and 5 mM

sodium sorrel (NaN_3) buffered to pH 7.0 with Buffer HEPES. Each sample was immersed in 10 mL of demineralizing solution for 8 h, followed by immersion in 10 mL of remineralizing solution for 16 h, with fresh solutions used for each cycle. This procedure was carried out for 14 days at room temperature. After preparing the dentin surface, the teeth were divided into six groups (n=6) according to the pretreatment to be used (Qi et al., 2012).

2.3 Adhesive procedure

All teeth underwent adhesive procedures. For this, 37% phosphoric acid (Condac 37% Phosphoric Acid Conditioner - FGM, Brazil) was applied for 15 s on dentin, then rinsed with water for 30 s, followed by drying of the dentin. The experimental primers were applied to the specimens using a microbrush (Microbrush International, WI, USA) for 60s, with the exception of the BOND group, then the excess was removed with absorbent paper and the Single Bond Universal adhesive system was used. Thus, a layer of adhesive was applied under the affected dentin with an applicator brush (Microbrush International, WI, USA) rubbing it for 20 seconds, then a light jet of air was applied for 5 seconds to evaporate the solvent and light curing for 20 seconds (Bluephase Ivoclar Vivadent, Brazil) with an irradiance of 1100 W/cm^2.

2.4. Restorative Procedure

For the microtensile test, the teeth were restored with Z-100 composite resin (3M ESPE, St. Paul, MN, USA), with 5 increments of 1.0 mm thick (Silva et al., 2015) each and light cured individually for 40 seconds (Bluephase Ivoclar Vivadent, Brazil) with an irradiance of 1100mW/cm2.

2.5. Microtensile bond strength testing

After storage in distilled water at 37 °C for 24 h, the teeth were sectioned longitudinally in the mesio-distal and buccal-lingual directions, through the bonded interface, using a diamond disk under abundant water cooling (IsoMet 4000, Buëhler, Lake Bluff, IL, USA), to obtain composite resin sticks and tooth structure, with a cross-sectional area of approximately 1.0mm².

The sticks were divided into two groups: tested immediately (24 hours of storage in distilled water at 37°C) and tested after 6 months (stored in distilled water at 37°C, with change of solution weekly). The area of the sticks was measured prior to the performance of the mechanical test, with a digital caliper (Starrett Industria e Comercio Ltda, São Paulo, SP, Brazil).

The sticks were individually fixed in a microtensile device (ODMT03d, Odeme Biothecnology, Joaçaba, SC, Brazil), with cyanoacrylate resin (Superbonder Gel, 3M, São Paulo, Brazil) and subjected to tensile force in a universal testing machine (Instron Corp, Canton, MA, USA) at a speed of 0.5 mm/min until failure occurs. The results of the mechanical

microtensile tests were obtained, in MPa, by dividing the load at the moment of failure (N) by the cross-sectional area of the toothpick (mm^2).

Bond strengths of sticks from the same tooth were averaged and the mean was used as statistical units. Data were analyzed using the statistical *program Statistical Packcage for the Social Sciences* (SPSS) version 22.0 for *Windows*, by two-way ANOVA (pre-treatment and storage time) and Tukey's test.($p<0.05$).

2.6. Micropermeability evaluation

Two teeth per group (n = 2) were bonded as previously described with the adhesive doped with 0.1wt% rhodamine-B (Sigma Aldrich, St. Louis, MO, USA) and assessed by confocal laser scanning microscopy (CLSM). In brief, the micropermeability of resin–dentin interfaces was evaluated using a 0.3 wt% aqueous fluorescein (Sigma Aldrich, St. Louis, MO, USA) solution. This dye was perfused for 3h under 15 cm H2O simulated pulpal pressure to test the sealing ability of the adhesive after different pre-treatments. The specimens were subsequently cut into 1 mm thick slabs, slightly polished with 2000 grit polishing paper and sonicated for 2 min.

The specimens were evaluated using CLSM (LSM 710, Carl Zeiss, Munchen, Germany) equipped with a 63×/1.4 NA oil immersion lens using 488-nm and 568-nm laser illumination. CLSM fluorescence images were obtained with a 1 μm z-step to section optically the specimens up to 20 μm below the surface. The z-stack scans were compiled into single projections. Each resin–dentin interface was entirely characterized and images were randomly captured along bonded interfaces representing the micropermeability characteristic from each group (Feitosa et al., 2014)

2.7 Antimicrobial evaluation

2.7.1 Specimen preparation

Dentin specimens were prepared in dimensions of 4x4x2 mm^3 using a low-speed, water-cooled diamond disk (Isomet 4000; Buehler, Lake Bluff, USA). Subsequently, they were polished with #320, #600 and #1200 silicon carbide sandpaper (Carbimet, Buehler, Lake Bluff, IL, USA). The samples were cleaned in an ultrasonic bath for 15 minutes, then sterilized in an autoclave for 15 minutes at 121°C. The samples were randomly distributed into six groups (n=9) and the experiment was performed in triplicate (Porto et al., 2021).

2.7.2 Application of pretreatments

In a 24-well plate, the specimens were immersed in 2ml of the pretreatment solutions for 60s. Two negative control groups with 100% ethanol and distilled water and a positive control group with 2% chlorhexidine were used.

2.7.3 Microbiological model of *Streptococcus mutans* biofilm formation *in vitro.*

Streptococcus mutans ATCC 25175 was cultured overnight at 37° C in a sterile brain–heart infusion broth (BHI CM0225; Oxoid LTD, SP, Brazil) in a partial atmosphere (5% CO2). After 18 h, the Gram test was conducted to verify the existence of *S. mutans* exclusively. The obtained bacterial suspension was adjusted to a specific optical density of 108 through the McFarland Scale. For the experiment, a 24-well plate (Cell Culture Plate-24 wells, Prolab, São Paulo, SP, Brazil) was used, in which each well contained 2.0 mL of tryptone-soybean broth and 1.0% of previously filtered sucrose and was inoculated with 0.1 mL (2 × 108 colony-forming units [CFU] mL−1) of the *S. mutans* ATCC 25175 culture. Finally, the specimens were inserted into each well. Bacterial inoculation was performed only on the 1st day, and the culture medium was replaced daily during 5 consecutive days. The 24 well plates were incubated at 37°C in 5% CO2 throughout the entire experimental period. At each transfer, the culture samples were cultured on BHI agar plates and incubated at 37°C in a 5% CO2 atmosphere to verify purity (Silva et al., 2020).

2.7.4 Collection of formed biofilm

A 0.9% saline solution (NaCl) was prepared at a ratio of 0.9 g/100 mL and preautoclaved (121°C, 15 min). On the 3rd experimental day, the biofilm formed on the specimens was removed and inserted into 5.0 mL Eppendorf® tubes (Eppendorf® AG, Alto da Lapa, SP, Brazil) containing 1.0 mL of the previously prepared saline solution. The tubes containing the collected biofilm and the saline solution were vortexed (Vortex TS-2000A VDRL Shaker-Biomixer, Curitiba, Brazil) to disperse bacterial cells (Silva et al., 2020).

2.7.5 Microbiological analysis

The suspension obtained in the Eppendorf® tubes was diluted in decimal series (1:10–1:100,000) with 0.9% saline solution (NaCl). The samples were plated in triplicate on BHI agar and incubated for 48 h at 37°C in a 5% CO_2 atmosphere. The representative colonies with typical *S. mutans* morphology were counted after 48 h, and the results were expressed in CFU.[4]

The microbiological experiment was performed in triplicate, and the data were transformed into a logarithm. Unidirectional analysis of variance (ANOVA) was also conducted, followed by the Tukey test.

3. RESULTS

Results for the immediate and 6-month dentin microtensile bond strength are shown in Table 2. Premature failure was not accounted when calculating the bond strength values. The statistical analysis showed that the factors group and aging time were statistically significant

($p<0.05$). However, the interaction between the factors was not significant ($p = 0,531$). The results showed that ethanol-treated group achieved higher bond strength ($p=0.028$) when compared to the adhesive-only group, but it did not show a significant difference among the RVT groups and CHX ($p>0.05$). All groups showed lower bond strengths after 6 months compared with immediate test ($p<0.001$).

The concentration of 0.001% resveratrol/ethanol solution showed lower CFU when compared to the other concentrations. However, this difference was not statistically significant compared to the ethanol, water and other concentrations of resveratrol/ethanol. There was a statistical difference in relation to the antimicrobial potential between chlorhexidine and the other groups ($p<0.001$) (Fig. 2).

Analyzing the images obtained through confocal microscopy, it was possible to perceive the formation of hybrid layers with different thicknesses, but a greater formation of the number of tags was observed in the groups: chlorhexidine, ethanol, RVT1 and RVT3, with greater depth of the tags in the groups ethanol and RVT1 (Fig. 3).

4. DISCUSSION

This study evaluated the effects of a resveratrol/ethanol solution applied as a primer on the durability of the caries-affected adhesive-dentin bond. The first and second hypotheses were rejected, since the ethanol solution significantly influenced the bond strength ($p=0.028$) and the chlorhexidine solution significantly reduced the biofilm formation of *S. mutans* ($p<0.001$).

Resveratrol is a naturally occurring polyphenolic antioxidant that has received great attention for its potential health benefits, including anticarcinogenesis, antiaging, and antimicrobial properties[9]. Recently, it has shown promising results as a dentin biomodifier (Porto et al., 2018, Peng et al., 2020, Porto et al., 2021, Atalayin et al., 2019), however, its effects on affected dentin have not been studied.

Although there are still few studies that show that RVT exerts a crosslinking activity on collagen fibers, it is reasonable to infer that it can generate some biological modification in collagen fibrils, maintaining or increasing the bond strength and integrity of the hybrid layer, as it is a polyphenol, it has hydroxyl groups in its composition, which may be able to induce exogenous cross-linking with dentinal collagen, mainly through hydrogen bonds between the carbonyl amide protein and the phenolic hydroxyl group (Porto et al., 2021). In addition, it is known that the presence of free radicals can also increase the processes of degradation and demineralization of the collagen matrix in dentin, so antioxidant polyphenols can be beneficial for maintaining the integrity of the hybrid layer (Neri et al., 2016). The same mechanisms in

the protection of dentin collagen are observed in other natural polyphenols such as epigallocatechin 3-gallate (Neri et al., 2016).

In this study, although resveratrol/ethanol solutions at concentrations of 0.001% and 0.002% showed higher mean values of bond strength compared to the BOND group (Table. 2), this difference was not significant. Our result differs from the studies by Porto et al., 2018 and Peng et al., 2020. In the first study, a significant increase in bond strength to dentin was observed after 120 days of storage in distilled water in groups previously treated with a solution containing 0,01% and 0,025% of resveratrol diluted in ethanol. The results of the second study showed that, with increasing pre-treatment concentration with resveratrol/ethanol, the bond strength values improved after thermocycling and aging with collagenase, in this case the concentrations of resveratrol used were 0,1%, 1% and 2%. Both studies evaluated the effect of pretreatment solutions on sound dentin, in our study we used the affected substrate, which may be a reason for the divergence in the results.

In caries-affected regions, bonding to dentin is more critical. Morphological studies show that a perfect hybrid layer is rarely formed in caries-affected dentin (Haj-ali et al., 2006). This substrate is more porous, due to hypomineralization. Thus, deeper demineralization occurs after acid conditioning, giving rise to a deeper zone of partially exposed collagen, making it more difficult for resin monomers to infiltrate. Thus, there is the presence of a greater number of non-infiltrated collagen fibrils exposed to degradation (Haj-ali et al., 2006, Lenzi et al., 2014). Another important factor is that, due to the low solubility of resveratrol, we used a lower concentration compared to the studies mentioned above, this difference in concentration may be another factor for the divergence of results to have occurred.

There is a concern about the use of chlorhexidine as bioactive agent when adhesive procedures are employed (Neri et al., 2016). Vivanco et al., 2020 observed that CHX positively affected the bond strength values, showing higher values in the pre-treated groups than in the control groups, regardless of whether the samples were submitted to thermo-mechanical cycling (TMC) or not. In the present study, there was no statistical difference with the use of pre-treatment with chlorhexidine and the other groups, this finding is corroborated by other studies (Mobarak et al., 2011, Castro et al., 2003).

In our study, ethanol presented the best bond strength values when compared to the adhesive group. Ethanol when applied to dentinal cavities has the ability to remove water from the dentin, keeping the collagen network stretched. Thus, it facilitates the interfibrillar spaces in the collagen matrix to be infiltrated by the adhesive monomers. Furthermore, it is able to create a more hydrophobic environment, reducing water absorption over time, which is a key

factor in adhesive bond degradation (Coelho et al., 2020). Corroborating our findings, other studies also demonstrated an increase in bond strength when ethanol was applied prior to infiltration of the adhesive (Hosaka et al., 2009, Venigalla et al., 2016, Mousavinasab et al., 2018, Nishitani et al., 2006).

When the bond strength was analyzed after 6 months in distilled water, a significant decrease in bond strength was observed in all groups. The bond strength obtained with the adhesive systems changes over time. The highest values of bond strength are related to the first 24 hours after application of the adhesive system. After this period, a reduction occurs as time passes, influenced by the time itself, storage medium, type of substrate and adhesive system used (Leloup et al., 2001, Sano et al., 2006).

Storage in distilled water is the most common artificial aging model to study the degradation of adhesive-dentin interfaces because it can simulate the humid intraoral environment in which restorations are always surrounded by saliva. A study by Deng et al., 2014 showed that direct water storage for 6 months significantly decreased μTBS values. During storage in water, the hydrolytic attack increases the disintegration of the collagen fibrils of the hybrid layer, and the associated resin in the demineralized dentin zone gradually collapses over time (Deng et al., 2014), this fact would justify the significant decline of the bond strength values after 6 months verified in our study.

Regarding the antimicrobial capacity against *S. mutans*, at the concentrations tested, resveratrol did not present antimicrobial activity, differing statistically from chlorhexidine. Thus, the second null hypothesis was rejected. . Our finding is supported by the study by O'Connor et al., 2011. However, other more recent studies demonstrate the opposite. Guo et al., 2021 incorporated resveratrol into a dental adhesive and found that *S. mutans* biofilm formation in the 1 and 10 mg/mL groups was significantly lower than in the control group, illustrating the effective inhibitory capacity for secondary caries prevention. In addition to it, Peng et al., 2020 used a series of resveratrol/ethanol solutions to pre-treat dentin slices and *S. mutans* culture, the results showed that the experimental group had less live bacteria than the control group, showing the excellent effects inhibitors on *S. mutans*. The mechanism by which resveratrol appears to have antimicrobial action is not yet fully understood, but one of the supposed pathways is that it could inhibit the production of glycolytic acid and the activity of glycosyltransferase, interfering with the adhesion and intercellular cohesion between cariogenic bacteria (Peng et al., 2020, Ban et al., 2010). However, conflicting results on the antimicrobial capacity of resveratrol against *S. mutans* and the variety of concentrations used between studies justify further investigation.

Only chlorhexidine was able to have an antimicrobial effect, corroborating the study by Borges et al., 2012. Chlorhexidine (CLX) is considered the "gold standard" of antimicrobial agents, as it has a broad spectrum of action and low cytotoxicity. It is known that chlorhexidine is capable of causing bacterial cellular osmotic imbalance, as it attracts and adsorbs its cationic molecules to the anionic cell surface of microorganisms, causing loss of intracellular components. It also has the ability to inhibit the action of glycosyltransferase and has an effect on sugar transport and acid production by oral bacteria (Borges et al., 2012, Hennessey et al., 1973, Delany et al., 1982, Gomes et al., 2006).

Confocal microscopy is widely used in the analysis of the area of union between the restorative material and the enamel or dentin (D'Alpino et al., 2006, D'Alpino et al., 2006, Aguiar et al., 2012). In this study, we performed the analysis in order to observe the morphology of the hybrid layer formed after the different pretreatments. During the analysis of the images, it was possible to perceive the formation of layers with different thicknesses, however it was noticed a greater formation of the number of tags in the groups: chlorhexidine, ethanol, RVT1 and RVT3, with greater depth of the tags in the ethanol groups and RVT1.

Resin tags are formed when adhesive resin flows into open dentinal tubules and ensure micromechanical interlocking by penetrating the tubules and forming a stable hybrid layer (Alhenaki et al., 2021). In our study, resin tags of varying depth were seen for all groups of adhesives (Fig.3), however it was not possible to attribute these findings to the bond strength values obtained, corroborating other studies that demonstrate that the depth of the tags does not essentially affect the strength joining the material (Anchieta et al., 2011, Carvalho et al., 2019).

The limitations of this study can not be ignored, resveratrol is an active molecule, which is easily oxidized by ambient oxygen and easily degraded in sunlight (Sessa et al., 2011). In addition, it has low solubility (Peng et al., 2020), which led us to use it in low concentrations. Our study is an *in vitro* research, therefore, it does not have all the variables present *in vivo*.

In fact, excellent results of bond strength and antimicrobial capacity associated with resveratrol are reported in the literature, however, some findings are controversial, and further studies are needed, with standardization of concentration, substrate and methodology, as well as the development of clinical studies to obtain consistent conclusions, in order to establish an ideal concentration capable of providing the promising activities of resveratrol on human dentin.

CONCLUSION

Within the limitations of this study, we concluded that pretreatment with resveratrol did

not impair the bond strength to caries-affected dentin and did not demonstrate significant antimicrobial potential against *S. mutans*.

REFERENCES

1. Guo R, Peng W, Yang H, Yao C, Yu J, Huang C. (2021). Evaluation of resveratrol-doped adhesive with advanced dentin bond durability. *J Dent*, 114.

2. De Munck, J.; Van Landuyt, K.; Peumans, M; Poitevin, A; Lambrechts, P; Braem, M. et al. (2005) A Critical Review of the durability of adhesion to tooth tissue: Methods and results. *Journal of Dental Research*, 84, 118- 132.

3. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt Kl. (2011) State of the art of self-etch adhesives. *Dent Mat*, 27,17-28.

4. Silva, P. G B., Terto, C. N. N., Andrade, R. C., et al. (2020) Antimicrobial evaluation, degree of solubility, and water sorption of universal dental adhesive incorporated with epigallocatechin-3-gallate: An *In-vitro* study. *European Journal of General Dentistry*, 9, 79-83.

5. Rodrigues MP, Cunha LSD, Vilela ABF, Schettini ACT, Bragança GF, França R, Soares CJ (2021). Selective carious tissue removal and glass ionomer liner reduction of pulp stress in bulk fill resin composite restorations. *Braz Oral Res*, 35:e119.

6. Neves AA, Coutinho E, Cardoso MV, Lambrechts P, Van Meerbeek B. Current concepts and techniques for caries excavation and adhesion to residual dentin (2011). *J Adhes Dent*, 13, 7-22.

7. Schwendicke F, Frencken JE, Bjørndal L, Maltz M, Manton DJ, Ricketts D, Van Landuyt K, Banerjee A, Campus G, Doméjean S, Fontana M, Leal S, Lo E, Machiulskiene V, Schulte A, Splieth C, Zandona AF, Innes NP. Managing Carious Lesions: Consensus Recommendations on Carious Tissue Removal (2016). *Adv Dent Res*, 28, 58-67.

8. Seseogullari-dirihan, R. et al. Use of crosslinkers to inactivate dentin MMPs. (2016) *Dent Mater*, 32, 423-432.

9. Vestergaard M, Ingmer H. Antibacterial and antifungal properties of resveratrol (2019). *Int J Antimicrob Agents*, 53, 716-723.

10. Porto ICCM, Nascimento TG, Oliveira JMS, Freitas PH, Haimeur A, França R. Use of polyphenols as a strategy to prevent bond degradation in the dentin-resin interface (2018). *Eur J Oral Sci*, 126, 146-158.

11. Peng W, Yi L, Wang Z, Yang H, Huang C. Effects of resveratrol/ethanol pretreatment on dentin bonding durability (2020). *Mater Sci Eng C Mater Biol*

12. Porto ICCM, Rocha ABB, Ferreira IIS, de Barros BM, Ávila EC, da Silva MC, de Oliveira MPS, Lôbo TLGF, Oliveira JMDS, do Nascimento TG, de Freitas JMD, de Freitas JD.

Polyphenols and Brazilian red propolis incorporated into a total-etching adhesive system help in maintaining bonding durability (2021). *Heliyon*, 19.

13. Humel MM, Oliveira MT, Cavalli V, Giannini M.; Effect of storage and disinfection methods of extracted bovine teeth on bond strength to dentin (2007). *Braz J Oral Sci*, 6, 1402-6.

14. Qi YP, LI N, Niu LN, Primus CM, Ling JQ, Pashley DH, Tay FR. Remineralization of artificial dentinal caries lesions by biomimetically modified mineral trioxide aggregate (2012). *Acta Biomater*, 8, 836-42.

15. Silva EM, Rodrigues CUS, Matos MPO, Carvalho TR, Santos GB, Amaral CM. Experimental etch-and-rinse adhesive systems containing MMP-inhibitors: Physicochemical characterization and resin-dentin bonding stability. (2015) *J Dent*, 43, 1491-7.

16. Feitosa VP, Sauro S, Ogliari FA, Ogliari AO, Yoshihara K, Zanchi CH, CorrerSobrinho L, Sinhoreti MA, Correr AB, Watson TF, Van Meerbeek B. Impact of hydrophilicity and length of spacer chains on the bonding of functional monomers (2014). *Dent Mater*, 30, 317-23.

17. Atalayin C, Tezel H, Ergucu Z, Unlu N, Armagan G, Dagci T, Kose T. The improvement of biocompatibility of adhesives : The effects of resveratrol on biocompatibility and dentin micro-tensile bond strengths of self-etch adhesives.(2019) *Clin Oral Investig*, 23, 3213-3218

18. Neri JR, Yamauti M, da Silveira FD, Mendonça JS, de Carvalho RM, Santiago SL. Influence of dentin biomodification with epigallocatechin-3-gallate on the bond strength of self-etch adhesive: Twelve-month results. (2016). *International Journal of Adhesion and Adhesive*, 71, 81-86.

19. Haj-ali R, Walker M, Williams K, Wang Y, Spencer P. Histomorphologiccharacterization of noncarious and caries-affected dentin/adhesive interfaces (2006). *J Prosthodont*, 15, 82–88.

20. Lenzi, T. L.; tedesco, T. K.; soares, F. Z. M.; loguercio, A. D.; rocha, R. O.;Chlorhexidine application for bond strength preservation in artificially-created caries-affected primary dentin (2014). *Int J Adhes Adhes*, 51–56.

21. Geng Vivanco R, Cardoso RS, Sousa ABS, et al. Effect of thermo-mechanical cycling and chlorhexidine on the bond strength of universal adhesive system to dentin (2020). *Heliyon*.

22. Mobarak EH. Effect of chlorhexidine pretreatment on bond strength durability of caries-affected dentin over 2-year aging in artificial saliva and under simulated intrapulpal pressure. (2011) *Oper Dent*, 36(6):649-60.

23. de Castro FL, de Andrade MF, Duarte Júnior SL, Vaz LG, Ahid FJ. Effect of 2% chlorhexidine on microtensile bond strength of composite to dentin (2003). *J Adhes Dent*, 5, 129-38.

24. Coelho A, Amaro I, Rascão B, Marcelino I, Paula A, Saraiva J, Spagnuolo G, Marques Ferreira M, Miguel Marto C, Carrilho E. Effect of Cavity Disinfectants on Dentin Bond

Strength and Clinical Success of Composite Restorations-A Systematic Review of In Vitro, In Situ and Clinical Studies. (2020) *Int J Mol Sci*.
25. Hosaka K, Nishitani Y, Tagami J, Yoshiyama M, Brackett WW, Agee KA, Tay FR, Pashley DH. Durability of resin-dentin bonds to water- vs. ethanol-saturated dentin. (2009) *J Dent Res*, 88(2):146-51.

26. Venigalla, B. S., Jyothi, P., Kamishetty, S., Reddy, S., Cherukupalli, R. C., & Reddy, D. AResin bond strength to water versus ethanol-saturated human dentin pretreated with three different cross-linking agents (2016). *Journal of conservative dentistry : JCD*, 19, 555–559.

27. Mousavinasab SM, Atai M, Barekatain M, Fattahi P, Fattahi A, Rakhshan V. Effects of ethanol concentrations of acrylate-based dental adhesives on microtensile composite-dentin bond strength and hybrid layer structure of a 10 wt% polyhedral oligomeric silsesquioxane (POSS)-incorporated bonding agent. (2018) *Dent Res J (Isfahan)*, 15(1):25-32.

28. Nishitani Y, Yoshiyama M, Donnelly AM, Agee KA, Sword J, Tay FR, Pashley DH. Effects of resin hydrophilicity on dentin bond strength. (2006) *J Dent Res*, 85(11):1016-21.

29. Leloup G, D'Hoore W, Bouter D, Degrange M, Vreven J. Meta-analytical review of factors involved in dentin adherence (2001). *J Dent Res*, 80, 1605-14.

30. Sano, H. Microtensile testing, nanoleakage and biodegradation of resin-dentin bonds (2006). *J Dent Res*, 85, 11-14.

31. Deng D, Yang H, Guo J, Chen X, Zhang W, Huang C. Effects of different artificial ageing methods on the degradation of adhesive-dentine interfaces (2014). *J Dent*, 42, 1577-85

32. O'Connor DJ, Wong RW, Rabie AB. Resveratrol inhibits periodontal pathogens in vitro. (2011). *Phytother Res*, 25, 1727-31

33. Ban SH, Kwon YR, Pandit S, Lee YS, Yi HK, Jeon JG. Effects of a bio-assay guided fraction from Polygonum cuspidatum root on the viability, acid production and glucosyltranferase of mutans streptococci (2010). *Fitoterapia*, 81, 30-4.

34. Borges FM, de Melo MA, Lima JP, Zanin IC, Rodrigues LK. Antimicrobial effect of chlorhexidine digluconate in dentin: In vitro and in situ study (2012). *J Conserv Dent*, 15, 22-26.

35. Hennessey TD. Some antibacterial properties of chlorhexidine (1973). *J Periodontal Res Suppl*.

36. Delany GM, Patterson SS, Miller CH, Newton CW (1982). The effect of chlorhexidine gluconate irrigation on the root canal flora of freshly extracted necrotic teeth. *Oral Surg Oral Med Oral Pathol*, 53, 518-523.

37. Gomes BPFA, Vianna ME, Sena NT, Zaia AA, Ferraz CCR, Souza FJ. In vitro evaluation of the antimicrobial activity of calcium hydroxide combined with chlorhexidine gel used as intracanal medicament (2006). *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 102, 544-550.

38. D'Alpino PH, Pereira JC, Svizero NR, Rueggeberg FA, Pashley DH. Factors affecting

use of fluorescent agents in identification of resin-based polymers (2006). *J Adhes Dent*, 8, 285-92.
39. D'Alpino PH, Pereira JC, Svizero NR, Rueggeberg FA, Pashley DH. Use of fluorescent compounds in assessing bonded resin-based restorations: a literature review (2006). *J Dent*, 34, 623-34.

40. Aguiar TR, Andre CB, Arrais CAG, Bedran-Russo AK, Giannini M. Micromorphology of resin–dentin interfaces using self-adhesive and conventional resin cements: A confocal laser and scanning electron microscope analysis (2012). *Inter J Adhes Adhes*, 38, 69-74.

41. Alhenaki AM, Attar EA, Alshahrani A, Farooq I, Vohra F, Abduljabbar T. Dentin Bond Integrity of Filled and Unfilled Resin Adhesive Enhanced with Silica Nanoparticles-An SEM, EDX, Micro-Raman, FTIR and Micro-Tensile Bond Strength Study (2021). *Polymers (Basel)*, 13, 1093

42. Anchieta RB, Oliveira FG, Sundfeld RH, Rahal V, Machado LS, Alexandre RS, Sundefeld ML, Rocha EP. Analysis of hybrid layer thickness, resin tag length and their correlation with microtensile bond strength using a total etch adhesive to intact dentin (2011). *Acta Odontol. Ltinoam*, 24, 272–278.

43. Carvalho, Eric Costa et al. Análise de interfaces de sistemas restauradores diretos em esmalte e em dentina humanos (2019). *Matéria*.

44. M. Sessa, R. Tsao, R. Liu, G. Ferrari, F. Donsi Evaluation of the stability and antioxidant activity of nanoencapsulated resveratrol during in vitro digestion (2011). *J. Agric. Food Che*m., 59, 12352-12360

Table 1. Groups

Groups	Description
BOND (negative control)	Single Bond Universal without pretreatment
ETN (experimental control)	100% ehtanol pretreatment + Single Bond Universal
CLX (positive control)	Pretreatment with 2% chlorhexidine digluconate (Cavity Cleanser, Bisco, Inc., Schaumburg, IL, EUA) + Single Bond Universal
RVT 1	Ehanol pretreatment containing 0,001% resveratrol w/v + Single Bond Universal
RVT 2	Ehanol pretreatment containing 0,002% resveratrol w/v + Single Bond Universal
RVT 3	Ehanol pretreatment containing 0,003% resveratrol w/v + Single Bond Universal

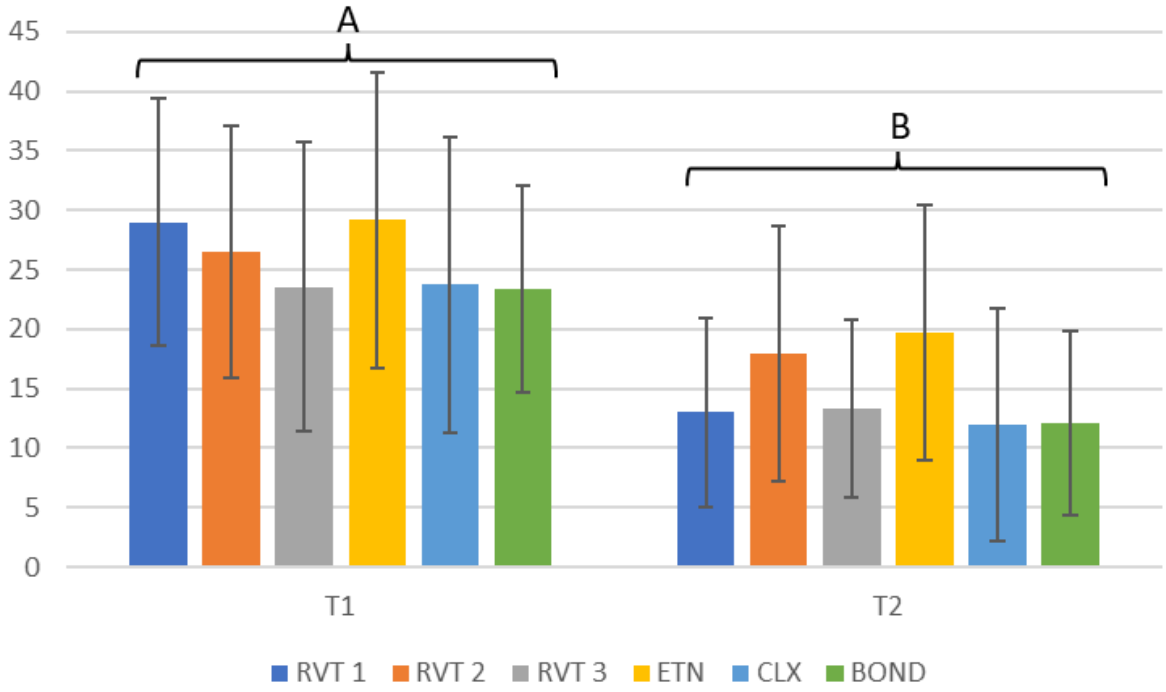

Figure 1. Graph representing the results of microtensile bond strength. Different letters represent statistical difference with respect to time. RVT1 - ehanol pretreatment containing 0,001% resveratrol; RVT 2 - ehanol pretreatment containing 0,002% resveratrol; RVT3 - ehanol pretreatment containing 0,003% resveratrol; CLX - pretreatment with 2% chlorhexidine digluconate; ETN - 100% ehtanol pretreatment; BOND - Single Bond Universal without pretreatment.

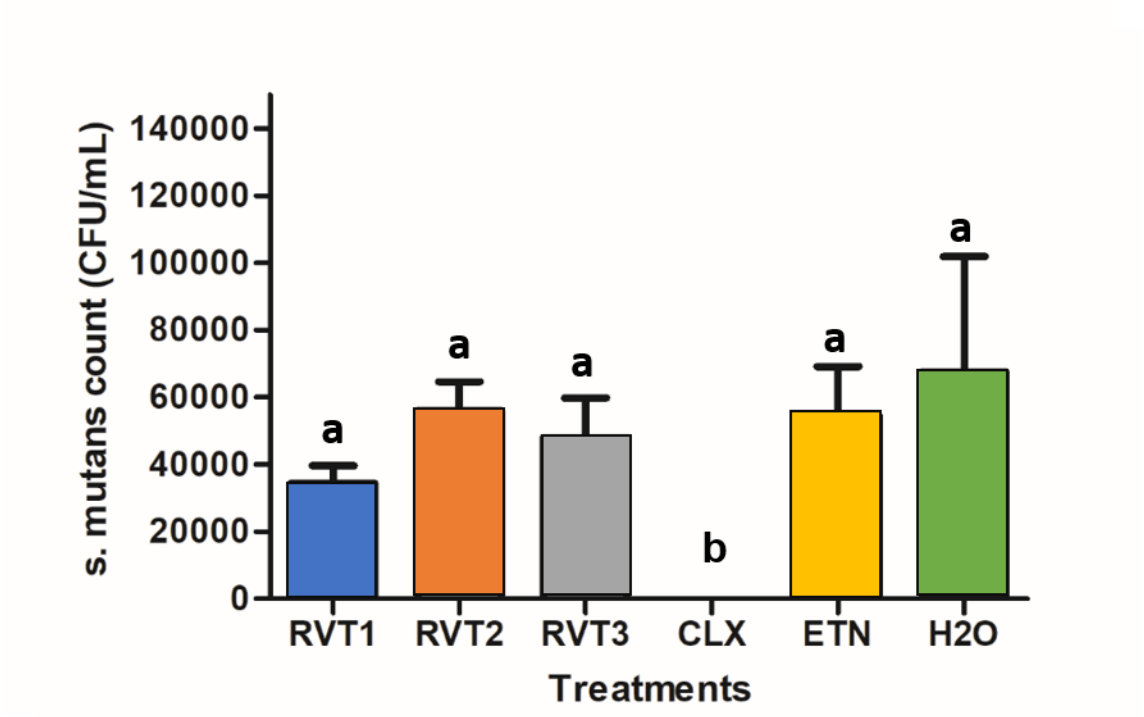

Figure 2. Graph representing the results of microbiological test. Data expressed in logarithm. Vertical lines represent standard deviations. Different lower case letters represent statistical difference ($P < 0.001$). RVT1 - ehanol pretreatment containing 0,001% resveratrol; RVT 2 - ehanol pretreatment containing 0,002% resveratrol; RVT3 - ehanol pretreatment containing 0,003% resveratrol; CLX - pretreatment with 2% chlorhexidine digluconate; ETN - 100% ehtanol pretreatment; H2O - pretreatment with distilled water.

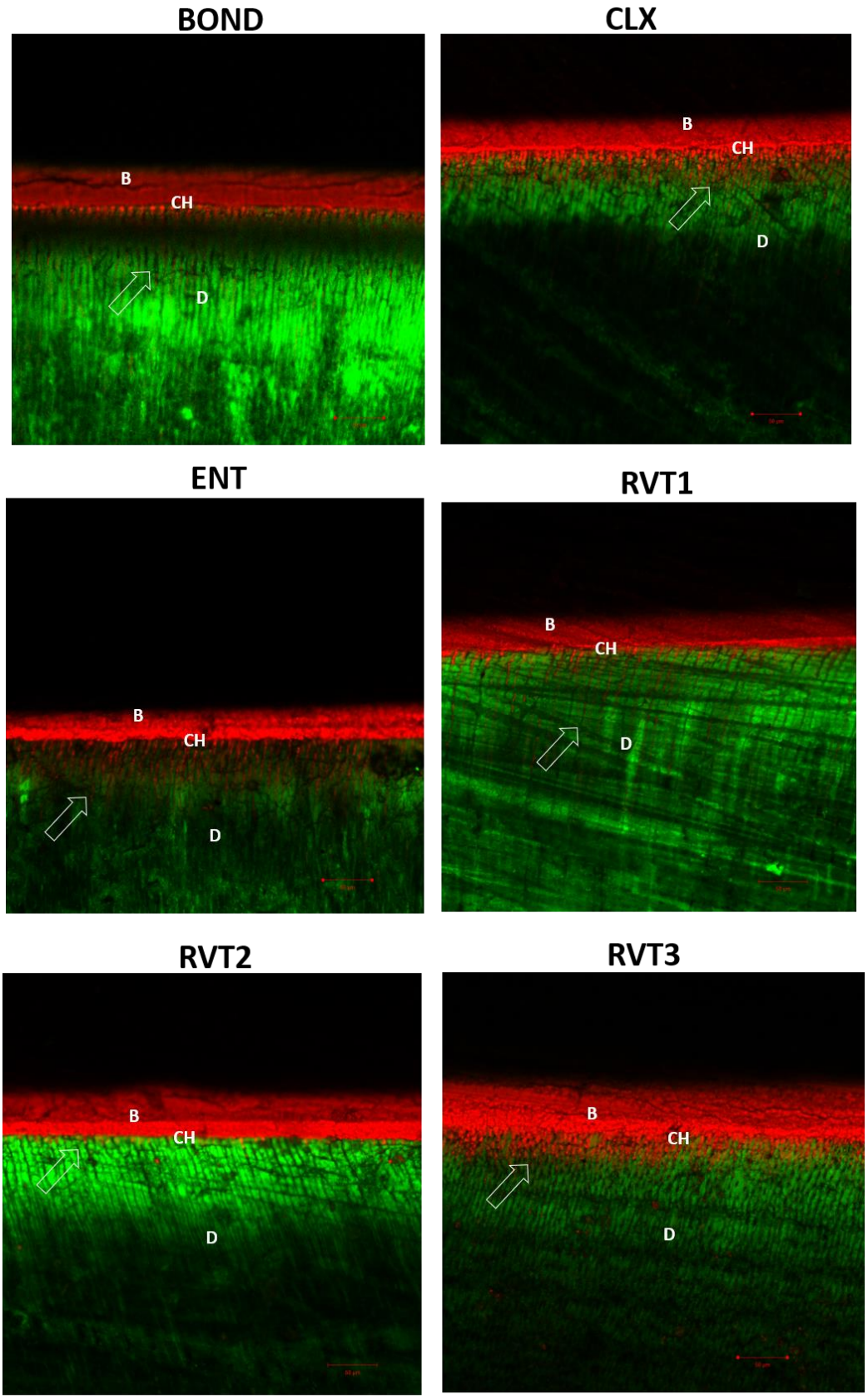

Figure 3. Confocal micrographs showing the mais features of the morphology of the hybrid layer. Open arrows indicate the presence of resin tags. CH – hybrid layer; D – dentin; A – bond; RVT1 - ehanol pretreatment containing 0,001% resveratrol; RVT 2 - ehanol pretreatment containing 0,002% resveratrol; RVT3 - ehanol pretreatment containing 0,003% resveratrol; CLX - pretreatment with 2% chlorhexidine digluconate; ETN - 100% ehtanol pretreatment; BOND - Single Bond Universal without pretreatment.

4. CONCLUSÃO

Dentro das limitações desse estudo, concluímos que o pré-tratamento com resveratrol, nas concentrações testadas, não prejudicou a resistência de união à dentina afetada por cárie e não demonstrou potencial antimicrobiano significativo contra o *S. mutans*.

REFERÊNCIAS

ARAUJO, J. F., et al. Remoção parcial do tecido cariado em dentes permanentes: uma revisão integrativa da literatura. **Rev. Bras. Odontol**, v. 74, n. 1, p. 31-5, 2017.

ATALAYIN, C.; ARMAGAN, G.; KONYALIOGLU, S.; KEMALOGLU, H.; TEZEL, H.; ERGUCßUZ.; KESER, A.; DAGCI, T.; ONAL, B. The protec-tive effect of resveratrol against dentin bonding agents-induced cytotoxicity**. Dent Mater J** v. 34,:p.766–773, 2015

ATALAYIN, C., TEZEL, H., ERGUCU, Z. et al. The improvement of biocompatibility of adhesives: The effects of resveratrol on biocompatibility and dentin micro-tensile bond strengths of self-etch adhesives **Clin Oral Invest** v. 23, p.213, 2019.

BARROS MMAF, DE QUEIROZ RODRIGUES MI, MUNIZ FWMG, RODRIGUES LKA. Selective, stepwise, or nonselective removal of carious tissue: which technique offers lower risk for the treatment of dental caries in permanent teeth? A systematic review and meta-analysis. **Clin Oral Investig**. v.24, n.2, p521-532, 2011.

CHAN, M. M. Antimicrobial effect of resveratrol on dermatophytes and bacterial pathogens of the skin. **Biochem. Pharmac.**, v. 63, p. 90-104, 2002.

COSTA, A. R., et al. Influence of Different Dentin Substrate (Caries-Affected, Caries-Infected, Sound) on Long-Term μTBS. **Braz. Dent. J.**, Ribeirão Preto , v. 28, n. 1, p. 16-23, 2017.

FATTAH, Z., JOWKAR, Z., REZAEIAN, S. Microshear Bond Strength of Nanoparticle-Incorporated Conventional and Resin-Modified Glass Ionomer to Caries-Affected Dentin. **Int J Dent**. 2021

FIRMINO, L. B. et al. Microbial Load After Selective and Complete Caries Removal in Permanent Molars: A Randomized Clinical Trial. **Brazilian Dental Journal** v. 29, n. 3, p. 290-295, 2018.

GUO R, PENG W, Yang H, YAO C, YU J, HUANG C. Evaluation of resveratrol-doped adhesive with advanced dentin bond durability. **J Dent**. 2021

HAJ-ALI, R.; WALKER, M.; WILLIAMS, K.; WANG, Y.; SPENCER, P. Histomorphologic characterization of noncarious and caries-affected dentin/adhesive interfaces. **J Prosthodont**. v. 15, p. 82–88, 2006.

ISOLAN CP, SARKIS-ONOFRE R, LIMA GS, MORAES RR. Bonding to Sound and Caries-Affected Dentin: A Systematic Review and Meta-Analysis. **J Adhes Dent**, v. 20, n. 1p.7-18, 2018

INNES, N. P. T. et al. Managing carious lesions: consensus recommendations on terminology. **Advances in dental research**, v. 28, n. 2, p. 49-57, 2016.

JOWKAR Z, FIROUZMANDI M, TABIBI S. The effect of proanthocyanidin and casein phosphopeptide-amorphous calcium phosphate on the bond strength durability to caries-affected dentin. **Clin Exp Dent Res**. v. 7, n. 3, p. 338-343, 2021

KHAMVERDI, Z.; REZAEI-SOUFI, L.; ROSTAMZADEH, T. The Effect of Epigallocatechin Gallate on the Dentin Bond Durability of Two Self-etch Adhesives. **J Dent (Shiraz)**. v. 16, n. 2, p. 68-74, 2015.

KRAFT, T. E; PARISOTTO, D.; SCHEMPP, C., EFFERTH, T. Fighting cancer with red wine? Molecular mechanisms of resveratrol. **Critical reviews in food science and nutrition**, v. 49, n. 9, p. 782-799, 2009.

LENZI, T. L.; TEDESCO, T. K.; SOARES, F. Z. M.; LOGUERCIO, A. D.; ROCHA, R. O.; Chlorhexidine application for bond strength preservation in artificially-created caries-affected primary dentin. **Int J Adhes Adhes**. p. 51–56, 2014.

MALTZ M, DE OLIVEIRA EF, FONTANELLA V, BIANCHI R. A clinical, microbiologic, and radiographic study of deep caries lesions after incomplete caries removal. **Quintessence Int.** v. 33, n. 2, p 151-9, 2002

NAKABAYASHI, N; KOJIMA, K; MASUHARA, E. The promotion of adhesion by the infiltration of monomers into tooth substrates. J Biomed Mater Res v. 16, p 265-273, 1982.

PADDICK JS, BRAILSFORD SR, KIDD EA, BEIGHTON D. Seleção fenotípica e genotípica da microbiota sobrevivente sob restaurações dentárias. **Appl Environ Microbiol** . v. 71, n. 5, p. 2467-2472, 2005.

PAULO, L.; FERREIRA, S.; GALLARDO, E.; QUEIROZ JA, D. F. Antimicrobial activity and effects of resveratrol on human pathogenic bactéria. **World J Microbiol Biotechnol**. v. 26, p. 1533–1538, 2010.

PENG, W.; YI, L.; WANG, Z.; YANG, H.; HUANG, C. Effects of resveratrol/ethanol pretreatment on dentin bonding durability. Materials Science & engineering. C, **Materials for Biological Applications**. 2020

PORTO, I. C. C. M.; NASCIMENTO, T. G.; OLIVEIRA, J. M. S.; FREITAS, P. H.; HAIMEUR, A.; FRANÇA, R. Use of polyphenols as a strategy to prevent bond degradation in the dentin–resin interface**. Eur J Oral Sci**., v. 126, n. 2, p. 146-158, 2018.

QI, Y. P.; LI, N.; NIU, L. N.; PRIMUS, C. M.; LING, J. Q.; PASHLEY, D. H.; TAY, F. R. Remineralization of artificial dentinal caries lesions by biomimetically modified mineral trioxide aggregate. **Acta Biomater**. v. 8, p.836-42, 2012.

RODRIGUES MP, CUNHA LSD, VILELA ABF, SCHETTINI ACT, BRAGANÇA GF, FRANÇA R, SOARES CJ. Selective carious tissue removal and glass ionomer liner reduction of pulp stress in bulk fill resin composite restorations. **Braz Oral Res**. 2021

SCHWENDICKE, F.; AL-ABDI, A.; MOSCARDÓ, A.P.; CASCALES, A. F.; SAUROCO, S. Remineralization effects of conventional and experimental ion-releasing materials in chemically or bacterially-induced dentin caries lesions. **Dental materials**, v. 35 p. 772–779, 2019.

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ANEXO A – PARECER CONSUBSTANCIADO DO CEP

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: AVALIAÇÃO ANTIMICROBIANA E INFLUENCIA NA ADESÃO À DENTINA AFETADA POR CÁRIE DO PRÉ-TRATAMENTO DENTINÁRIO COM RESVERATROL.

Pesquisador: Caroline Nágila do Nascimento Terto

Área Temática:

Versão: 2

CAAE: 43017621.7.0000.5054

Instituição Proponente: Programa de Pós-Graduação em Odontologia

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.597.231

Apresentação do Projeto:

O objetivo dessa pesquisa será avaliar in vitro, o potencial antimicrobiano e a influência na adesão à dentina afetada por cárie do pré-tratamento dentinário com uma solução de resveratrol/etanol nas concentrações de 1%, 2% e 3% p/v. O Resveratrol (RVT) é um composto fenólico encontrado em uvas e amoras com ação antioxidante que apresenta biocompatibilidade em dentina. Serão utilizados vinte e quatro dentes (n = 6), terceiros molares hígidos que serão submetidos a indução de dentina afetada pelo método de ciclagem de pH. Microtração (n=6) e micropermeabilidade dentinária (n=2), serão avaliados em uma máquina de ensaios universais e microscopia confocal a laser, respectivamente. A determinação do ângulo de contato (n=6) será realizada dispersando uma gota de água sobre a superfície tratada e observando o ângulo de contato obtido, utilizando fotografias digitais de alta resolução e o programa Image J. Para o teste microbiológico (n=3) serão confeccionados espécimes de dentina, submetidos a ciclagem de pH, que serão divididos em seis grupos, incluindo dois grupos controle negativo com água destilada e etanol 100% e um grupo controle positivo com clorexidina 2%. Os espécimes serão submetidos a um desafio cariogênico in vitro, onde serão imersos em TSB contendo extrato de levedura com sacarose a 10% e inoculados com S. mutans UA 159. Após o período experimental de 5 dias, o biofilme formado será coletado, diluições em série decimais das suspensões serão semeadas em BHI ágar e a relação de unidades formadoras de colônia será estabelecida. Os dados serão analisados estatisticamente com o nível de significância estabelecido em 5%.

Objetivo da Pesquisa:

-Objetivo Primário:

Avaliar, in vitro, o potencial antimicrobiano e a influência na adesão à dentina afetada por cárie do pré tratamento dentinário com uma solução de resveratrol/etanol nas concentrações de 1%, 2% e 3% p/v.

- Objetivo Secundário:

-Analisar ‘‘in vitro’’ o potencial antimicrobiano do pré-tratamento de resveratrol/etanol nas concentrações de 1%, 2% e 3% p/v.

-Analisar, “in vitro”, a influência do pré-tratamento de resveratrol/etanol nas concentrações de 1%, 2% e 3% p/v sobre a resistência de união imediata

e, após 6 meses de armazenamento, de um sistema adesivo universal à dentina afetada por cárie.

Analisar a morfologia da camada híbrida promovida por um sistema adesivo universal, após aplicação do pré-tratamento de resveratrol/etanol, nas concentrações de 1%, 2% e 3% p/v, sobre à dentina afetada por cárie, por meio de microscopia confocal a laser.

Analisar, fotografias digitais de alta resolução do ângulo de contato promovido entre o sistema adesivo universal e a dentina afetada por cárie, após o pré-tratamento de resveratrol/etanol nas concentrações de1%, 2% e 3% p/v, por meio do programa Image J.

Avaliação dos Riscos e Benefícios:

-Riscos:

Mínimos. Por tratar-se de um estudo laboratorial in vitro, pode oferecer riscos aos pesquisadores decorrentes do uso de material biológico. Entretanto, todos os cuidados de biossegurança serão adotados, com o intuito de evitar tal risco.

- Benefícios:

Possível desenvolvimento de materiais que possam favorecer tratamentos odontológicos menos invasivos e que possam reduzir os custos públicos e privados com as substituições de restaurações.

Comentários e Considerações sobre a Pesquisa:

Trata-se de uma pesquisa laboratorial utilizando dentes hígidos(3molares), obtidos por meio de doação após as exodontias em consultórios particulares. O objetivo é avaliar in vitro, o potencial antimicrobiano e a influência na adesão à dentina afetada por cárie do pré-tratamento dentinário com uma solução de resveratrol/etanol nas concentrações de 1%, 2% e 3% p/v. Serão utilizados vinte e quatro dentes (n = 6), sendo 6 grupos de estudo: SBU (controle negativo 1), ETN (controle negativo 2),CLX2 (controle positivo),RVT 1, RVT 2 e RVT 3.

Considerações sobre os Termos de apresentação obrigatória:

-Projeto

-Carta de apreciação do CEP

-Termo de dispensa de TCLE: Inadequado

-Termo de doação dos dentes

-Orçamento: Financiamento próprio

-Cronograma: vigência do projeto será até dezembro de 2021.

Conclusões ou Pendências e Lista de Inadequações:

-Termo de dispensa do TCLE: A justificativa do termo de dispensa deve estar condizente com a pesquisa e não por não ter riscos aos participantes. Solicitação não atendida do parecer anterior.

-Quantos dentes serão utilizados? Deixar claro o n amostral. Preencher também no formulário, pois neste tem 0(zero)indivíduos, contudo os dentes serão de seres humanos.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1697164.pdf	02/03/2021 00:36:06		Aceito
Outros	TERMO_DE_DOACAO_DE_DENTES.docx	02/03/2021 00:34:49	Caroline Nágila do Nascimento Terto	Aceito
Solicitação Assinada pelo Pesquisador Responsável	CARTA_SOLILICITANDO_APRECIACAO_CEP_UFC.pdf	02/02/2021 18:59:16	Caroline Nágila do Nascimento Terto	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	DISPENSA_DE_TCLE.docx	02/02/2021 18:57:34	Caroline Nágila do Nascimento Terto	Aceito
Orçamento	DECLARACAO_DE_ORCAMENTO.docx	02/02/2021 18:56:44	Caroline Nágila do Nascimento Terto	Aceito
Declaração de Pesquisadores	DECLARACAO_DOS_PESQUISADORES_ENVOLVIDOS_NA_PESQUISA.pdf	02/02/2021 18:56:15	Caroline Nágila do Nascimento Terto	Aceito
Declaração de Instituição e Infraestrutura	AUTORIZACAO_DO_LOCAL_DE_REALIZACAO_DA_PESQUISA.pdf	02/02/2021 18:36:41	Caroline Nágila do Nascimento Terto	Aceito
Cronograma	CRONOGRAMA.docx	02/02/2021 18:35:03	Caroline Nágila do Nascimento Terto	Aceito
Projeto Detalhado	PROJETO_COMITE_DE_ETICA.docx	02/02/2021	Caroline Nágila do	Aceito

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/ Brochura Investigador	PROJETO_COMITE_DE_ETICA.docx	18:34:42	Nascimento Terto	Aceito
Folha de Rosto	folhaDeRosto_caroline_signed.pdf	02/02/2021 18:28:36	Caroline Nágila do Nascimento Terto	Aceito

Situação do Parecer:
Pendente

Necessita Apreciação da CONEP:
Não

FORTALEZA, 17 de Março de 2021

Assinado por:
FERNANDO ANTONIO FROTA BEZERRA
(Coordenador(a))

Endereço: Rua Cel. Nunes de Melo, 1000
Bairro: Rodolfo Teófilo **CEP:** 60.430-275
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Telefone: (85)3366-8344 **E-mail:** comepe@ufc.br

ANEXO B – DIRETRIZES PARA PUBLICAÇÃO NA REVISTA ARCHIVESORAL BIOLOGY

GUIDE FOR AUTHORS

Editors-in-Chief:

Professor S W Cadden, Dundee, Scotland
Dr Fionnuala T. Lundy, Northern Ireland, UK

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- paeleodontology

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All authors should have made substantial contributions to all of the following: (1) the conception anddesign of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

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