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

MARIA ELISA MARTINS MOURA

AVALIAÇÃO DO POTENCIAL DE REMINERALIZAÇÃO E DA RESISTÊNCIA DE UNIÃO DE MATERIAIS RESTAURADORES BIOATIVOS EM DENTINA AFETADA POR CÁRIE

FORTALEZA
2021

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

Área de Concentração: Clínica Odontológica.

Orientador: Prof. Dr. Vicente de Paulo Aragão Saboia.

FORTALEZA

2021

Dados Internacionais de Catalogação na Publicação
Universidade Federal do Ceará
Biblioteca Universitária
Gerada automaticamente pelo módulo Catalog, mediante os dados fornecidos pelo(a) autor(a)

M888a Moura, Maria Elisa Martins.
AVALIAÇÃO DO POTENCIAL DE REMINERALIZAÇÃO E DA RESISTÊNCIA DE UNIÃO DE MATERIAIS RESTAURADORES BIOATIVOS EM DENTINA AFETADA POR CÁRIE / Maria Elisa Martins Moura. – 2021.
81 f. : il. color.

Tese (doutorado) – Universidade Federal do Ceará, Faculdade de Farmácia, Odontologia e Enfermagem, Programa de Pós-Graduação em Odontologia, Fortaleza, 2021.
Orientação: Prof. Dr. Vicente de Paulo Aragão Saboia.

1. Dentina cariada. 2. Remoção seletiva de cárie. 3. Adesivos dentários. I. Título.
CDD 617.6

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A Deus.

Aos meus pais, Adolfo e Mirian.

AGRADECIMENTOS ESPECIAIS

A Deus e ao meu bondoso São José, pois sem Eles nada disso seria possível.

Aos meus amados pais, Adolfo e Mirian, que sempre foram meu alicerce. Meus exemplos de dedicação e amor. Obrigada por sempre me incentivar a seguir os meus sonhos com resiliência e perseverança. Ao meu irmão, Daniel Levi que nunca mediu esforços para me apoiar e ser meu grande incentivador.

Ao meu marido Helton, pela cumplicidade e companheirismo. Obrigada por apoiar incondicionalmente os meus sonhos, por ser minha força e paz nos momentos mais importantes da minha vida. Amo você!

À minha filha Alice, que mesmo ainda dentro do meu ventre foi capaz ser meu impulso e força para finalizar esse projeto de vida.

Aos meus amigos e familiares, agradeço por estarem ao meu lado, comemorando as vitórias e me amparando nos momentos difíceis.

Ao Dr. Vicente, orientador e grande incentivador. Obrigada pelos conselhos e oportunidades! Sou muito grata por ter me acolhido como sua orientada de uma forma tão gentil.

Ao Dr. Salvatore Sauro, por ter me recebido na Universidad Cardenal Herrera e ter me possibilitado tamanho crescimento pessoal e profissional.

À minha grande amiga Ana Laura, agradeço a amizade, o companheirismo e toda a dedicação. Deborah e Diana, obrigada pela parceria nesta reta final. Vocês também são parte importante desta vitória.

Ao Grupo de Pesquisa, agradeço por toda ajuda e incentivo.

Aos amigos de doutorado, obrigada pela companhia e amizade nessa jornada.

AGRADECIMENTOS

À Universidade Federal do Ceará, por meio do Reitor Prof. Dr. Cândido Albuquerque.

À Faculdade de Farmácia, Odontologia e Enfermagem (FFOE/UFC), na pessoa de sua diretora Profª. Drª. Lidiany Karla Azevedo Rodrigues.

Ao curso de Odontologia, na pessoa de seu coordenador, Profª. Drª. Thyciana Rodrigues Ribeiro.

Ao Programa de Pós-Graduação em Odontologia da Universidade Federal do Ceará, na pessoa da sua coordenadora Profª. Drª. Cristiane Sá Roriz Fonteles.

Aos membros da banca examinadora, pela disponibilidade, além da presteza em avaliar e enriquecer este trabalho.

À secretaria da Pós-Graduação em Odontologia da Universidade Federal do Ceará, por todo o auxílio.

À Coordenação do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), pela concessão da bolsa de estudo e pela oportunidade tão enriquecedora na pós-graduação.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES, pelo fomento na pós-graduação.

RESUMO

A Odontologia Minimamente Invasiva engloba uma abordagem conservadora das lesões de cárie e orienta condutas para remoção seletiva e o manuseio de materiais adesivos para restauração da cavidade. A presente tese é constituída por dois capítulos, dos quais o primeiro avalia o potencial de adesivos experimentais incorporados com cálcio e fosfato associados a uma resina bioativa em induzir a formação de minerais em dentina desmineralizada. Foram usados três adesivos experimentais: Exp. 1 (autocondicionante 1 passo), Exp. 2 (autocondicionante 2 passos), Exp. 3 (convencional 2 passos), e um controle, DenTASTIC (convencional 2 passos) (Dentastic™ UNO™). A espectroscopia de infravermelho com transformada de Fourier (ATR-FTIR) avaliou a dentina totalmente desmineralizada e infiltrada com os adesivos (n=5) ou com adesivos/materiais restauradores (n=5) (Activa BioATIVE-RESTORATIVE ou Clearfil Majesty Flow) após 0, 15, 30 e 45 dias. Discos de adesivos (n=5) foram usados para detectar picos de formação de apatita em 0, 15, 30 e 60 dias. O processo de remineralização em dentina afetada por cárie (CAD) foi avaliado usando Microdureza (MH) e Microscopia Confocal (MC) após 0 e 3 meses. O capítulo 2 investigou a ação de materiais bioativos após remoção químico-mecânica da cárie (Papacárie Duo Gel®), por meio da análise da resistência de união (RU) e análise em MEV. Foram selecionados molares humanos hígidos (n = 40) e com lesões de cárie oclusais (n = 40). Foi utilizado um adesivo controle (DenTASTIC) e três experimentais: Exp. A (convencional 3 passos), Exp. B (autocondicionante 2 passos), Exp. C (autocondicionante 1 passo) e dois materiais restauradores (Activa ou Clearfil). Os dentes restaurados (n=5) foram submetidos ao teste RU e análise das interfaces adesivas em MEV. Os dados foram submetidos à análise estatística ($p<0,05$). Os resultados do experimento 1 mostraram que a remineralização parcial da dentina ocorreu após 7 dias com Exp. 2/Activa, enquanto Exp. 1 e Exp. 3 alcançaram este resultado após 2 semanas. Todos os adesivos experimentais mostraram sinal de bioatividade, mas apenas Exp. 2 obteve picos de formação de apatita. No *baseline*, na profundidade de 50µm e 100µm para ambos os materiais restauradores, os grupos Exp. 3 e DenTASTIC obtiveram o maior valor de MH. Após 3 meses, para 50µm de profundidade, Activa/Exp. 1 apresentou maior valor de MH ($p<0,01$). Não houve diferença entre os grupos nesta análise considerando 100µm de profundidade após 3 meses ($p>0,05$). MC mostrou remineralização em vários estágios para todos os grupos, exceto para DenTASTIC. O experimento 2 revelou que para a dentina hígida, após 1 ano, apenas Exp. C foi capaz de manter e até melhorar a RU quando combinado com os compósitos Activa e Clearfil, respectivamente. Quando aplicados em CAD, Exp. A, Exp. B e

Exp. C obtiveram os melhores resultados de RU, independentemente dos materiais restauradores ($p<0,01$). Como conclusão, os adesivos experimentais associados ou não a uma resina bioativa mostraram-se promissores para a remineralização da dentina desmineralizada e foram capazes de manter, ou mesmo melhorar, a RU à CAD, independentemente da estratégia adesiva.

Palavras-chave: Dentina cariada; Remoção seletiva de cárie; Adesivos dentários

ABSTRACT

Minimally Invasive Dentistry encompasses a conservative approach to caries lesions and guides procedures for selective removal and handling of adhesive materials to restore the cavity. The present thesis consists of two chapters in which the first assesses the potential of experimental adhesives incorporated with calcium and phosphate associated with a bioactive resin to induce the formation of minerals in demineralized dentin. Three experimental adhesives were used: Exp. 1 (self-etch 1 step), Exp. 2 (self-etch 2 steps), Exp. 3 (etch and rinse 2 steps) and a control, DenTASTIC (etch and rinse 2 steps) (Dentastic ™ UNO ™). Fourier transform infrared spectroscopy (ATR-FTIR) was used to assess dentin that was completely demineralized and infiltrated with adhesives (n=5) or with adhesives/restorative materials (n= 5) (Activa BioATIVE-RESTORATIVE or Clearfil Majesty Flow) after 0, 15, 30 and 45 days. Adhesive discs (n=5) were used to detect peaks of apatite formation at 0, 15, 30 and 60 days. The caries affected dentin (CAD) remineralization process was evaluated using Microhardness (MH) and Confocal Microscopy (CM) after 0 and 3 months. Chapter 2 investigated the action of bioactive materials after chemical-mechanical removal of caries (Papacárie Duo Gel®), through analysis of bonding strength (BS) and analysis in Scanning Electron Microscope (SEM). Human sound molars (n = 40) and with occlusal caries lesions (n=40) were selected. A control adhesive (DenTASTIC) and three experimental ones were used: Exp. A (etch and rinse 3 steps), Exp. B (self-etch 2 steps), Exp. C (self-etch 1 step) and also two restorative materials (Activa or Clearfil). The restored teeth (n=5) were submitted to the BS test and analysis of the adhesive interfaces in SEM. The data were submitted to statistical analysis ($p<0.05$). The results of experiment 1 showed that partial dentin remineralization occurred after 7 days with Exp. 2/Activa, while Exp. 1 and Exp. 3 achieved this result after 2 weeks. All experimental adhesives showed a sign of bioactivity, but only Exp. 2 obtained peaks in apatite formation. The groups Exp. 3 and DenTASTIC obtained the highest MH value in depth of 50µm and 100µm for both restorative materials at baseline. After 3 months, for 50µm of depth, Activa/Exp. 1 showed a higher MH value ($p <0.01$). There was no difference between groups in this analysis considering 100µm of depth after 3 months ($p> 0.05$). CM showed remineralization in several stages for all groups, except for DenTASTIC. In sound dentin, Experiment 2 revealed only Exp. C was able to maintain and even improve BS when combined with Activa and Clearfil composites, respectively after 1 year. When applied in CAD, Exp. A, Exp. B and Exp. C obtained the best BS results, regardless of the restorative materials ($p<0.01$). In conclusion, the experimental adhesives associated or not with a bioactive resin proved to be promising for the remineralization of demineralized dentin and were able to maintain or even improve BS to CAD, regardless of the adhesive strategy.

Keywords: Carious dentin; Selective caries removal; Dental Adhesives

SUMÁRIO

Introdução Geral

1. INTRODUÇÃO GERAL

A Odontologia Minimamente Invasiva (MID) é uma filosofia que engloba uma abordagem completa do cuidado e do gerenciamento da cárie. A MID descreve um manejo ultraconservador das lesões cavitadas e condutas preventivas não invasivas para as lesões incipientes (BANERJEE, 2013, SCHWENDICKE *et al*., 2016). Para que essa filosofia seja empregada é fundamental o conhecimento de três fatores: a histologia dos tecidos envolvidos, técnicas operacionais para remoção seletiva de cárie e o manuseio de materiais adesivos para restauração da cavidade (BANERJEE, 2013).

A cárie em dentina pode ser dividida em dentina amolecida, coriácea, firme e dura. A dentina afetada por cárie (CAD) é caracterizada por ter um aspecto coriáceo e firme (INNES et al., 2016). No entanto, o comprometimento dessa camada pelos ácidos bacterianos ainda é reversível e, devido a sua capacidade de recuperação, esta deve ser mantida durante o preparo cavitário (ERHARDT et al., 2008, ABUNA et al., 2021).

Com a evolução dos materiais adesivos e das técnicas de remoção de cárie, as cavidades macrorretentivas foram substituídas por cavidades menos invasivas (TYAS *et al*., 2000; TJÄDERHANE, TEZVERGIL-MUTLUAY, 2019). A remoção convencional da cárie envolve o uso de brocas, porém isso provoca algumas desvantagens como a remoção da dentina amolecida, mas não infectada, resultando em uma perda excessiva de tecido remineralizável (BANERJEE, WATSON, KIDD, 2000; SCHWENDICKE *et al*., 2016). O método químico-mecânico para remoção de cárie foi desenvolvido para superar essas deficiências. Além de ser mais confortável para o paciente, este é capaz de preservar melhor a dentina hígida e a CAD, pois reúne características atraumáticas e ação bactericida e bacteriostática (BUSSADORI, CASTRO, GALVÃO, 2005).

O Papacárie (Fórmula e Ação, São Paulo, Brasil) é um produto que atua como agente de remoção químico-mecânico da cárie (BUSSADORI, CASTRO, GALVÃO, 2005) e contém a enzima papaína, uma protease de ampla atividade proteolítica extraída da árvore *Carica Papaya* (NEVES *et al*., 2015). A papaína age seletivamente na dentina infectada porque esses tecidos carecem de uma antiprotease plasmática chamada α-1-antitripisina, permitindo que a papaína quebre as moléculas de colágeno parcialmente degradadas e preserve as regiões de colágeno íntegro (BUSSADORI *et al*., 2011).

Os estudos que avaliam a durabilidade de união dos materiais adesivos à CAD mostram que há uma degradação hidrolítica recorrente na interface restauradora (ERHARDT *et al.*, 2008; HE *et al.*, 2019). As principais causas são a polimerização deficiente devido a umidade residual dentro das fibrilas de colágeno, degradação enzimática provocada pelas enzimas metaloproteinases de matriz (MMP's) e a perda mineral que impacta nas propriedades mecânicas finais (VAN MEERBEEK *et al.*, 2020). É de interesse da MID desenvolver técnicas restauradoras com potencial de remineralização, de forma que ocorra o estímulo para a manutenção e a recomposição mineral perdida. O processo de remineralização envolve a liberação de íons fluoreto, cálcio e fosfato que interagem quimicamente com os constituintes dos tecidos desmineralizados (MOUSAVINASAB *et al.*, 2011; VAN MEERBEEK *et al.*, 2020).

Diversas estratégias como uso de materiais resinosos com liberação de flúor, fosfato de cálcio amorfo (ACP) ou adesivos contendo vidro bioativo têm sido empregadas para auxiliar no restabelecimento mineral da CAD e, consequentemente, no aumento da durabilidade de união entre a dentina desmineralizada e os materiais restauradores (NIU *et al.*, 2014; CHENG *et al.*, 201512; TJÄDERHANE, TEZVERGIL-MUTLUAY, 2019). O processo de remineralização também pode ocorrer quando adesivos bioativos simplificados são utilizados e, através da sua permeabilidade natural, permitem a passagem de íons até a dentina subjacente (TOLEDANO *et al.*, 2015; SAURO *et al.*, 2019).

O ACTIVA BioActive-Restorative (Pulpdent Corp., Watertown, EUA) é um cimento ionômero de vidro modificado por resina enriquecido com biovidro e incorporado a uma resina polimérica. Estudos recentes têm demonstrado que esse material tem a capacidade de liberar íons remineralizantes sem efeitos adversos (PAMEIJER *et al.*, 2015; PORENCZUK *et al.*, 2019; VAN MEERBEEK *et al.*, 2020). Segundo a fabricante, esse material restaurador é o primeiro material odontológico bioativo com matriz de resina iônica que mimetiza as propriedades físicas e químicas dos dentes naturais (PORENCZUK *et al.*, 2019).

A deposição mineral é uma abordagem promissora para preservar a camada híbrida de enzimas colagenolíticas e do desafio ácido bacteriano. Uma possibilidade seria associar sistemas adesivos incorporados com minerais a materiais restauradores bioativos e avaliar se o potencial de bioatividade seria potencializado. Dessa forma, uma recuperação mineral mais rápida e eficiente poderia ser promovida. Sabendo disso, este estudo propõe analisar a capacidade dos adesivos experimentais incorporados com Ca-PO associados a uma resina bioativa em induzir a formação mineral na dentina desmineralizada. Além disso, avaliar a resistência de união em dentina afetada por cárie após o uso de um agente de remoção químico-mecânico.

Proposição

2. PROPOSIÇÃO

2.1 Objetivo Geral

Formular adesivos experimentais incorporados com minerais (Ca e PO) e avaliar o seu potencial remineralizador e resistência adesiva em dentina afetada por cárie, quando associados ou não a uma resina bioativa.

2.2 Objetivos Específicos

- Avaliar, através da análise da Espectroscopia de Infravermelho com Transformada de Fourier (FTIR), se os adesivos experimentais incorporados com minerais (Ca e PO), associados ou não a uma resina bioativa, são capazes de promover formação mineral em dentina totalmente desmineralizada.

- Avaliar, através do teste de Microdureza, se os adesivos experimentais incorporados com minerais (Ca e PO), associados ou não a uma resina bioativa, são capazes de remineralizar a dentina afetada por cárie *in vitro*.

- Avaliar, através do ensaio de Microtração (μTBS), se os adesivos experimentais incorporados com minerais (Ca e PO), associados ou não a uma resina bioativa, são capazes de preservar a resistência de união em dentina afetada por cárie.

- Avaliar o padrão de fratura dos espécimes após o ensaio de Microtração (μTBS).

- Avaliar a integridade das interfaces restauradoras através da análise em Microscópio Eletrônico de Varredura (MEV).

Capítulo

3. CAPÍTULO

Esta tese está baseada no artigo 46 do Regimento Interno do Programa de Pós-graduação em Odontologia da Universidade Federal do Ceará que regulamenta o formato alternativo para dissertações de Mestrado e teses de Doutorado e permite a inserção de artigos científicos de autoria ou coautoria do candidato. Por se tratar de pesquisas envolvendo seres humanos, ou partes deles, o projeto de pesquisa deste trabalho foi submetido à apreciação do Comitê de Ética em Pesquisa da Universidade Federal do Ceará, através da submissão no site da plataforma Brasil, tendo sido aprovado (Anexo). Assim sendo, esta tese é composta por 2 capítulos citados abaixo:

- **Capítulo 1**

Título: Remineralization of caries affected dentin via bioactive restoration materials.

Periódico: Dental Materials*

- **Capítulo 2**

Título: Bond strength of bioactive materials in caries affected dentin after a chemo-mechanical removal agent.

Periódico: Journal of Dentistry**

Normas das Revistas:

* https://www.elsevier.com/journals/dental-materials/0109-5641/guide-for-authors

** https://www.elsevier.com/journals/journal-of-dentistry/0300-5712/guide-for-authors

Capítulo *1*

REMINERALIZATION OF CARIES AFFECTED DENTIN VIA BIOACTIVE RESTORATION MATERIALS

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REMINERALIZATION OF CARIES AFFECTED DENTIN VIA BIOACTIVE RESTORATION MATERIALS

ABSTRACT

Purpose. To evaluate the potential of experimental adhesives incorporated with Ca-PO associated with or without bioactive resin to induce mineral formation in demineralized dentin.

Methods. Three experimental adhesives were used: Exp. 1 (self-etch/1 step), Exp. 2 (self-etch/2 steps), Exp. 3 (etch and rinse/ 2 steps) and a control DenTASTIC™UNO™ (DenTASTIC). The Fourier transform infrared spectroscopy (ATR-FTIR) was evaluated in demineralized dentin infiltrated with those adhesives (n = 5) and specimens of dentin infiltrated with adhesives/restorative materials (n = 15) (ACTIVA BioACTIVE-RESTORATIVE or CLEARFIL MAJESTY Flow) at 0 to 45 days. Discs of adhesives (n = 5) were used to detect peaks of apatite formation at 0 to 60 days. The remineralization process in caries affected dentin (CAD) was assessed using Microhardness (MH) and Confocal Laser Scanning Microscope (CLSM) after 0 to 3 months.

Results. For ATR-FTIR analysis showed that partial remineralization of demineralized dentin occurred after 7 days with Exp. 2/Activa, while Exp. 1 and Exp. 3 adhesives achieved this result after 2 weeks. Exp. 1, Exp. 2 and Exp. 3 showed a clear sign of bioactivity, but only Exp. 2 obtained peaks of apatite formation. There was increase of MH after 3 months, in 100µm of depth, when either Activa or Clearfil were applied, regardless of the adhesive. CLSM showed remineralization in many stages for all groups, except for DenTASTIC which composition has no ions.

Conclusions. The experimental adhesives incorporated with Ca-PO associated or not with a bioactive resin proved to be promising for treatment of CAD, according to Minimally Invasive Dentistry approach.

1. INTRODUCTION

The clinical restorative approach most accepted nowadays is the Minimal Invasive Dentistry (MID), which is defined as a set of techniques and concepts for an ultra-conservative handling of caries injuries. To apply this philosophy, the knowledge of three factors is fundamental: histology of the involved tissues, handling of the adhesive materials for the cavity restoration and operational techniques for the selective caries removal [1].

The dentin caries can be divided in two histopathological zones: infected dentin and caries affected dentin (CAD). The infected dentin is a tissue that is irreversibly damaged while CAD is a tissue compromised by bacterial acids but still presents capability of recovering. Studies that evaluate the durability of adhesive materials bonded to CAD show that there is a recurrent hydrolytic degradation at the adhesive interface [2, 3]. The main reported problems are deficient polymerization due to residual humidity inside collagen fibrils, enzymatic degradation motivated by matrix metalloproteinases (MMP) enzymes and mineral loss that impact on final mechanical properties [4].

It is of MID interest to develop restorative techniques using materials with remineralization potential. This approach suggests no complete removal of carious tissue in deep caries with pulp exposure high risk in which CAD on the cavity-bottom may remain and be remineralized [4]. That can be possible through bioactive materials which involve antibacterial and anti-enzymatic effects and/or remineralization [5, 6].

The remineralization process involves fluoride, calcium and phosphate ions release from bioactive materials that interact chemically with demineralized tissues constituents [7, 8]. However, decrease in ions release may occur over time, therefore for long term protection it is important to keep the adhesive remineralization ability [9] through rechargeable biomaterials [3, 10]. Knowing that, an alternative would be to incorporate minerals (Ca and P) into adhesives combined with potential bioactive resins.

The present study aims to evaluate the ability of experimental adhesives incorporated with Ca-PO associated with a bioactive resin to induce mineral formation in demineralized dentin after storage in artificial saliva. The tested null hypothesis are (1) the experimental adhesives containing Ca-PO associated with a bioactive resin would not promote mineral formation in the demineralized dentin and (2) the caries affected dentin *in vitro* would not have its hardness recovered after the restorative protocol using the bioactive adhesive materials.

2. MATERIALS AND METHODS

2.1 Adhesive resin blending

Four adhesive systems were used: DenTASTIC™UNO™ (DenTASTIC) (Pulpdent Corporation, Watertown, USA) (composition under license from patent) as a commercial control and three experimental resin-based were formulated: Experimental 1 (Exp. 1) (universal self-etch adhesive) (Urethane dimethacrylate (UDMA), Hydroxy-ethyl methacrylate (di-HEMA phosphate), Tri-ethyl glycol dimethacrylate (TEGDMA), ethanol, water, camphorquinone (CQ) photoinitiator, ethyl-dimethylamino-benzoate (EDAB), 2% haloisite and 1% tri-calcium phosphate), Experimental 2 (Exp. 2) (self-etch 2 steps adhesive) (primer: di-HEMA phosphate, ethanol, water, TEGDMA, UDMA, CQ, EDAB, 2% haloisite and 1% tri-calcium phosphate and bond: UDMA, TEGDMA, HEMA, ethanol, CQ, EDAB, 2% halosite, 1% tri-calcium phosphate) and Experimental 3 (Exp. 3) (etch and rinse adhesive 2 steps) (UDMA, TEGDMA, HEMA, acetona, CQ, EDAB, 1% tri-calcium phosphate).

2.2.1 Totally demineralized dentin infiltrated with adhesives

Dentin discs (5 mm diameter x 2 mm thick) were prepared from extracted human third molars obtained under approval of Institutional Ethics Committee (protocol 3.964.912). Five dentin discs per group of adhesives (Exp. 1, Exp. 2, Exp. 3 and DenTASTIC) used in this study were sectioned from the mid-coronal dentin of human molars and totally demineralized with 0.5 M ethylenediamine tetra-acetic acid (EDTA; pH=7.4) for 7 days at 25°C. The demineralization of the specimens was checked by ATR-FTIR (Spectrum-One, Perkin Elmer, London, UK). Adhesive infiltration was performed for 2 hours, while for the primer and bond adhesive it was performed 1 hour in primer and 1 hour in bond by constant agitation at 25°C in the dark [11]. Excess resin was removed by strong air-blowing of the specimens for 3 seconds on each side at a minimum distance of 10 cm on each side. LED-curing was performed for 20 sec (1500 mW/cm^2; Radii Plus, SDI Limited, Victoria, Australia).

2.2.2 Totally demineralized dentin with adhesives and in contact with restorative materials

Further fifteen dentin discs (5 mm diameter x 2 mm thick) per group of adhesives (Exp. 1, Exp. 2, Exp. 3 and DenTASTIC) used in this study. The infiltration of the adhesives occurred as previously described. The specimens subsequently received 3 different layers, 2mm each, of restorative materials ACTIVA BioACTIVE-RESTORATIVE (Activa) (Pulpdent Corporation, Watertown, USA) or conventional composite CLEARFIL MAJESTY Flow (Clearfil) (Kuraray Medical Inc, Tokyo, Japan).

2.2.3 Discs of adhesives

Five discs (4 mm diameter x 1 mm thick) were created per group of adhesives (Exp. 1, Exp. 2, Exp. 3 and DenTASTIC) using silicon moulds. The solvent was evaporated by air-blowing the specimens for 5 sec and finally LED-curing for 20 sec. The specimens were polished after incubation in dry and dark condition for 24 hours. The specimens were then checked (T0 - baseline) by ATR-FTIR and immersed in AS. ATR-FTIR analysis were performed 0 to 60 days.

2.2.4 Fourier transform infrared spectroscopy (ATR-FTIR)

ATR-FTIR analysis were performed 0 to 45 days. The specimens were saved in 5 ml of Artificial Saliva (AS) (Dulbecco's Phosphate Buffered Saline (DPBS, Sigma-Aldrich, St. Louis, USA) and every 3 days replaced with fresh one. The analyses were realized in three different positions of each specimen in the region of 650 to 4000 cm^{-1} with a resolution of 4 cm^{-1} and 8 scans per spectrum. A moderate pressure was applied (5 psi) to establish good contact between the specimen and the surface. All spectra were subjected to normalization, baseline correction, and smoothing processes and were averaged into a single spectrum by the FTIR software.

2.3 Preparation of artificial caries affected dentin

A high-speed system was used in the preparation of standardized cavities (depth = 2 mm, length = 6 mm and wide = 4 mm). The size of the cavities was checked with a digital caliper. Cavity preparation began at the center of the occlusal surface of each molar. The #8 tungsten carbide burs (KG Sorensen, Cotia, Brazil) with an initial depth of 1.5 mm were used initially and then a #3131 diamond burs (KG Sorensen, Cotia, Brazil) to obtain the final size of 2mm. Both were used under continuous cooling. The burs were replaced after 5 cavity preparations. To complete this step, the teeth were immersed in an ultrasonic bath (distilled water) for 280 seconds to remove the debris from the cavity preparation. By the visual examination of the samples with a stereoscopic magnifying glass (Olympus SZ 40-50; Tokyo, Japan) at 10x magnification was checked if the pulp chamber was exposed by the preparation of the cavity and, if it was, the tooth discarded. The presence of enamel also was evaluated.

When present, it was removed so that the cavity was only in the dentin substrate. The teeth were completely covered with varnish except for the dentin surface (the surrounding enamel also was covered with varnish) to delimit the area of exposure to the acid solution that simulated the carious lesion. It was applied a protocol published by [12] to prepare samples of caries affected dentin. A depth of 150±50 µm of partially demineralized dentin was created on the occlusal surface of the selected teeth through pH cycling. The demineralizing solution consisted of 1.5 mmol/L of $CaCl_2$, 0.9 mmol/L of KH_2PO_4, 50 mmol/L of acetic acid, 5 mmol / L NaN_3, adjusted to pH 4.8. The remineralizing solution consisted of 1.5 mmol/L of $CaCl_2$, 0.9 mmol/L of NaH_2PO_4, 0.13 mmol/L of KCl, 5 mmol/L NaN3, adjusted to pH 7 with HEPES buffer. Each sample was immersed in 10mL of demineralizing solution for 8 hours followed by immersion in 10mL of remineralizing solution for 16 hours. Cycling was carried out for 14 days and new solutions were used in each cycle.

2.4 Microhardness Analysis

The Microhardness (MH) Analysis performed on a Microhardness Apparatus (HMV, Shimadzu, Kyoto, Japan) with a Vickers diamond indenter, set at a 10g of load for 30 sec [13].Vickers hardness was measured along 3 lines in each section, starting from the cavity floor toward the pulp. Four measurements were taken along each line: underlying dentin, 50µm and 100µm in depth.

2.5 Confocal laser scanning microscopy (CLSM)

One slab from each tooth and from each group was used to ascertain the interface demineralization at time 24 hours and 3 months of artificial saliva storage for all the specimens after the microhardness test. The slabs were immersed in a 1 wt% aqueous rhodamine/xylenol orange dye solution for 12 hours and subsequently rinsed with copious amounts of water in an ultrasonic bath for 2 min. The micropermeability/remineralization along the interfaces was examined using a Confocal Laser Scanning Microscope (Microscope Confocal Espectral Leica, Wetzlar, Germany), equipped with a 63X/1.4 NA oil-immersion lens and different LED illumination. Confocal laser scanning microscopy reflection and fluorescence images was obtained with a 1-um z-step to section optically the specimens to a depth of up to 20 µm below the surface. The z-axis scan of the interface surface was arbitrarily pseudo-colored for better exposure and compiled into both single and topographic projections using Microscope Imaging

Software. The configuration of the system was standardized and used at the same settings for the entire investigation.

2.6 Statistical Analysis

The data were analyzed in Microsoft Excel and exported to the Statistical Package for the Social Sciences Software, in which the analyzes were performed adopting a 95% confidence. After evaluation by the Shapiro-Wilk normality test, data were expressed as means and standard deviations and compared at different depths using Student's t test (time) or ANOVA-1-way/Bonferroni (adhesive system).

3. RESULTS

3.1 Fourier transform infrared spectroscopy

The ATR-FTIR analysis showed that the mineralized control specimens (sound dentin) within spectra recorded in the region of 800–1800 cm^{-1} presented phosphate bands at 885–1180 cm^{-1}, representative of mineral components, assigned to the phosphate stretching between 550 and 600 cm^{-1}. Amide bands from 1180 to 1725 cm^{-1} from organic components were also detected in sound mineralized dentin. After demineralization, the entire inorganic phase disappeared from all specimens.

3.1.1 Dentin infiltrated with adhesives

For Exp. 1 and Exp. 2 at time 0 it was possible to detect the presence of Ca-PO (MCP - peaks of 1082-1030cm^{-1}) in dentin, which disappeared during the first 15 days of storage in AS. However, after 45 days of storage in AS, it was possible to see in both adhesives that the totally demineralized dentin substrate presented sign of phosphorylation and therefore, remineralization (Orthophosphate optical absorption band (peaks of 1034 cm^{-1}) and absorption bands at 1081 cm^{-1} (orthophosphate stretching vibrations were particularly visible then, along with other phosphate compounds typically present in dentin, 563 cm^{-1}). The presence of halloysite nanotubes was visible at 531cm^{-1} (Figure 1 – FTIR Exp. 1 and Figure 2 - FTIR Exp. 2).

For Exp. 3 between time 0 and the first 15-30 days of storage in AS it was possible to see the peaks of Ca-PO as Exp. 1 and Exp. 2. After 45 days this adhesive was not able to induce some phosphorylation in the demineralized dentin collagen once peaks of orthophosphate (peaks of 1031-1032 cm^{-1}) were not detected (Figure 3 – FTIR Exp. 3).

It was not possible to detect the presence of Ca-PO in dentin (peaks of 1082-1030cm^{-1}) nor signs of phosphorylation over time. (Figure 4 – FTIR DenTASTIC)

3.1.2 Dentin infiltrated with adhesives/restorative materials

Signs of phosphorylation and remineralization (peaks of 1034 cm^{-1}) were detected after 7 and 15 to 45 days for Activa and Clearfil, respectively (Figure 5 – FTIR Exp. 1/Activa and Exp. 1/ Clearfil).

While for Activa the signs of phosphorylation (peaks of 1034 cm^{-1} and 1081 cm^{-1}) were detected since the 7th day, for Clearfil those signs appeared only from the 15th day on. (Figure 6 – FTIR Exp. 2/Activa and Exp. 2/Clearfil).

Exp. 3 in combination with Activa resulted in little phosphorylation and remineralization (peaks of 1034 cm^{-1} and 1081cm^{-1}) and no signs of mineralization were seen when Clearfil was used. DenTASTIC with either Activa or Clearfil was not able to induce any signs of phosphorylation even after 45 days. (Figure 7 – FTIR Exp. 3/Activa, Exp. 3/Clearfil, DenTASTIC/Activa and DenTASTIC/Clearfil).

3.2.3 Discs of adhesives

Exp. 1 and Exp. 2 showed at time 0 the presence of Ca-PO (MCP) in the adhesive (peaks of 1080-960 cm^{-1}). On the 60th day of analysis, minerals in Exp. 2 changed in phase and this adhesive showed peaks of 1137, 955/960 cm^{-1} and 563 cm^{-1}, representative for apatite formation. (Figure 8 – FTIR Exp. 1 and Exp. 2).

Exp. 3 and in particular DenTASTIC were not tested for a period longer than 15 days as they almost dissolved and degraded after AS storage. Exp. 3 showed at time 0 the presence of Ca-PO (MCP - peaks of 1080-960 cm^{-1}). These peaks disappeared after a period of 15 days and on the 30th day the discs of Exp. 3 had a gel-like consistency, while the discs of DenTASTIC degraded completely, so it was impossible to test them. (Figure 9 – FTIR Exp. 3).

3.2 Microhardness (MH) Analysis

For MH analysis, results of different depths, adhesive systems and restorative materials were evaluated.

In the underlying dentin, at baseline, DenTASTIC/Activa and Exp. 3/Activa showed highest values of MH, followed by Exp. 2/Activa and Exp. 1/Activa, which presented the worst results ($p<0.01$). The specimens restored with Exp. 3/Clearfil, at baseline, obtained higher values of MH than the other groups, which were similar ($p>0.05$). After 3 months, a decrease in the values of MH was seen and all groups were similar. (table 1 - underlying dentin microhardness).

At baseline, considering the depth of 50µm and 100µm for both restorative materials, the groups Exp. 3 and DenTASTIC obtained the highest value of MH followed by Exp. 1 and Exp. 2. After 3 months, for 50µm depth, Exp. 1/Activa showed higher value of MA than other groups, which were similar ($p<0.01$). For Clearfil, Exp. 2 had the lowest value, followed by Exp. 1. Exp. 3 and DenTASTIC were statistically similar and showed the best results. After aging (3 months in artificial saliva), all groups presented significant increase in MH, except the group Exp. 2/Clearfil which maintained its initial value. There was no result difference among the groups in this analysis considering 100µm depth (table 2 - 50µm depth and table 3 - 100µm depth).

3.3 Confocal laser scanning microscopy

It was possible to see approximately 120-150 microns of highly demineralised dentin and a partially demineralised caries dentin in all groups at baseline (Image (A) Baseline Confocal). After 3 months storage in AS, it was possible to see that the specimens bonded with Exp. 1 and restored either with Activa or Clearfil were remineralized on some regions along the interface (Image (B) and (C) – Exp. 1/Activa and Exp. 1/Clearfil). Exp. 2 combined with both restorative materials was able to induce remineralization along the entire interface (Image (D) and (E) – Exp. 2/Activa and Exp. 2/Clearfil). For Exp. 3/Activa were observed scarce zones of slight remineralization and when Clearfill was used only degradation areas were seen close to the adhesive layer (Image (F) and (G) – Exp. 3/Activa and Exp. 3/Clearfil). Most areas of non-remineralized dentin along the interface were seen for DenTASTIC/Activa while for

DenTASTIC/Clearfill impressive signs of degradation were shown. (Image (H) and (I) – DenTASTIC/Activa and DenTASTIC/Clearfil).

4. DISCUSSION

Adhesion on dentin substrate is complex and can be even more critical in caries affect dentin. Once demineralized, the underlying dentin layer is susceptible to enzymatic degradation due to the action of metalloproteinases and cathepsins, compromising the bond durability in this [13-16]. Minimally Invasive Dentistry seeks to develop restorative techniques that induce caries affected dentin to remineralization in a way there is no complete removal of caries tissue, especially in deep caries injuries with pulp exposure high risk [4]. In the present study, the ATR-FTIR and Confocal Microscopy analyses revealed that the use of adhesives incorporated with Ca-PO, associated or not with a bioactive resin in a restorative protocol, have the ability to partially remineralize caries affected dentin. Thus, we must reject the first null hypotheses of this study.

The biggest challenge for successful mineralization in CAD is the relationship between the mineral and the collagen structure [17]. Two mechanisms may explain the remineralization of the partially demineralized dentin. The classical remineralization concept claims that crystal formation begins with the nucleation of the crystal, forming aggregations of mineral clusters between collagen fibrils [18]. The other way would be the non-classical crystallization pathway, which consists of a particle-mediated mineralization. The crystals are gathered in nanometric units, known as pre-enucleation clusters, which bind to Non-Collagen Polyanionic (NCP) or biomimetic analogs of these proteins involved in mineralization [19, 20]. These clusters aggregate into larger amorphous calcium phosphate nanoparticles and then penetrate into the intrafibrillar compartments of a collagen matrix and form a stable crystalline phase [20, 21].

Although the latter technique may be considered ideal for dentin remineralization, as it covers interfibrilar and intrafibrilar remineralization, one of its limitations is the long clinical application time and the need for NCP analogues [22]. However, recent studies confirmed that type I collagen may play an active role in remineralization, acting as a guide and modulator without the need of other substances [23, 24].

In the present study, the ATR-FTIR analysis showed that the partial remineralization of fully demineralized dentin occurred after 7 days with Exp. 2 adhesive associated with Activa, while Exp. 1 and Exp. 3 adhesives achieved this result after 2 weeks. Considering these data, the incorporation of minerals in the adhesive, associated or not with a bioactive composite resin, can be useful for remineralization, which is in accordance with previous studies [3, 25-27]. The aforementioned analysis assumed that the precipitates formed are calcium phosphate, although the exact composition and nature of the crystallinity cannot be verified by ATR-FTIR. The adhesives Exp. 1, Exp. 2 and Exp. 3 showed peaks of Ca-PO (1080-960 cm-1) showing a clear sign of bioactivity, however, only in Exp. 2 were detected peaks of 1137 cm-1, 960 cm-1 and 565 cm-1 which are the most representative for apatite formation.

In the purpose to evaluate functional remineralization, which consists in recovering of lost biomechanical and chemical properties of the substrate [28], MH was measured before and after aging (3 months in artificial saliva). For MH, CAD layers were stratified in underlying dentin, 50µm, and 100µm below the adhesive-dentin interface for obtaining a detailed analysis of the proposed protocol response. The second null hypotheses must be partially rejected once MH was not recovered in underlying dentin after the restorative protocol using the bioactive adhesive materials.

In underlying dentin, the Exp. 3 group obtained the highest value of MH in the baseline but it was not maintained after 3 months. In this layer, all groups exhibited a statistically significant decrease in MH values after aging ($p < 0.05$). It is known that caries affect dentin closer to the adhesive layer, where there is more significant demineralization and destruction of collagen, interacts differently with remineralizing materials compared to the inner portion of the dentin (healthier). This part of the lesion is more easily remineralized and has the ability to recover its mechanical properties in a shorter period than superficial layers [29]. It is assumed that some of the collagen fibrils within the most affected portion (underlying dentin) may have been irreversibly damaged during demineralization [24]. In the present study, this layer was soft and, consequently, it was impossible to perform the indentations. This phenomenon was described by Bresciani et al. (2010), which also evaluated remineralization of underlying dentin after 3 months. We suggest that for remineralizing this layer longer times of evaluation and the use substances that can modulate this process may be required.

In the inner portion (100µm), demineralization is less severe, and, in clinical situations, the activation of proteases may not happen in such an aggressive way, leaving the collagen less damaged [12].

When Activa or Clearfil were applied over the adhesive layer, MH in the inner layer (100µm) increased regardless of the adhesive. This happened also at 50µm, except to Clearfil/ Exp. 2. A previous study that evaluated Activa observed that it could promote the spread of ions through the adhesive layer when used with simplified adhesives [3]. Through X-ray powder diffraction analysis, Porenczuk et al. (2019) showed that Activa contains fluoride, silica, aluminum and calcium in its composition which can explain its results obtained in present work. It has been speculated that the dentin repair steps occur at different time intervals, with mineral capture being the first step, followed by the remineralization of the interfibrillar and intrafibrillar collagen [12, 27]. Several factors can interfere with mineral release, including the buffer capacity and the evaluation period [5].

The preparation of artificial caries affected dentin adopted was adequate once it is possible to see a CAD layer of about 120-150µm in the baseline specimens [11] (Image A). The confocal microscopy showed remineralization in many stages for all groups, except for DenTASTIC which has no ions in its composition (Image B-I). Some authors suggest that an effective remineralization can only be achieved if mineral released ions were mediated by NCP or its analogues, but the incorporation of these substances in a restorative protocol is not viable yet [22, 23]. NCP analogues are hard to dissolve in the adhesive blend and may interfere in the chemical union between calcium and functional monomers, jeopardizing the bonding effectiveness of adhesives [30]. On the other hand, CAD remineralization using only Ca-PO into experimental adhesives associated or not with a bioactive resin is achievable like showed in the present study. Thus, the adhesive composition simplification is an important factor to be considered while evaluating restorative treatments since the excess of components may interfere negatively on their quality over time.

5. CONCLUSION

The use of experimental adhesives incorporated with Ca-PO associated or not with a bioactive resin proved to be promising for using in caries affected dentin, according to

Minimally Invasive Dentistry approach. However, this alternative is not yet sufficient for a complete recovery of the underlying dentin microhardness near hybrid layer.

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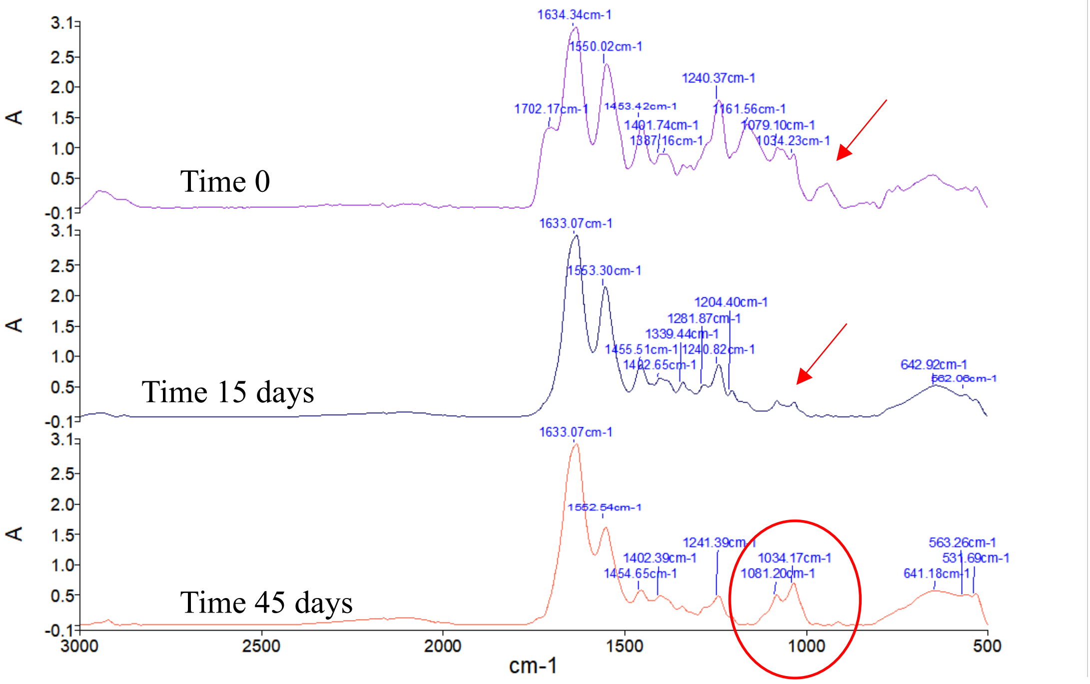

Figure 1 – Representative Fourier transform infrared spectroscopy (ATR-FTIR) peaks of totally demineralized dentin infiltrated with adhesives – Exp. 1 (Ca-PO (peaks of 1082-1030 cm^{-1}), orthophosphate optical absorption band (peaks of 1034 cm^{-1}) and orthophosphate stretching vibrations—v3 (peaks of 1081 cm^{-1})

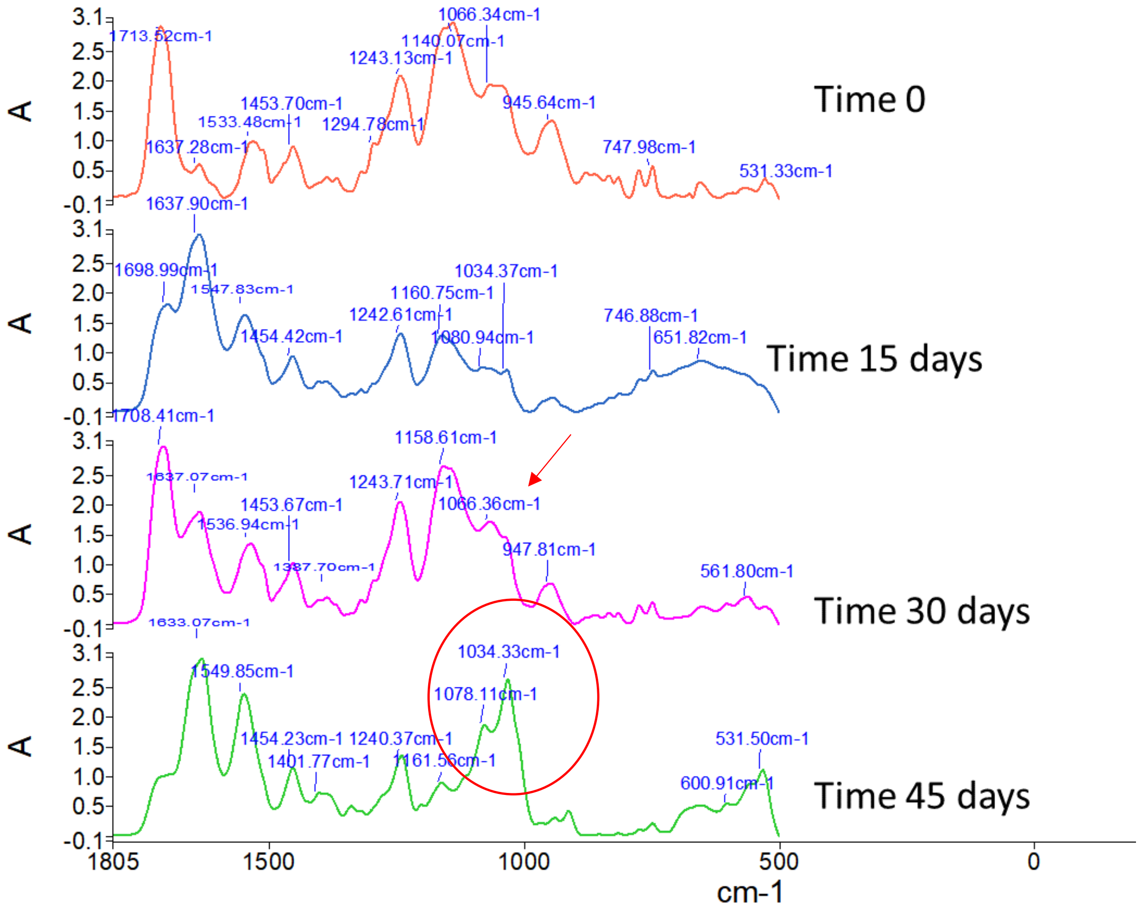

Figure 2 – Representative Fourier transform infrared spectroscopy (ATR-FTIR) peaks of totally demineralized dentin infiltrated with adhesives – Exp. 2 (CA-PO (peaks of 1082-1030 cm^{-1}), orthophosphate optical absorption band (peaks of 1032cm^{-1}), orthophosphate stretching vibrations—ν3 (peaks of 1078cm^{-1}), stretching of pyrophosphates (peaks of 646cm^{-1}).

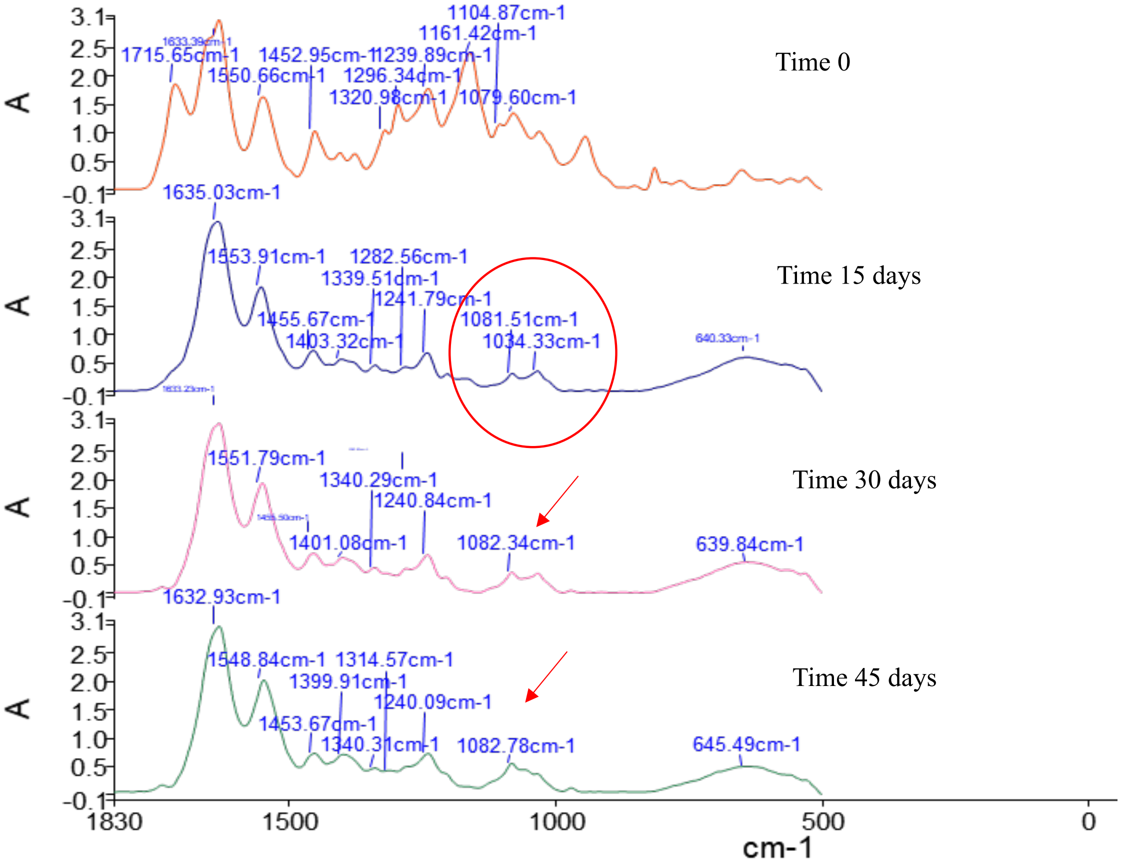

Figure 3 – Representative Fourier transform infrared spectroscopy (ATR-FTIR) peaks of totally demineralized dentin infiltrated with adhesives – Exp. 3 (orthophosphate stretching vibrations—ν3 (peaks of 1082cm^{-1}), orthophosphate optical absorption band (1034cm^{-1}) and pyrophosphates (peaks of 646cm^{-1}).

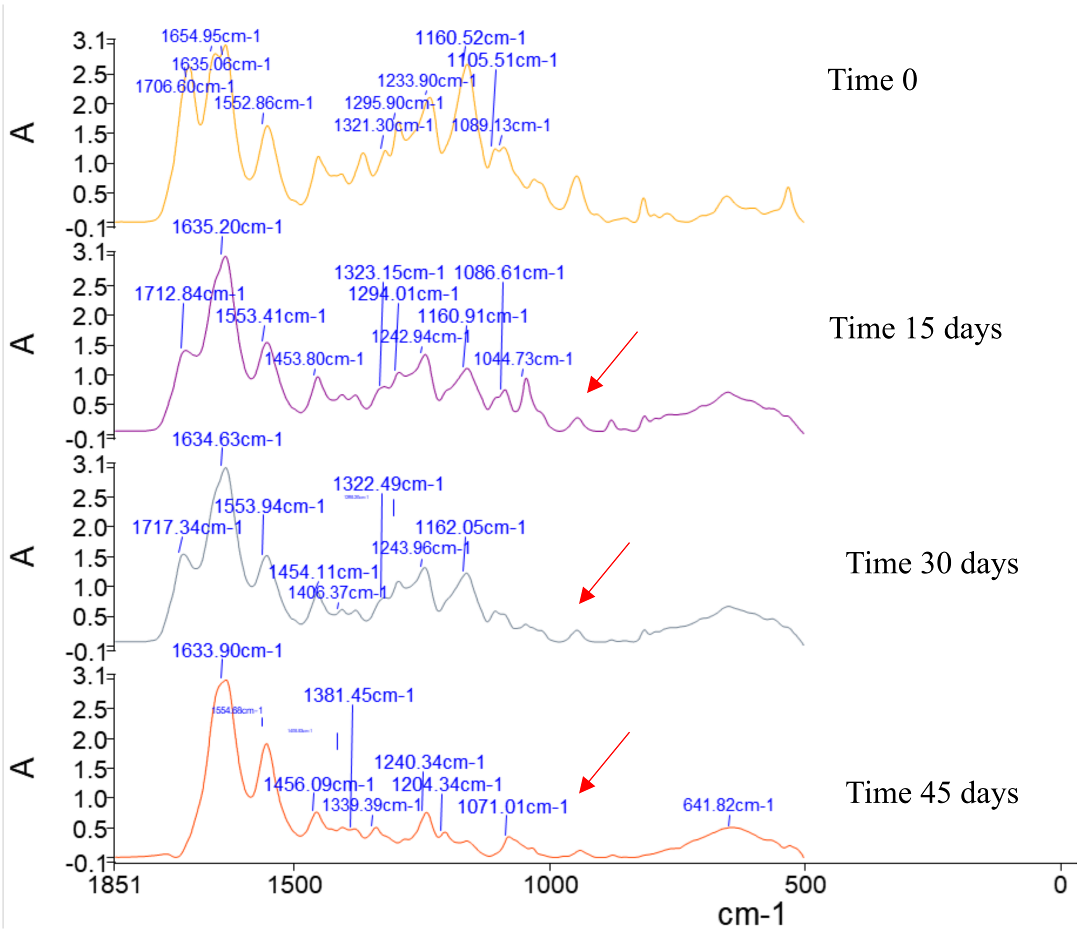

Figure 4 – Representative Fourier transform infrared spectroscopy (ATR-FTIR) peaks of totally demineralized dentin infiltrated with adhesives – DenTASTIC.

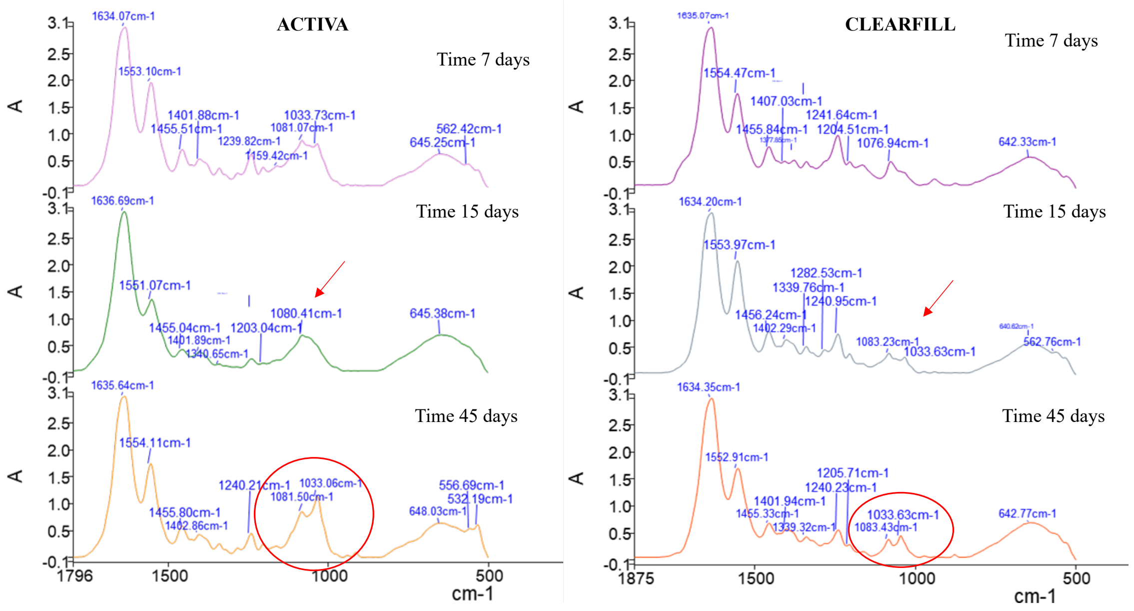

Figure 5 - Representative Fourier transform infrared spectroscopy (ATR-FTIR) peaks of totally demineralized dentin infiltrated with adhesives and restored with composites –Exp. 1/Activa and Exp. 1/Clearfil (orthophosphate optical absorption band (1034cm^{-1}), orthophosphate stretching vibrations—v3 (peaks of 1081cm^{-1}).

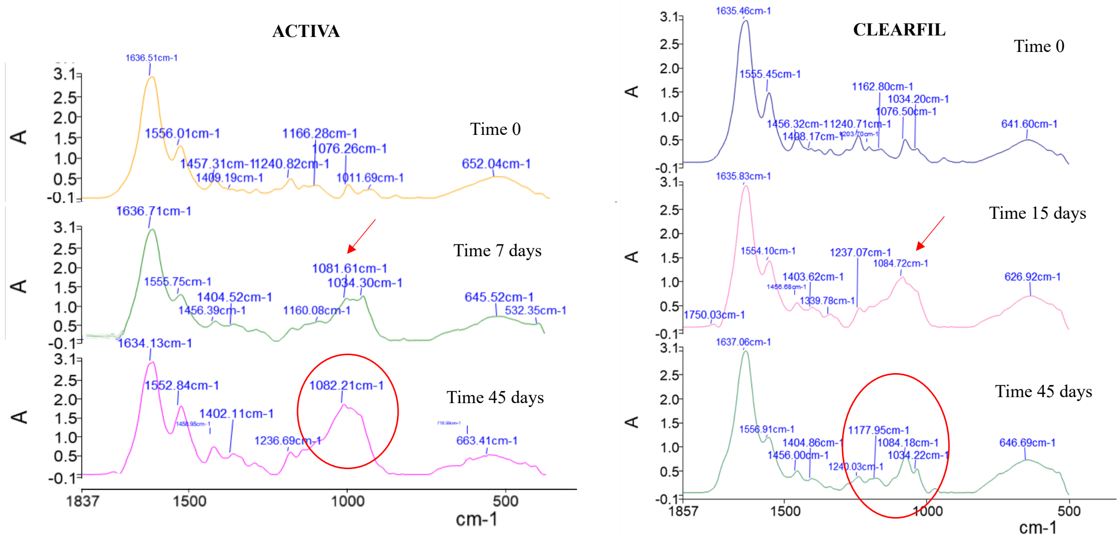

Figure 6 - Representative Fourier transform infrared spectroscopy (ATR-FTIR) peaks of totally demineralized dentin infiltrated with adhesives and restored with composites – Exp. 2/Activa and Exp. 2/Clearfil (orthophosphate stretching vibrations—v3 (peaks of 1082cm^{-1}), orthophosphate optical absorption band (1034cm^{-1}) and pyrophosphates (peaks of 646cm^{-1}).

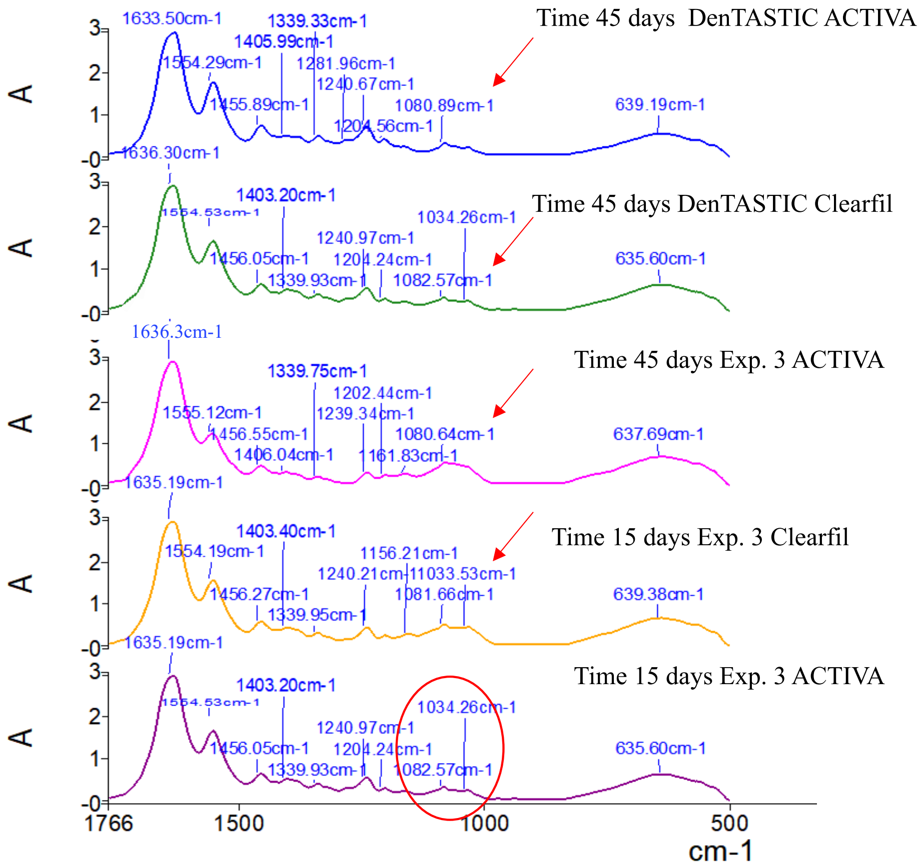

Figure 7 - Representative Fourier transform infrared spectroscopy (ATR-FTIR) peaks of totally demineralized dentin infiltrated with adhesives and restored with composites – Exp. 3/Activa, Exp. 3/ Clearfil, DenTASTIC/Activa and DenTASTIC/Clearfil (orthophosphate stretching vibrations—ν3 (peaks of 1081cm^{-1}), orthophosphate optical absorption band (1034cm^{-1}).

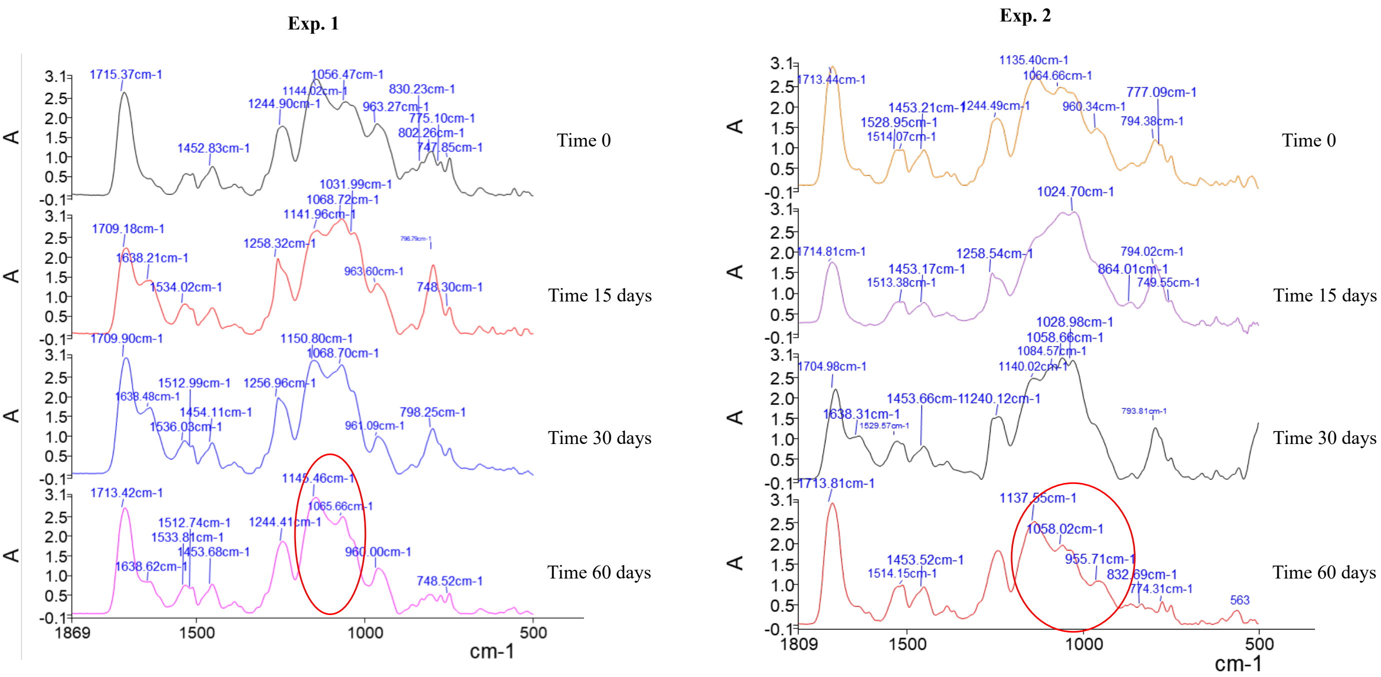

Figure 8 - Representative Fourier transform infrared spectroscopy (ATR-FTIR) peaks of discs of adhesives – Exp. 1 e Exp. 2 (Ca-PO (peaks of 1082-1030 cm^{-1}), orthophosphate optical absorption band (peaks of 1034 cm^{-1}) and orthophosphate stretching vibrations—ν3 (peaks of 1081 cm^{-1}) and representative ones for apatite formation (peaks of 1137, 955/960 cm^{-1} and 563 cm^{-1}).

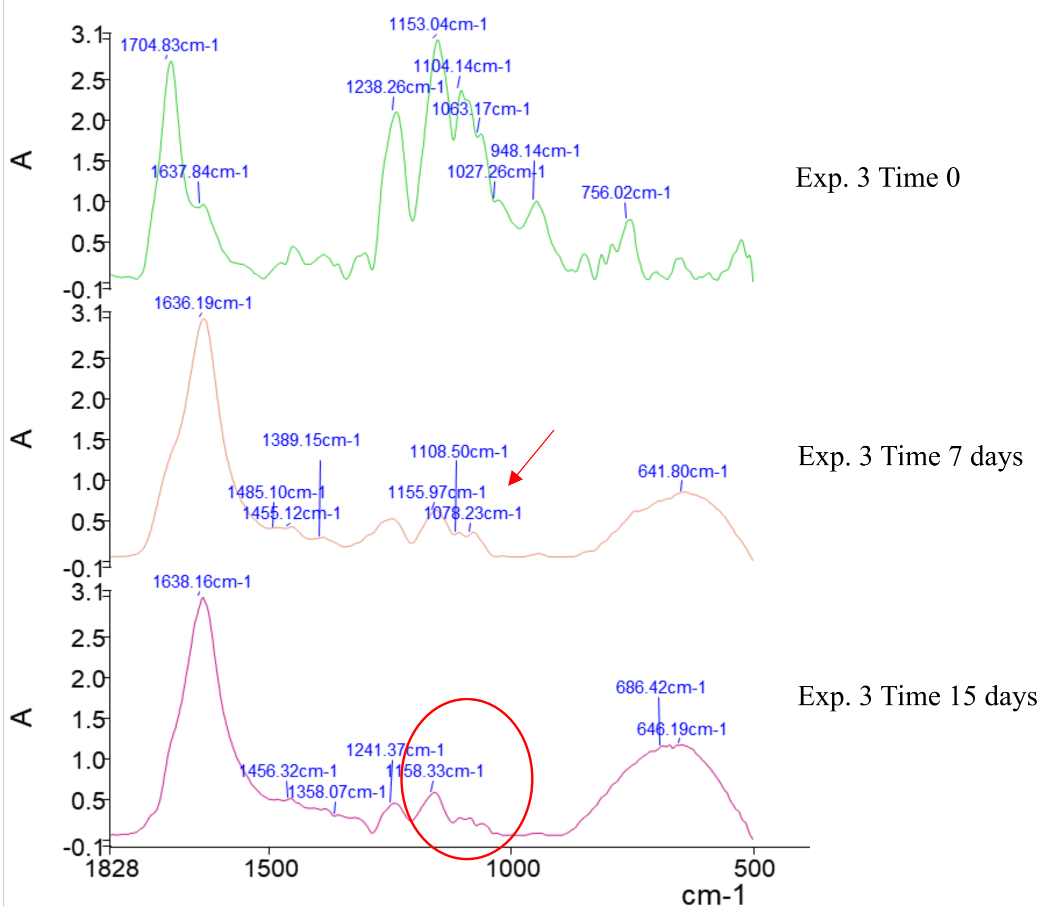

Figure 9 - Representative Fourier transform infrared spectroscopy (ATR-FTIR) peaks of discs of adhesives – Exp. 3 (Ca-PO (peaks of 1080-960 cm^{-1}).

Table 1 - Mean of Vickers Microhardness values (VMH) in underlying layer at baseline and 3 months.

	Activa			**Clearfill**		
	Baseline	**3m**	**p-Valor**†	**Baseline**	**3m**	**p-Valor**†
Adhesives						
Exp. 1	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	1,000	5.00 ± 1.00^{aA}	0.00 ± 0.00^{aB}	***<0,001***
Exp. 2	5.33 ± 1.21^{bA}	0.00 ± 0.00^{aB}	***<0,001***	5.17 ± 1.83^{aA}	0.00 ± 0.00^{aB}	***0,002***
Exp. 3	15.67 ± 5.75^{cA}	0.00 ± 0.00^{aB}	***<0,001***	20.83 ± 7.47^{bA}	0.00 ± 0.00^{aB}	***<0,001***
DenTASTIC	17.60 ± 4.72^{cA}	0.00 ± 0.00^{aB}	***<0,001***	14.17 ± 5.46^{aA}	0.00 ± 0.00^{aB}	***<0,001***
p-Valor*	***<0,001***	1,000		***<0,001***	1,000	

* ANOVA / Bonferroni test, different lowercase letters = difference between groups.

† Student's t test, Different capital letters = difference between assessment moments.

Data expressed as mean ± SD.

Table 2 - Mean of Vickers Microhardness values (VMH) in 50µm depth at baseline and 3 months.

	Activa			**Clearfil**		
	Baseline	**3m**	**p-Valor**	**Baseline**	**3m**	**p-Valor**
Adhesives						
Exp. 1	26.00±7.85^{aA}	96.00±14.14^{aB}	***<0,001***	20.67±4.55^{aA}	69.33±9.29^{aB}	***<0,001***
Exp. 2	20.17±4.12^{aA}	47.33±14.98^{bB}	***0,003***	17.83±3.19^{aA}	22.00±9.17^{bA}	0,327
Exp. 3	30.83±11.72^{bA}	39.75±18.23^{bB}	0,369	36.00±10.92^{bA}	108.67±36.53^{cB}	***0,002***
DenTASTIC	42.00±9.78^{bA}	54.00±16.99^{bB}	0,189	29.17±5.91^{bA}	113.00±26.29^{cB}	***<0,001***
p-Valor	***0,003***	***0,022***		***0,001***	***0,005***	

* ANOVA / Bonferroni test, different lowercase letters = difference between groups.

† Student's t test, Different capital letters = difference between assessment moments.

Data expressed as mean ± SD.

Table 3 - Mean of Vickers Microhardness values (VMH) in 100µm depth at baseline and 3 months.

	Activa			**Clearfil**		
	Baseline	**3m**	**p-Valor**	**Baseline**	**3m**	**p-Valor**
Adhesives						
Exp. 1	39.00±10.30^{aA}	234.00±70.26^{aB}	***<0,001***	35.83±2.48^{aA}	242.00±110.58^{aB}	***0,001***
Exp. 2	35.67±6.44^{aA}	294.83±77.09^{aB}	***<0,001***	30.50±4.14^{aA}	336.83±52.64^{aB}	***<0,001***
Exp. 3	64.83±7.88^{bA}	256.17±109.89^{aB}	***0,002***	52.17±8.98^{bA}	259.50±55.98^{aB}	***<0,001***
DenTASTIC	61.00±6.96^{bA}	251.83±91.21^{aB}	***<0,001***	46.67±5.09^{bA}	260.17±25.83^{aB}	***<0,001***
p-Valor	***<0,001***	0,684		***<0,001***	0,110	

* ANOVA / Bonferroni test, different lowercase letters = difference between groups.

† Student's t test, Different capital letters = difference between assessment moments.

Data expressed as mean ± SD.

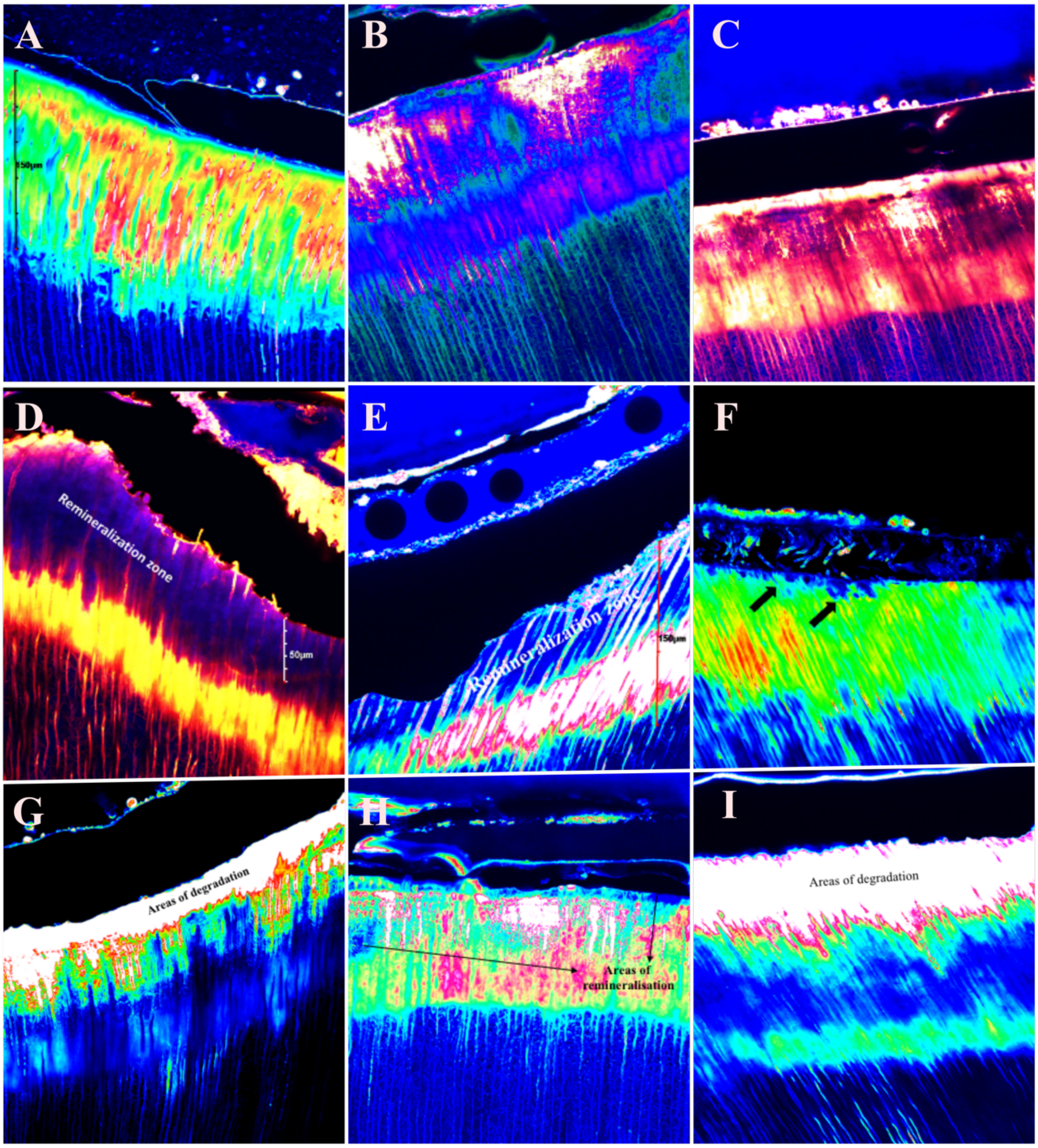

Figure 9 – Representative CLSM scans (63X 1.4NA oil immersion objective) of the caries affected dentin and resin interface. Letter **(A)** Baseline - Simulated caries lesion in dentine (120 - 150µm of highly), **(B)** Exp. 1/Activa, **(C)** Exp. 1/Clearfil **(D)** Exp. 2/Activa, **(E)** Exp. 2/Clearfil, **(F)** Exp. 3/Activa - areas of remineralizations (arrows), **(G)** Exp. 3/Clearfil, **(H)** DenTASTIC/Activa and **(I)** DenTASTIC/Clearfil.

Capítulo *2*

BOND STRENGTH OF BIOACTIVE MATERIALS IN CARIES AFFECTED DENTIN AFTER A CHEMO-MECHANICAL REMOVAL AGENT

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BOND STRENGTH OF BIOACTIVE MATERIALS IN CARIES AFFECTED DENTIN AFTER A CHEMO-MECHANICAL REMOVAL AGENT

ABSTRACT

Objectives. This study aimed to evaluate the use of bioactive adhesive materials in caries affected dentin (CAD) after a chemo-mechanical removal agent through analysis of resin-dentin bond strength and Scanning Electron Microscope (SEM) images.

Methods. Extracted human sound molars (n=40) and molars presenting occlusal carious lesions (n=40) were selected. The Papacárie Duo Gel® (Fórmula e Ação) was the method to remove all the soft tissue. Three experimental adhesives, A (Etch and rinse/ 3 steps; Exp. A), B (self-etch/ 2 steps; Exp. B), C (self-etch/ 1 step; Exp. C) and a commercial control DenTASTIC™UNO™ (Etch and rinse/ 2 steps; DenTASTIC) were applied and two restorative materials were used to the restorative protocol (ACTIVA BioACTIVE-RESTORATIVE or CLEARFIL MAJESTY Flow). Specimens restored (n = 5) were sectioned for microtensile bond strength testing and SEM images examination after submitted to 1 year aging.

Results. In sound dentin (SD), DenTASTIC/Activa obtained higher values of µTBS than the other groups at baseline and after 1 year. After 1 year, only Exp. C was able to maintain and improve adhesive performance when combined with Activa and Clearfil composites, respectively. When applied on CAD, bioactive adhesives (Exp. A, Exp. B and Exp. C) showed the best results of µTBS, regardless of the restorative materials ($p<0.01$).

Conclusions. The experimental adhesives incorporated with minerals were able to maintain or even improve the bond strength to CAD regardless of the adhesive strategy.

Clinical Significance. The biomaterial used to perform the restorative procedure in CAD can directly influence the long-term of bonding.

1. INTRODUCTION

Minimally Invasive Dentistry (MID) is a philosophy that encompasses a complete approach to caries care and management. MID describes an ultraconservative surgical handling of cavitated lesions and non-invasive preventive measures for incipient lesions [1]. With the evolution of adhesive materials and caries removal techniques, macro-retentive cavities have been replaced by less invasive ones [14]. Conventional caries removal involves the use of drills which presents, as main disadvantage, the removal of softened but uninfected dentin, resulting in excessive loss of recoverable tissue[15]. On the other hand, a chemical-mechanical method for caries removal, that combines atraumatic characteristics, bactericidal and bacteriostatic action, may overcome this deficiency since it better preserves the tissue that still able to be reminezalized, besides being more comfortable for the patient [16].

A product based on papain (Papacárie - Fórmula e Ação, São Paulo, Brazil), a protease with broad proteolytic activity extracted from the tree *Carica Papaya* [17], was launched in Brazil for the use as a chemical-mechanical method. Papacárie eliminates both local anesthesia and the use of drills while conserving sound tissue and has proved to be easy to handle, simple, cheap and safety [16, 17].

The maintenance of caries-affected dentin (CAD) is based on the assumption that dentin is a vital tissue and perfectly capable of responding to stimuli that promote its remineralization[18]. However, the presence of CAD in the adhesive interface is still a challenge for restorative dentistry [19]. The changes caused by caries process, such as loss of mineral content, increased porosity of the intertubular dentin, dissolution of apatite crystals and degradation of the unprotected collagen can negatively impact the performance of adhesives in CAD [17, 19].

Combined with a less invasive preparation, the use of restorative materials that may induce a recovery of CAD is a crucial factor for the longevity of the restorations[8, 9]. Materials that present bioactive potential, especially those that promote the diffusion of Ca and PO ions, have been formulated to use with this technique. The process of remineralization occurs when simplified adhesives are used and, through their natural permeability, allow the passage of ions [3, 10].

This study aimed to evaluate the use of bioactive adhesive materials after removing CAD with Papacárie through analysis of resin-dentin bond strength and Scanning Electron Microscope (SEM) images. The null hypothesis tested is that CAD would not have its bond strength recovered after the use of Papacárie associated with a restorative protocol using bioactive adhesive materials.

2. MATERIALS AND METHODS

2.1 Dentin bonding procedures

From a pool of extracted elements stored for less than 6 months in 1% aqueous chloramine solution under refrigeration were selected sound molars (n=40) and with occlusal carious lesions (n=40). This study complied with all ethical aspects of donation and use of biological human tissue and has been approved by the local university ethical committee (protocol 3.964.912). For sound molars, each tooth was horizontally cut using a slow-speed water-cooled diamond saw (Isomet; Buehler, Lake Bluff, USA) in order to remove occlusal enamel crown and the roots. Exposed dentin surfaces were grounded using 600-grit SiC abrasive papers under water irrigation for 30s to create a standardized smear-layer. For carious teeth, Papacárie Duo Gel® (PapaCárie Duo Gel, Fórmula e Ação, São Paulo, Brazil) was used following the manufacturer’s instructions. In short, the gel was be applied to the cavity for approximately 30-40 seconds and then hand instruments were used to remove softened tissue. This procedure was repeated until all the soft tissue had been removed. The vitrous aspect of the cavity appeared when the cavity was free from caries, then the cavity was washed with water for 20 seconds and proceed with a restorative procedure. For the sound group, only the restorative procedure was performed (table 1).

2.2 Microtensile bond strength (μTBS)

Resin-bonded specimens were sectioned in resin-dentin sticks (0.9 mm x 0.9 mm) for microtensile bond strength testing. Sticks from the most peripheral area presenting residual enamel were excluded. After this procedure, half of the sticks per tooth was tested after 24h in Artificial Saliva (AS) (Dulbecco’s Phosphate Buffered Saline (DPBS, Sigma-Aldrich, St. Louis, USA). Whereas the other half was submitted to AS immersion (aging) for 1 year (replaced with fresh one every 1 month). The sticks were attached to a jig with a cyanoacrylate cement (Super Bonder gel, Loctite, Henkel Corp., Rocky Hill, USA) and tested to tensile failure in a universal testing machine (EMIC, Sao Jose do Rio Preto, Brazil) with a 500N load cell and 0.5 mm/min crosshead speed. The exact cross-sectional area of each tested stick was measured with a digital caliper. The μTBS results were calculated and expressed in MPa. The μTBS values obtained from the sticks of the same resin-bonded tooth were averaged and the mean bond strength of each individual tooth was used as one unit for statistical analysis. Five resin bonded teeth (n =5) were evaluated for each sub-group.

After the µTBS, the fractographic analysis of each fractured stick was determined using a stereoscopic magnifying glass (Olympus SZ 40-50; Tokyo, Japan) at 100x magnification. The fractures were classified as adhesive, mixed, and cohesive in composite or in dentin.

2.3 Analysis of the adhesive interface under SEM

Two resin-dentin sticks were selected from each bonded tooth and storage condition during the cutting procedure (n=10). These sticks were immersed in 50wt% ammoniacal silver nitrate ($AgNO_3$ (aq)) solution in total darkness for 24h [20]. Subsequently, the specimens were rinsed with distilled water to remove the excess of silver nitrate and immersed in photo-developing solution for 8h under light to reduce silver ions into metallic silver grains. The silver-impregnated sticks were embedded in epoxy resin and polished using 600, 1200, 2000 grit SiC papers and diamond pastes (Buehler, Lake Bluff, IL, USA) with 3, 1, and 0.25 µm particle sizes, and ultrasonically cleaned of 15min after each abrasive/polishing step. Specimens were finally air-dried, dehydrated overnight in silica gel under vacuum, coated with carbon and analyzed using SEM (Inspect 50, FEI, Amsterdam, Netherlands) and observed in backscattered electron mode at 20 kV.

2.4 Statistical Analysis

The data were tabulated in Microsoft Excel and exported to the Statistical Packcage for the Social Sciences software, in which the analyzes were performed adopting a 95% confidence. After evaluation by the Shapiro-Wilk normality test, data were expressed as means and standard deviations using Student's t test (evaluation moments) or ANOVA-1-way / Bonferroni (groups of study). The ANOVA-3-way / Bonferroni test was used to assess the interaction between these three factors (time, adhesive and restorative materials). The percentage frequencies of fracture patterns were analyzed.

3. RESULTS

3.1 Microtensile bond strength (µTBS)

In SD, the specimens restored with DenTASTIC/Activa obtained higher values of µTBS than the other groups using this composite at baseline and after 1 year. For the specimens restored with Clearfil composite, experimental adhesives A and B showed higher µTBS than the other groups using this composite. However, after 1 year aging, only Exp. C was able to maintain and improve adhesive performance when combined with Activa and Clearfil composites, respectively (table 2).

When Activa's composite was used in CAD, Exp. A and Exp. B showed the best values of µTBS regardless of evaluation time, while for Clearfil composite there was no significant difference ($p>0.05$) comparing adhesives system nor time evaluation. After 1 year aging when the Activa's composite was used, Exp. A maintained and Exp. B increased the values of µTBS, respectively. Considering Clearfil composite, Exp. A achieved better results than the other adhesives (table 3). Ultimately, when applied on CAD, bioactive adhesives (Exp. A, Exp. B and Exp. C) showed the best results of µTBS, regardless of the restorative materials ($p<0.01$) (table 4).

In SD, the fractographic analysis showed predominance of adhesive failures for all groups regardless of evaluation time. These results were observed also for CAD, but only at baseline. After 1-year, cohesive fractures in dentin were the most prevalent in CAD groups (table 5 and 6).

3.2 Analysis of the adhesive interface under SEM

All analyses were performed after 1 year aging. Sound dentin showed more preserved interfaces regardless composite. The interfaces produced with Exp. A/Activa or Exp. B/Activa and CAD achieved partial preservation (Fig. 1D and 1F), while those with DenTASTIC/Activa or Exp. C/Activa showed more GAPs (Fig. 1A and 1H). Figure 2H depicts absence of tubules on CAD surface probable indicating some extent of degradation.

4. DISCUSSION

Minimally invasive dentistry highlights the importance of diagnosis, prevention and rehabilitation of the tissues involved, promoting maximum preservation of substrates, particularly of the CAD [4, 21]. The bond strength of this tissue to dentin is affected by the hydrolysis of inorganic components and by the enzymatic degradation promoted by matrix metalloproteinases [1, 4]. Materials that promote mineral recovery of demineralized dentin can be an alternative to prevent or minimize these damages and improve bonding durability [10, 22]. In the present study, the use of experimental bioactive adhesives after removal of caries with Papacárie promoted the preservation of adhesive bond strength after 1 year of aging in artificial saliva, which obligate us to reject the null hypothesis of this study.

There are various methods for carious tissue removal, including hand excavators, tungsten carbide burs, ceramic burs, air abrasion, sonoabrasion, chemomechanical carious tissue removal, polymer burs, and lasers [23]. However, not only one method, but association among some of them is recommended to remove the softened dentin and leave the dentin uninfected, avoiding resulting in excessive loss of tissue that can be remineralized [15, 23]. Besides, the clinical experience of the operator is essential to determine the amount of tissue that must be removed[17]. The chemical-mechanical removal of caries can be employed without the use of local anesthesia or drills, preserving dental tissues and might reduce pain and discomfort during treatment and could thus positively affect dental anxiety, especially when treating children [23, 24].

Papacárie is a papain-based gel developed and intended for the chemical-mechanical removal of caries. This product contains the enzyme papain which acts exclusively on the breakdown of partially degraded collagen, without damaging intact collagen fibrils [16]. Papacárie's selective interaction is due to the lack of an α-1-antitrypsin antiprotease, which inhibits its action on sound collagen-based tissues [25]. The use of Papacárie results in a residual dentin with microbiological characteristics similar to those found after removal by the traditional method [25-28]. Another important advantage is that its technique does not produce aerosols, which may be relevant for clinical use in a pandemic context.

The remineralization process involves the release of fluoride, calcium and phosphate ions that chemically interact with the constituents of the demineralized tissues, increasing the bond strenght [7, 29]. In this study, the evaluation of the bond strength of experimental adhesives with bioactive potential revealed that the Exp. C adhesive was capable to maintain and even improve its bond strength to sound dentin after aging. Exp. C adhesive contains 10-MDP, an acid monomer formed by a methacrylate group and a functional ester group of hydrophilic phosphoric acid that can ionically bond to the hydroxyapatite calcium. Among the different functional monomers, 10-MDP has the greatest potential for chemical binding to calcium [4, 30]. The greater mineral availability in the sound dentin bonded to Exp. C, may have favored the bonding of the monomer and, consequently, the adhesive bond strength, regardless of the restorative material [4, 19].

In the evaluation of bond strength to CAD, Exp. A and Exp. B adhesives, when combined with Activa composite obtained the highest μTBS values. A previous study demonstrated a change in the surface topography due to a change in the smear layer when products based on the content of NaOCl are used [31, 32]. Self-etching adhesives tend to be more sensitive to smear layer characteristics than conventional adhesives [19], however, this was not observed in the present study. The papain enzyme, that is selective and digests only degraded collagen, seems to be crucial in the preservation of organic components, which may explain the μTBS results obtained by the experimental adhesive in the self-etching strategy (Exp. B) (table 3).

In an overview, when applied in CAD, the experimental bioactive adhesives were statistically superior to control ($p<0.001$) (table 4). Supposedly, experimental bioactive adhesives in direct contact with the substrate released ions favoring mineral recomposition and, consequently, improving the resin-dentin bond. These results highlight the importance of using bioactive materials in the adhesive strategy, since CAD is the most frequent substrate for performing restorative procedures in clinical practice [19, 33]. Changes on dentin, caused by the caries process, such as loss of mineral content, increased porosity and degradation of collagen, can negatively impact in the adhesives performance, resulting in poor hybridization and consequently loss of adhesion [18, 19]. However, this deficiencies on dentin were not sufficient to decrease the bond strength of the mineral contain adhesives, which corroborates with previous studies that evaluated bond strength to CAD [24, 31, 34].

Regarding the restorative material used in the adhesive protocol, Clearfil showed better performance than Activa ($p <0.05$) (table 4). Activa is a glass ionomer cement modified by resin enriched with bioglass and incorporated into a polymeric resin with the property of releasing calcium, phosphate and fluorite [4, 5]. Originally, the instructions for use of Activa recommended that it should be applied in a self-adhesive mode. However, due to the low values of bond strength, the company adapted the instructions recommending applying the material after using adhesive systems [4, 35]. Although the manufacturer's new recommendations for Activa have been followed in the present study, this material still showed lowest values of μTBS which compromised its ability to preserve the resin-dentin interface.

The fractographic analysis in SD corroborates previous studies that pointed out the adhesive layer as the most fragile region of the resin-dentin bonding interface once most of the fractures occurred within it [31, 34]. When assessing CAD fracture patterns at baseline, the most prevalent fractures were mixed and adhesive. On the other hand, after 1 year, the most frequent ones were cohesive in dentin. CAD has lower mechanical properties and less cohesive strenght than SD once they are dependent of the intertubular dentin mineral content, which was compromised during the caries and aging process. Thus, one of the weakest links in the resin-CAD interface is related to the cohesive strenght of the dentin itself (Figure 2H) [31, 36, 37].

In SEM images of the groups restored with Activa and Clearfil composites, it is possible to observe that sound dentin is totally covered by the adhesive layer, while in CAD it is possible to observe an irregular dentin layer on the surface that suggests ultrastructural changes within this tissue. These results were also found in other studies that observed that CAD promotes a distinct demineralized zone when compared to sound dentin after aging. Despite these morphological changes, Exp. A and Exp. B adhesives were able to maintain the integrity of the adhesive interface (Figure 1D and 1F), which is in agreement with the results of bond strength. The presence of this degraded and irregular layer of dentin aforementioned has been already this described as a consequence of alterations in the collagen structure and partial demineralization associated with the carious process [18, 31, 38].

Despite being a challenging tissue to adhere to because of degraded collagen and and loss of minerals, CAD is a substrate that can recover its mechanical properties after remineralization. The use of chemo-mechanical methods of caries removal combined with bioactive materials seems to be crucial for long term preservation of adhesive interfaces.

5. CONCLUSION

The experimental adhesives incorporated with minerals were able to maintain or even improve the bond strength to CAD regardless of the adhesive strategy. The present study highlights the need of randomized clinicals trials on this promising subject.

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Table 1: Restorative procedure.

Material	**Composition**		**Protocol**
DenTASTIC™UNO™ (DenTASTIC) **(Etch and rinse – 2 step)**	under license from patent		Apply phosphoric acid etch to the cavity prep for 15 seconds Rinse and leave dentin moist Apply two coats of DenTASTIC UNO to the wet dentin surface (gentle stream of air between the coats) LED-cure* for 20 seconds
Experimental A (Exp. A) **(Etch and rinse – 3 step)**	***Primer*** UDMA, TEGDMA, HEMA, Acetone, CQ, EDAB, 1% tri-calcium phosphate.	***Bond*** UDMA, TEGDMA, HEMA, CQ, EDAB, 2% halosita, 1% tri-cálcio phosphate.	Apply phosphoric acid etch on the cavity prep for 15 seconds dentin. Rinse and leave dentin moist Apply primer (10 second) (gentle stream of air between the coats) Apply for 10 second LED-cure* for 20 seconds.
Experimental B (Exp. B) **(self-etch – 2 step)**	***Primer*** di-HEMA phosphate, Ethanol, Water, TEGDMA, UDMA, CQ, EDAB, 2% haloisite and 1% tri-calcium phosphate.	***Bond*** UDMA, TEGDMA, HEMA, CQ, EDAB, 2% haloisite, 1% tri-calcium phosphate.	Apply primer (two coats – 10 second each) (gentle stream of air between the coats) Apply bond for 10 second. LED-cure* for 20 seconds.
Experimental C (Exp. C) **(self-etch – 1 step)**	UDMA, di-HEMA phosphate, TEGDMA, 10-MDP, Beta-Tricalcium Phosphate, Ethanol, water, CQ, EDAB, 2% haloisite, 1% tri-calcium phosphate		Apply adhesive with (two coats – 10 second each) (gentle stream of air between the coats) LED-cure* for 20 seconds.
ACTIVA™ BioACTIVE – RESTORATIVE™ (Activa)	Blend of diurethane and methacrylates with modified polyacrylic acid (44.6%); reactive glass filler (21.8 wt%); inorganic filler (56 wt%), patented rubberized resin (Embrace) and water.		Place a mix tip on the Activa syringe. Dispense Activa in increments of up to 4mm, keeping mix tip submerged in the material. Light-cure* for 20 seconds between each layer.
CLEARFIL MAJESTY FLOW (Clearfill)	TEGDMA, Silanated barium glass filler, Silanated colloidal silica, CQ and EDAB.		Place the Clearfil into the cavity in increments of up to 2mm. Light-cure* for 20 seconds between each layer.

Urethane dimethacrylate (UDMA), Tri-ethyl glycol dimethacrylate (TEGDMA), Hydroxy-ethyl methacrylate (HEMA), camphorquinone (CQ), ethyl-dimethylamino-benzoate (EDAB), 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) *(1500 mW/cm2; Radii Plus, SDI Limited, Victoria, Australia).

Table 2: Microtensile bond strength results (MPa) of sound dentin.

Composite	**Activa**			**Clearfil**		
	Baseline	**1 year**	**p-Valor†**	**Baseline**	**1 year**	**p-Valor†**
Adhesive						
DenTASTIC	33.35±4.45^{aA}	19.59±3.72^{aB}	***0,001***	28.98±7.62^{aA}	21.96±9.61^{aA}	0,236
Exp. A	19.79±4.53^{bA}	13.96±3.20^{bB}	***0,047***	34.62±9.13^{bA}	26.26±7.41^{bA}	0,150
Exp. B	22.46±5.46^{bA}	12.12±1.87^{bB}	***0,004***	35.25±11.44^{bA}	34.66±3.84^{bA}	0,916
Exp. C	17.89±5.81^{bA}	14.08±4.99^{bA}	0,299	14.25±3.24^{cA}	20.64±2.46^{aB}	***0,020***
p-Valor*	***0,001***	***0,017***		***0,017***	***0,025***	

* ANOVA / Bonferroni test, different lowercase letters = difference between groups.

† Student's t test, Different capital letters = difference between assessment times.

Data expressed as mean ± SD.

Table 3: Microtensile bond strength results (MPa) of caries affected dentin.

Composite	**Activa**			**Clearfil**		
	Baseline	**1 year**	**p-Valor†**	**Baseline**	**1 year**	**p-Valor†**
Adhesive						
DenTASTIC	20.52±5.11^{bA}	33.41±8.71^{bB}	***0,021***	29.70±6.26^{aA}	38.03±17.59^{aA}	0,348
Exp. A	39.40±18.29^{aA}	47.68±11.17^{aA}	0,412	21.67±3.74^{aA}	51.63±11.45^{aB}	***0,002***
Exp. B	42.84±6.00^{aA}	58.86±7.48^{aB}	***0,006***	42.96±18.36^{aA}	57.93±19.36^{aA}	0,245
Exp. C	20.85±11.96^{bA}	28.33±4.24^{bA}	0,276	39.84±21.95^{aA}	54.43±9.62^{aA}	0,210
p-Valor*	***0,015***	***<0,001***		0,131	0,162	

* ANOVA / Bonferroni test, different lowercase letters = difference between groups.

† Student's t test, Different capital letters = difference between assessment times.

Data expressed as mean ± SD.

Table 4: ANOVA-3-way/Bonferroni Analysis

	F	**p-Valor**	**Post-Test**
Sound			
Composite	40,97	***<0,001***	Activa < Clearfil
Adhesive	7,90	***<0,001***	DenTASTIC = Exp. B = Exp. A > Exp. C
Time	18,74	***<0,001***	Baseline > 1 year
Composite * Adhesive * Time	0,93	0,449	
Caries Affected Dentin			
Composite	10,46	***0,002***	Activa < Clearfil

Adhesive	7,42	***<0,001***	DenTASTIC < Exp. B = Exp. A = Exp. C
Time	30,14	***<0,001***	Baseline < 1 year
Composite * Adhesive * Time	0,77	0,545	

*p<0,05

Table 5: The percentage frequencies of fracture patterns in sound dentin.

	Activa		Clearfil	
	Baseline	**1 year**	**Baseline**	**1 year**
DenTASTIC				
A	69,0%	76,2%	52,8%	62,3%
M	10,3%	14,3%	8,3%	3,8%
D	6,9%	4,8%	22,2%	9,4%
R	13,8%	4,8%	16,7%	24,5%
Exp. A				
A	47,2%	69,1%	51,2%	73,9%
M	30,6%	23,6%	9,8%	10,9%
D	2,8%	1,8%	12,2%	10,9%
R	19,4%	5,5%	26,8%	4,3%
Exp. B				
A	75,0%	73,9%	47,8%	53,4%
M	25,0%	0,0%	10,9%	6,9%
D	0,0%	13,0%	10,9%	10,3%
R	0,0%	13,0%	30,4%	29,3%
Exp. C				
A	60,0%	66,0%	80,0%	98,1%
M	25,7%	6,4%	16,0%	0,0%
D	0,0%	12,8%	0,0%	0,0%
R	14,3%	14,9%	4,0%	1,9%

A: Adhesive, M: Mixed, D: Dentin, R: Resin

Table 6: The percentage frequencies of fracture patterns in caries affected dentin.

	Activa		Clearfil	
	Baseline	**1 year**	**Baseline**	**1 year**
DenTASTIC				
A	35,3%	2,4%	19,5%	4,2%
M	31,4%	61,0%	29,3%	25,0%
D	25,5%	26,8%	31,7%	70,8%

R	7,8%	9,8%	19,5%	0,0%
Exp. A				
A	40,0%	2,6%	45,7%	4,5%
M	22,5%	39,5%	28,6%	22,7%
D	25,0%	57,9%	20,0%	72,7%
R	12,5%	0,0%	5,7%	0,0%
Exp. B				
A	44,8%	21,7%	50,0%	0,0%
M	27,6%	13,0%	23,3%	13,0%
D	10,3%	65,2%	20,0%	87,0%
R	17,2%	0,0%	6,7%	0,0%
Exp. C				
A	51,6%	8,0%	42,4%	5,3%
M	25,8%	48,0%	24,2%	21,1%
D	16,1%	44,0%	3,0%	73,7%
R	6,5%	0,0%	30,3%	0,0%

A: Adhesive, M: Mixed, D: Dentin, R: Resin

ACTIVA

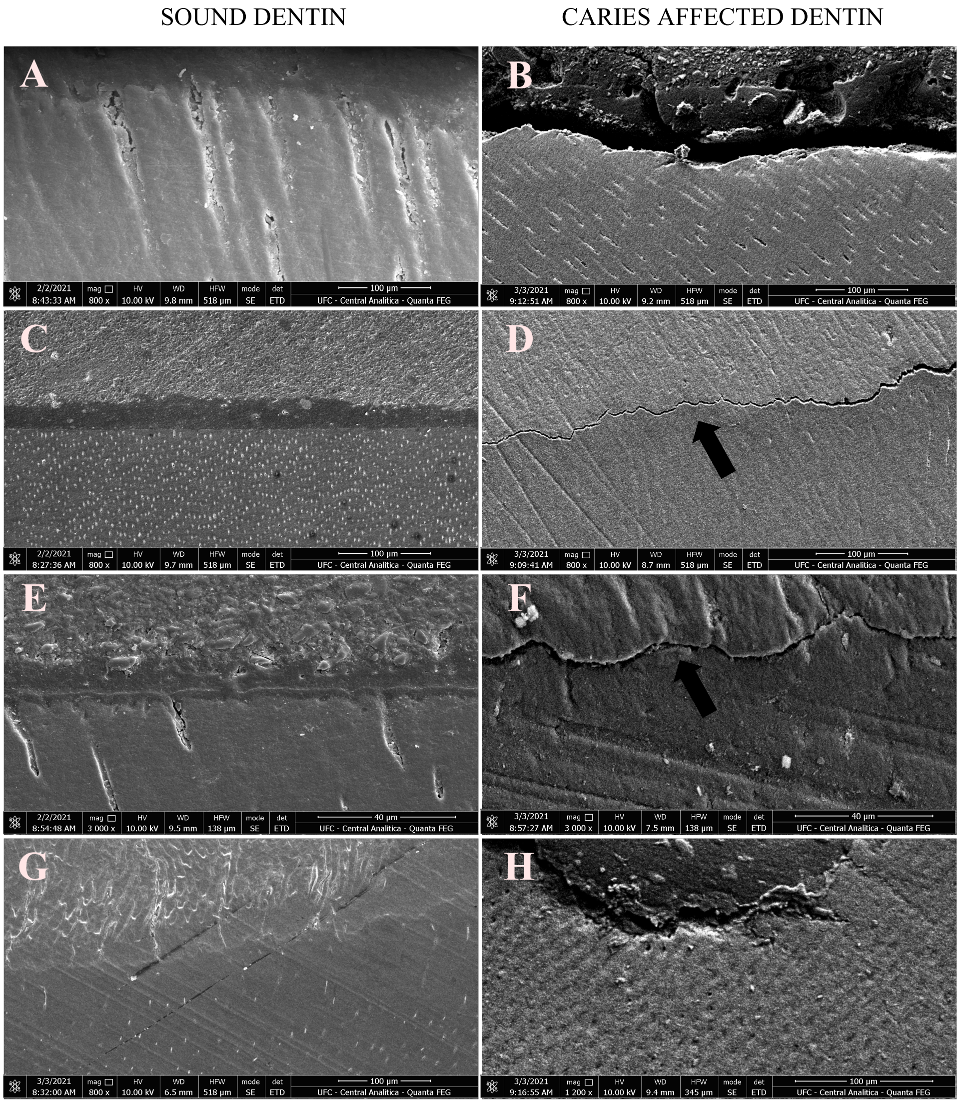

Figure 1: SEM micrographs of resin-dentin interfaces after 1 year. In sound dentin, most of the surface is covered by an adhesive layer. In caries affected dentin, the microstructural aspect of dentin is clearly modified. The black arrow showed partial preservation of interface. (**A:** DenTASTIC/Activa in SD; **B:** DenTASTIC/Activa in CAD; **C:** Exp. A/Activa in SD; **D:** Exp. A/Activa in CAD; **E:** Exp. B/Activa in SD; **F:** Exp. B/Activa in CAD; **G:** Exp. C/Activa in SD; **H:** Exp. C/Activa in CAD).

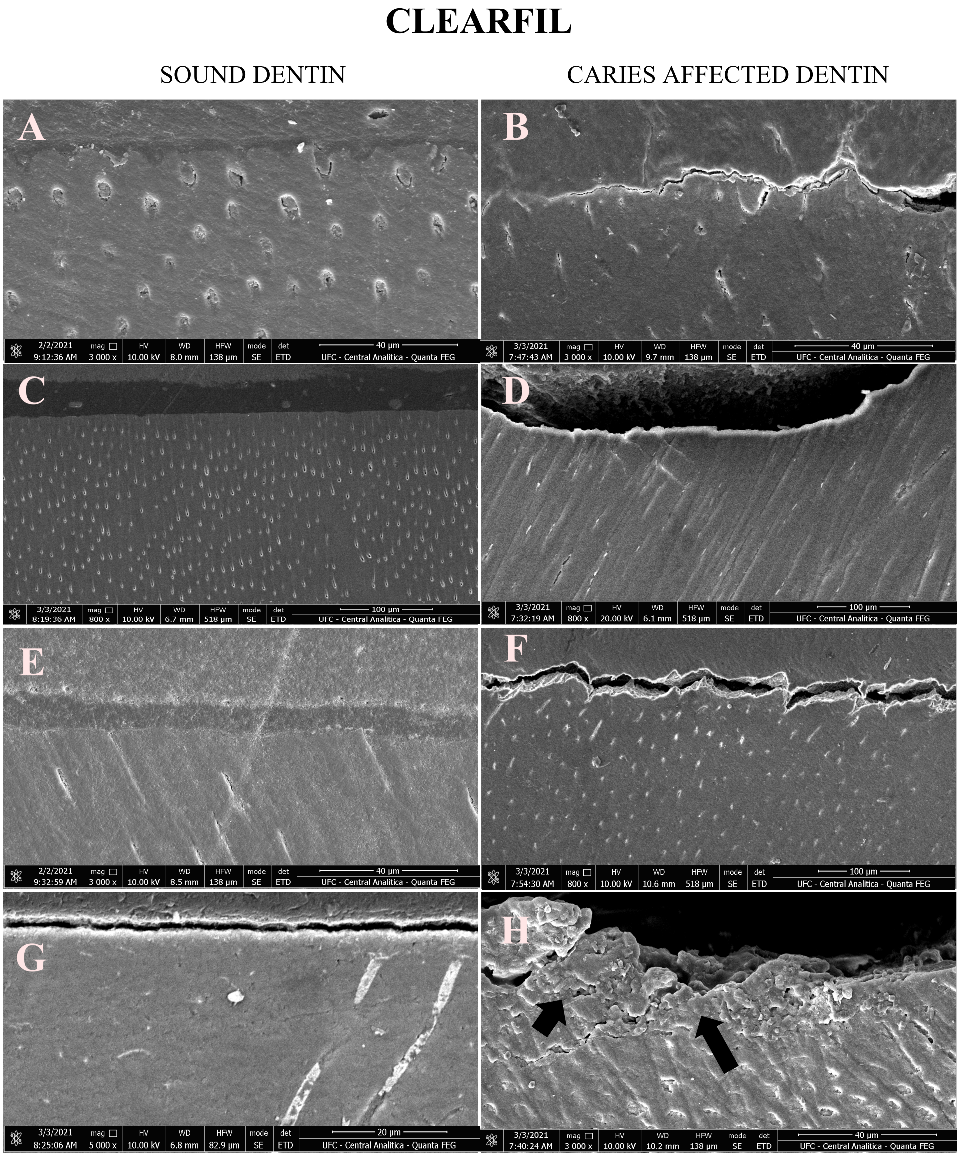

Figure 2: SEM micrographs of resin-dentin interfaces after 1 year. In sound dentin, most of the surface is covered by an adhesive layer although some dentin is exposed. The black arrow depicts absence of tubules on CAD surface probable indicating some extent of degradation. (**A:** DenTASTIC/Clearfil in SD; **B:** DenTASTIC/Clearfil in CAD; **C:** Exp. A/Clearfil in SD; **D:** Exp. A/Clearfil in CAD; **E:** Exp. B/Clearfil in SD; **F:** Exp. B/Clearfil in CAD; **G:** Exp. C/Clearfil in SD; **H:** Exp. C/Clearfil in CAD).

Conclusão Geral

4. CONCLUSÃO GERAL

De acordo com os resultados obtidos no presente estudo, é possível concluir que:

- O uso de adesivos experimentais incorporados com Ca-PO, associados ou não a uma resina bioativa, mostrou-se promissor para uso em dentina afetada por cárie. No entanto, esta alternativa ainda não é suficiente para a recuperação completa da microdureza da dentina subjacente à camada híbrida.
- Os adesivos experimentais incorporados com minerais foram capazes de manter ou até mesmo melhorar a resistência de união da dentina afetada por cárie independente da estratégia adesiva.

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Apêndice

APÊNDICE - TERMO DE DOAÇÃO DE DENTES

Pelo presente instrumento que atende às exigências legais, o Sr.(a): Eliane Ferreira Sampaio, após ter tomado conhecimento do protocolo da pesquisa “REMINERALIZAÇÃO DE DENTINA AFETADA POR CÁRIE VIA MATERIAIS RESTAURADORES BIOATIVOS” que tem como objetivo de formular e avaliar as propriedades físico-químicas, resistência de união e potencial remineralizador de adesivos experimentais incorporados com nanotúbulos de haloisita associado a uma resina bioativa em uma dentina afetada por cárie. DOA a cirurgiã-dentista Maria Elisa Martins Moura 100 dentes (terceiros molares), declarando, sob as penas da lei, que os dentes objeto da presente doação foram extraídos por indicação terapêutica, cujos históricos circunstanciados fazem parte dos prontuários dos pacientes de quem se originam.

Data: ___/___/___
Assinatura: ____
RG:____

Anexo

ANEXO – APROVAÇÃO DO COMITÊ DE ÉTICA EM PESQUISA

UFC - UNIVERSIDADE FEDERAL DO CEARÁ /

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: REMINERALIZAÇÃO DE DENTINA AFETADA POR CÁRIE VIA MATERIAIS RESTAURADORES BIOATIVOS

Pesquisador: Maria Elisa Martins Moura

Área Temática:

Versão: 2

CAAE: 27901020.8.0000.5054

Instituição Proponente: Departamento de Clínica Odontológica

Patrocinador Principal: CONSELHO NACIONAL DE DESENVOLVIMENTO CIENTIFICO E TECNOLOGICO-CNPQ

DADOS DO PARECER

Número do Parecer: 3.964.912

Apresentação do Projeto:

Na nova era da odontologia adesiva a união dos materiais restauradores ao substrato dentário está sendo investigada a nível nanométrico. Somado a esse conceito, a odontologia minimamente invasiva enfatiza suas práticas na remoção parcial de dentina cariada e, dessa forma, preserva a dentina que foi apenas afetada pelo processo carioso, mas que ainda possui potencial de remineralização. Com esse objetivo, surgiram os materiais

bioativos que simulam a formação de apatita e preenchem os pequenos gaps, selando as margens contra a microinfiltração e repondo mineral perdido durante o processo carioso. A haloisita compõe o mineral Caolim que é utilizado na indústria de porcelana, papel e borracha. Os nanotúbulos de haloisita (HNT) são estruturas em forma de cone envoltos por uma camada externa de sílica tetraédrica com uma porção interna octaédrica de alumina. Sabe-se que os HNTs possuem propriedades importantes já embasadas na literatura científica e são utilizados em outras áreas da medicina, principalmente na indústria farmacêutica devido ao lúmen dos HNTs ser capaz de absorver macromoléculas, incluindo drogas, DNA e proteínas. A ideia de usar nanopartículas de haloisita oferece possibilidades promissoras para o estudo da engenharia de cristais e aspectos fundamentais do processo de biomineralizaçao. A proposta deste trabalho é formular e avaliar as propriedades físico-químicas, resistência de união e potencial remineralizador de adesivos experimentais incorporados com nanotúbulos de haloisita

Endereço: Rua Cel. Nunes de Melo, 1000
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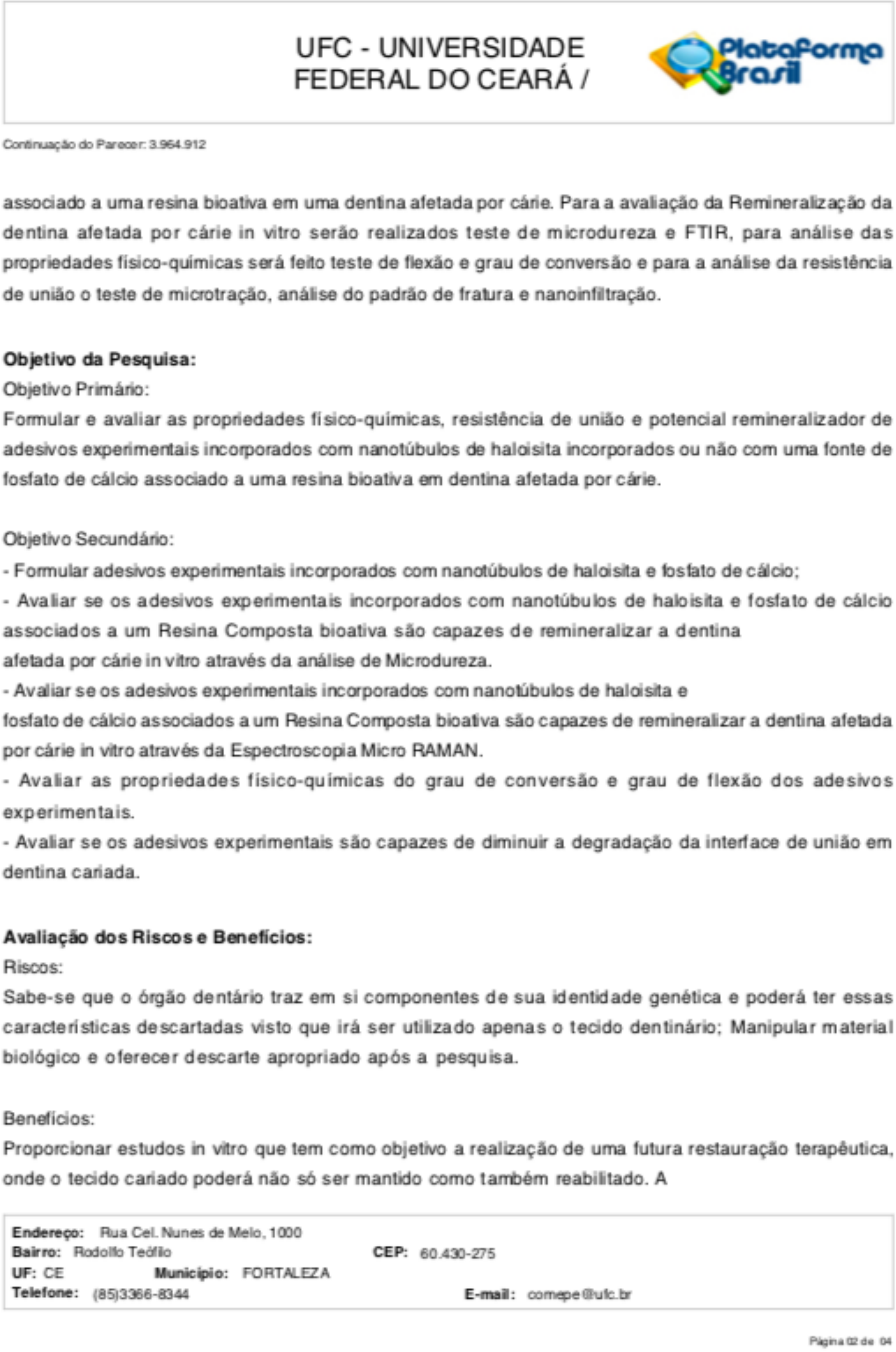
UF: CE **Município:** FORTALEZA
Telefone: (85)3366-8344 **E-mail:** comepe@ufc.br



Continuação do Parecer: 3.964.912

associado a uma resina bioativa em uma dentina afetada por cárie. Para a avaliação da Remineralização da dentina afetada por cárie in vitro serão realizados teste de microdureza e FTIR, para análise das propriedades físico-químicas será feito teste de flexão e grau de conversão e para a análise da resistência de união o teste de microtração, análise do padrão de fratura e nanoinfiltração.

Objetivo da Pesquisa:

Objetivo Primário:

Formular e avaliar as propriedades físico-químicas, resistência de união e potencial remineralizador de adesivos experimentais incorporados com nanotúbulos de haloisita incorporados ou não com uma fonte de fosfato de cálcio associado a uma resina bioativa em dentina afetada por cárie.

Objetivo Secundário:

- Formular adesivos experimentais incorporados com nanotúbulos de haloisita e fosfato de cálcio;
- Avaliar se os adesivos experimentais incorporados com nanotúbulos de haloisita e fosfato de cálcio associados a um Resina Composta bioativa são capazes de remineralizar a dentina
afetada por cárie in vitro através da análise de Microdureza.
- Avaliar se os adesivos experimentais incorporados com nanotúbulos de haloisita e
fosfato de cálcio associados a um Resina Composta bioativa são capazes de remineralizar a dentina afetada por cárie in vitro através da Espectroscopia Micro RAMAN.
- Avaliar as propriedades físico-químicas do grau de conversão e grau de flexão dos adesivos experimentais.
- Avaliar se os adesivos experimentais são capazes de diminuir a degradação da interface de união em dentina cariada.

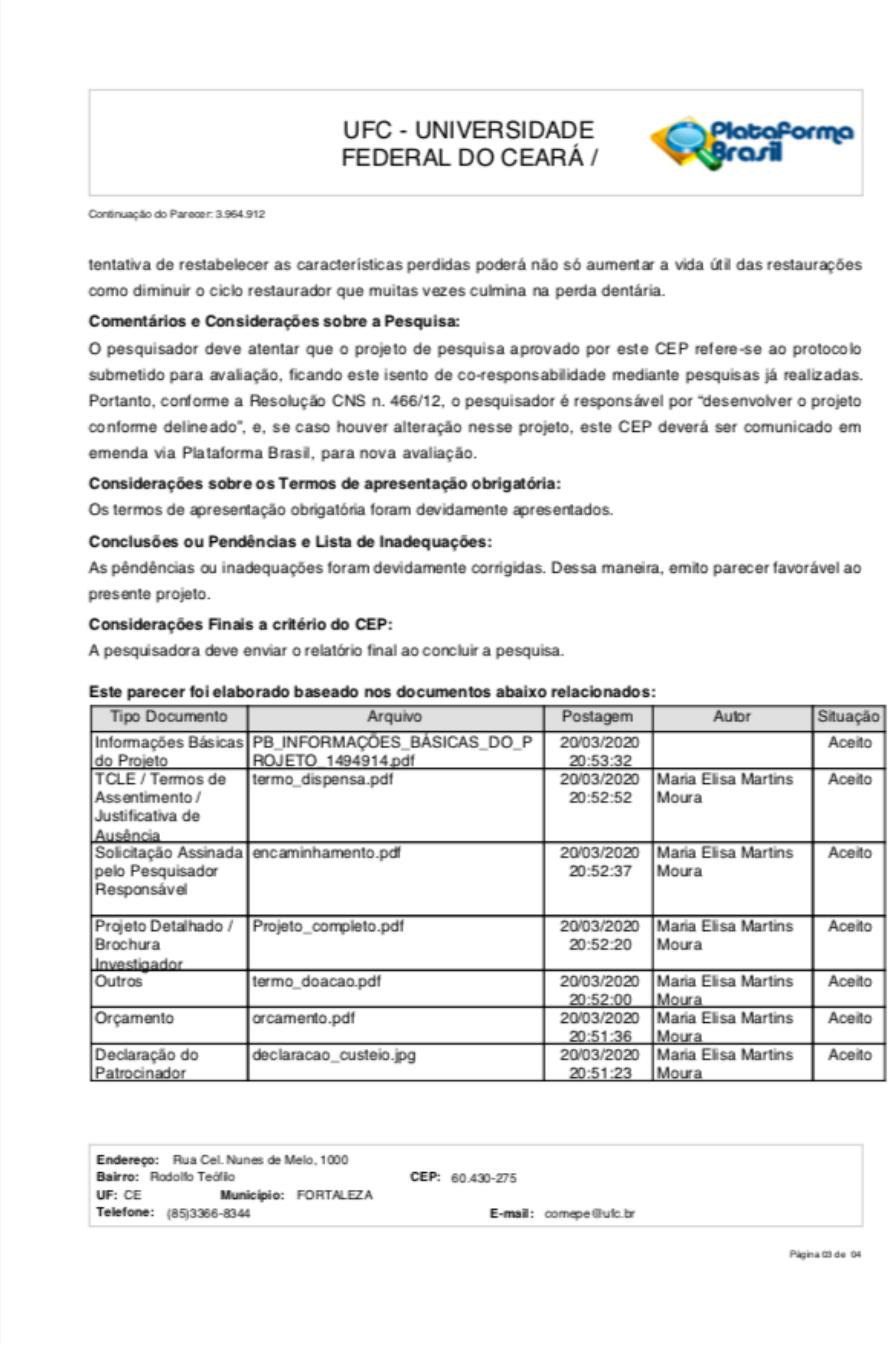
Avaliação dos Riscos e Benefícios:

Riscos:

Sabe-se que o órgão dentário traz em si componentes de sua identidade genética e poderá ter essas características descartadas visto que irá ser utilizado apenas o tecido dentinário; Manipular material biológico e oferecer descarte apropriado após a pesquisa.

Benefícios:

Proporcionar estudos in vitro que tem como objetivo a realização de uma futura restauração terapêutica, onde o tecido cariado poderá não só ser mantido como também reabilitado. A



tentativa de restabelecer as características perdidas poderá não só aumentar a vida útil das restaurações como diminuir o ciclo restaurador que muitas vezes culmina na perda dentária.

Comentários e Considerações sobre a Pesquisa:

O pesquisador deve atentar que o projeto de pesquisa aprovado por este CEP refere-se ao protocolo submetido para avaliação, ficando este isento de co-responsabilidade mediante pesquisas já realizadas. Portanto, conforme a Resolução CNS n. 466/12, o pesquisador é responsável por "desenvolver o projeto conforme delineado", e, se caso houver alteração nesse projeto, este CEP deverá ser comunicado em emenda via Plataforma Brasil, para nova avaliação.

Considerações sobre os Termos de apresentação obrigatória:

Os termos de apresentação obrigatória foram devidamente apresentados.

Conclusões ou Pendências e Lista de Inadequações:

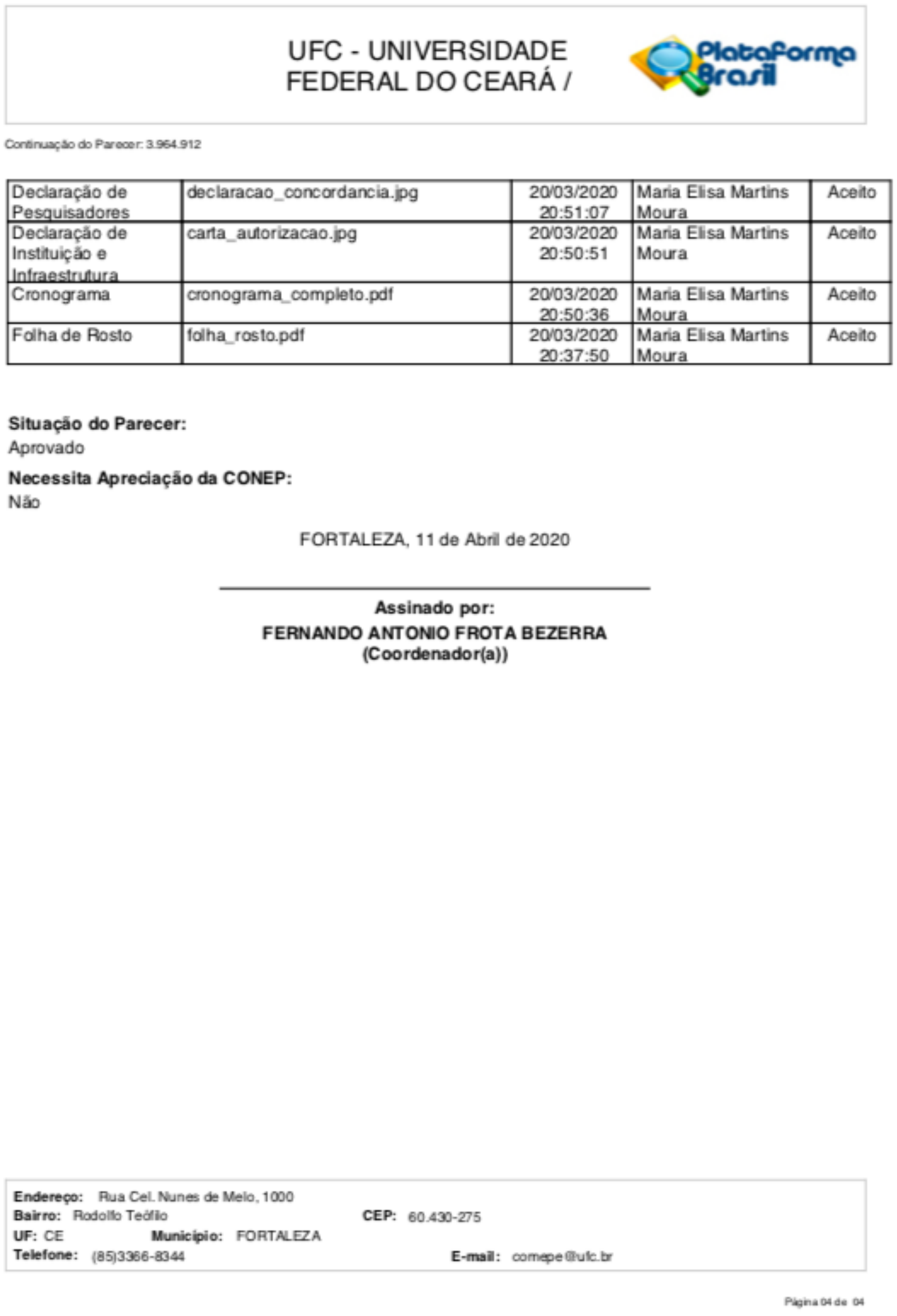
As pêndencias ou inadequações foram devidamente corrigidas. Dessa maneira, emito parecer favorável ao presente projeto.

Considerações Finais a critério do CEP:

A pesquisadora deve enviar o relatório final ao concluir a pesquisa.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1494914.pdf	20/03/2020 20:53:32		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	termo_dispensa.pdf	20/03/2020 20:52:52	Maria Elisa Martins Moura	Aceito
Solicitação Assinada pelo Pesquisador Responsável	encaminhamento.pdf	20/03/2020 20:52:37	Maria Elisa Martins Moura	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_completo.pdf	20/03/2020 20:52:20	Maria Elisa Martins Moura	Aceito
Outros	termo_doacao.pdf	20/03/2020 20:52:00	Maria Elisa Martins Moura	Aceito
Orçamento	orcamento.pdf	20/03/2020 20:51:36	Maria Elisa Martins Moura	Aceito
Declaração do Patrocinador	declaracao_custeio.jpg	20/03/2020 20:51:23	Maria Elisa Martins Moura	Aceito



Continuação do Parecer: 3.964.912

Declaração de Pesquisadores	declaracao_concordancia.jpg	20/03/2020 20:51:07	Maria Elisa Martins Moura	Aceito
Declaração de Instituição e Infraestrutura	carta_autorizacao.jpg	20/03/2020 20:50:51	Maria Elisa Martins Moura	Aceito
Cronograma	cronograma_completo.pdf	20/03/2020 20:50:36	Maria Elisa Martins Moura	Aceito
Folha de Rosto	folha_rosto.pdf	20/03/2020 20:37:50	Maria Elisa Martins Moura	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

FORTALEZA, 11 de Abril de 2020

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