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MATERIAIS INTELIGENTES NA ODONTOLOGIA MINIMAMENTE INVASIVA: REMOÇÃO, RECUPERAÇÃO E RESTAURAÇÃO DA DENTINA

Paula Maciel Pires

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Tese submetida ao Programa de Pós-graduação em Odontologia (Área de Concentração: Odontopediatria) da Faculdade de Odontologia da Universidade Federal do Rio de Janeiro como parte dos requisitos para qualificação de doutoramento (Área de Concentração: Odontopediatria).

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DEDICATÓRIA

Dedico ao amor,
Que foi toda minha força motriz.

É preciso esquecer os vendavais, o looping da montanha-russa, o giro incessante da ciranda e também as crenças limitantes impostas por águas passadas.
E hoje, mais que nunca, confiar.
De olhos fechados, mas cheios de certezas. No instinto, na travessia, no encaixe exato do enrosco. Nas respostas que ansiava tanto por chegar e no tempo das ideias, supostamente esquecidas.
Lembrar.
Que sempre que o medo inventar de fazer um motim, o amor permanecerá ali. Como única certeza de que tá tudo bem.

À minha mãe,
que transpassa todo amor.

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À banca avaliadora: **Profa. Maria Augusta Visconti, Profa. Carina Maciel Silva Boghossian, Profa. Larissa Maria Assad Cavalcante, Profa. Sara Gemini Piperni, Profa. Maristela Barbosa Portela.** E em especial, à **Profa. Andréa Fonseca Gonçalves e Prof. Matheus Melo Pithon** pelas considerações feitas durante minha qualificação de Doutorado.

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"O senhor saiba: eu toda minha vida pensei por mim, sou nascido diferente. Eu sou eu mesmo. Divêrjo de todo mundo... Eu quase que nada não sei. Mas desconfio de muita coisa. O senhor concedendo, eu digo: pra pensar longe, sou cão mestre – o senhor solte em minha frente uma ideia ligeira, e eu rastreio essa por fundo de todos os matos, amém!"

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RESUMO

PIRES, Paula Maciel. **Materiais inteligentes na odontologia minimamente invasiva: remoção, recuperação e restauração da dentina.** Rio de Janeiro, 2022. Tese (Doutorado em Odontologia – Área de Concentração: Odontopediatria) – Faculdade de Odontologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2022.

O objetivo da presente tese foi analisar as características das interações de materiais inteligentes quando aplicados em dentina, avaliando as técnicas de remoção, a recuperação e a restauração do tecido cariado. Para isso, foram realizados 6 estudos englobando os conceitos atuais da odontologia de mínima intervenção. O primeiro estudo consiste em uma revisão bibliométrica, que realizou um levantamento nas bases de dados MEDLINE, Scopus, Web of Science, Cochrane Library, Lilacs/BBO e Embase, evidenciando o potencial bioativo dos materiais estudados e a necessidade de testá-los em dentina totalmente ou parcialmente desmineralizada através de estudos laboratoriais. O segundo estudo visou comparar *in vitro* diferentes técnicas para remoção de cárie artificialmente produzidas: o uso de broca, cureta e dois agentes químico-mecânicos (Papacárie Duo Gel®, Fórmula e Ação e Brix3000®, Brix Medical Science). Para isso, foram realizados escaneamentos em micro-CT antes e após a remoção de cárie com o intuito de comparar o volume e a densidade mineral da dentina, demonstrando que não houve diferença entre as técnicas quanto a quantidade de tecido removido ($p>0,05$). Além disso, nesse mesmo estudo, foi avaliado o potencial de cimentos de polialquenoato (Poly Zinc®, Prevest DenPro e Ketac Molar®, 3M ESPE) na recuperação da densidade mineral da dentina remanescente, mostrando que o cimento de policarboxilato de zinco apresentou um desempenho melhor (33,6%) quando comparado ao cimento de ionômero de vidro (6%; $p<0,01$). Quanto à seletividade *in vitro* dos agentes químico-mecânicos à base de papaína, um terceiro estudo foi conduzido, testando as alterações na morfologia e rugosidade superficial da dentina hígida antes e após a aplicação dos géis, comprovando que esses géis são específicos e seguros, sendo que os valores da rugosidade superficial não apresentaram diferenças estatísticas ($p>0,05$). No quarto estudo foi avaliado *in vitro* a interação de materiais restauradores liberadores de íons aplicados sobre a dentina artificialmente cariada. Excetuando o compósito resinoso testado (Aura®, SDI), tanto o cimento de ionômero de vidro (Fuji IX®, GC) quanto os cimentos a base de silicato de cálcio (Endo-pass®, DEI e Theracal®, Bisco) provocaram precipitação mineral na interface com a dentina. Esses resultados foram confirmados através de análises quantitativas ($\alpha = 5\%$) e qualitativas. O quinto estudo analisou *in vitro* dois cimentos de ionômero de vidro modificados por resina (CIVMR; Ionolux®, VOCO e ACTIVA®, Pulpdent) quando aplicados sobre sistemas adesivos universais simplificados (Futurabond®, VOCO e Scotchbond®, 3M) com o objetivo de comparar a força de união a longo prazo desses materiais aplicados sobre dentina. Foi possível constatar que a composição dos sistemas adesivos influencia mais do que o protocolo de aplicação, seja a aplicação convencional ou autocondicionante. Entretanto, quando bem empregados, os CIVMR, podem melhorar a longevidade de adesão desses sistemas adesivos ($p<0,05$). O sexto estudo consiste em uma revisão de literatura narrativa que apresenta os materiais liberadores de íons disponíveis no mercado, seus mecanismos ação e suas indicações clínicas. Com isso, pode-se concluir que

grande parte dos estudos sobre materiais bioativos ainda são limitados a experimentos *in vitro* e que, apesar de apresentarem resultados positivos quanto a sua bioatividade em recuperar densidade e promover precipitação mineral, esses resultados ainda não podem ser extrapolados clinicamente. Quanto as técnicas de remoção seletiva de cárie, talvez conhecer os conceitos biológicos, fisiológicos e químicos dos processos seja mais importante do que a técnica aplicada em si. Isso também serve no emprego dos materiais bioativos já disponíveis no mercado, que de acordo com a sua composição, a indicação clínica pode variar.

Palavras-chave: Biomateriais, Cárie dentária, Dentina, Odontologia minimamente invasiva, Remineralização dentária.

ABSTRACT

PIRES, Paula Maciel. **Smart materials in minimally invasive dentistry: dentin removal, recovery and restoration.** Rio de Janeiro, 2022. Tese (Doutorado em Odontologia – Área de Concentração: Odontopediatria) – Faculdade de Odontologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2022.

The aim of the present study was to analyze the type of interactions between smart materials and dentin by studying the techniques for carious tissue removal, dentin recovery and tissue restoration. In order to do this, 6 studies were carried out, involving the current concepts of minimally invasive dentistry. The first study consisted of a bibliometric review, in which we carried out a survey in MEDLINE, Scopus, Web of Science, Cochrane Library, Lilacs/BBO and Embase databases, on the bioactive potential of restorative materials and their effect in sound, completely or partially demineralized dentin through laboratory studies. The second study aimed to compare *in vitro* different techniques for dentin caries removal: drill, hand excavator and two chemical-mechanical agents (Papacárie Duo Gel®, Fórmula e Ação and Brix3000®, Brix Medical Sciences). In order to do this, micro-CT scans were performed before and after caries removal to compare the volume and mineral density of dentin, and the results showed no difference among the techniques regarding the amount of tissue removed ($p>0.05$). At the same study, the potential of polyalkenoate cements (Poly Zinc®, Prevest DenPro and Ketac Molar®, 3M ESPE) to recover mineral density of the remaining dentin was evaluated, showing that the zinc polycarboxylate cement presented higher performance (33.6%) compared to the glass ionomer cement (6%; $p<0.01$). To test the *in vitro* selectivity of the chemical-mechanical agents based on papain, a third study was conducted to test the changes in the morphology and surface roughness of sound dentin before and after the application of papain gels. The results showed that they are specific and safe, as the surface roughness values after application were not statistically significant different ($p>0.05$). The fourth study evaluated *in vitro* the interaction of ion-releasing restorative materials applied on artificially carious dentin. With the exception of the resin composite (Aura®, SDI), both glass ionomer cement (Fuji IX®, GC) and calcium silicate-based cements (Endo-pass®, DEI and Theracal®, Bisco) resulted in mineral precipitation at the interface with dentin. These results were confirmed through quantitative ($\alpha= 5\%$) and qualitative analyses. The fifth study analyzed *in vitro* two resin-modified glass ionomer cements (RMGIC; Ionolux®, VOCO and ACTIVA®, Pulpdent) when applied with simplified universal adhesive systems (Futurabond®, VOCO and Scotchbond®, 3M) with the aim to compare the long-term bond strength between these materials and dentin. It was possible to verify that the composition of the adhesive systems influenced more than the application protocol (conventional or self-etch mode). However, RMGIC can improve the longevity of adhesion of these adhesive systems ($p<0.05$). The sixth study consisted of a narrative critical literature review presenting the ion-releasing materials available on the market, their mechanisms of action and their clinical indications. It can be thus concluded that most studies on bioactive materials are still limited to *in vitro* experiments and, despite showing positive results regarding bioactivity in recovering density and promoting mineral precipitation, these results still can not be extrapolated to clinical applications. Regarding the selective potential of caries removal techniques, the knowledge of biological, physiological and chemical concepts of the processes is more important than the technique

used. This can be also applied to the use of bioactive materials available on the market, which according to their composition, may present different clinical indications and applications.

Keywords: Biomaterials, Dental Caries, Dentin, Minimally invasive dentistry, Tooth Remineralization.

RESUMEN

PIRES, Paula Maciel. **Materiales inteligentes en odontología mínimamente invasiva: remoción, recuperación y restauración de la dentina.** Rio de Janeiro, 2022. Tese (Doutorado em Odontologia – Área de Concentração: Odontopediatria) – Faculdade de Odontologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2022.

El objetivo de la presente tesis fue analizar las características de las interacciones de los materiales inteligentes cuando se aplican a la dentina para estudiar las técnicas de remoción, recuperación y restauración del tejido. Para ello se realizaron 6 estudios que engloban los conceptos actuales de la odontología de mínima intervención. El primer estudio consiste en una revisión bibliométrica, que realizó una revisión en las bases de datos MEDLINE, Scopus, Web of Science, Cochrane Library, Lilacs/BBO y Embase, evidenciando el potencial bioactivo de los materiales estudiados y la necesidad de probarlos en dentina totalmente o parcialmente desmineralizada a través de estudios de laboratorio. El segundo estudio tuvo como objetivo comparar *in vitro* diferentes técnicas para la remoción de caries producidas artificialmente: el uso de material rotatorio, cureta y dos agentes químico-mecánicos (Papacárie Duo Gel® y Brix3000®). Para ello, se realizaron análisis en micro-CT antes y después de la remoción de caries con el fin de comparar el volumen y la densidad mineral de la dentina, demostrando que no hubo diferencia entre las técnicas en la cantidad de tejido removido ($p>0,05$). Además, en el mismo estudio se evaluó el potencial de los cementos de polialquenoato (Poly Zinc®, Prevest DenPro y Ketac Molar®, 3M ESPE) en la recuperación de la densidad mineral de la dentina remanente, demostrando que el cemento de policarboxilato de zinc presentó un comportamiento mejor (33,6%) en comparación con el cemento de ionómero de vidrio (6%; $p<0,01$). Con relación la selectividad *in vitro* de los agentes químico-mecánicos a base de papaína, se realizó un tercer estudio probando los cambios en la morfología y rugosidad superficial de la dentina sana antes y después de la aplicación de los geles, demostrando que estos geles son específicos y seguros, siendo que los valores de rugosidad superficial no presentaron diferencias estadísticas ($p>0.05$). El cuarto estudio evaluó *in vitro* la interacción de materiales restauradores liberadores de iones aplicados sobre dentina cariada artificialmente. Excepto por el compuesto de resina (Aura®, SDI), tanto el cemento de ionómero de vidrio (Fuji IX®, GC) como los cementos a base de silicato de calcio (Endo-pass®, DEI y Theracal®, Bisco) causaron precipitación mineral en la interfaz con la dentina. Estos resultados se confirmaron mediante análisis cuantitativos ($\alpha = 5\%$) y cualitativos. El quinto estudio analizó *in vitro* dos cementos de ionómero de vidrio modificados con resina (CIVMR; Ionolux®, VOCO y ACTIVA®, Pulpdent) aplicados sobre sistemas adhesivos universales simplificados (Futurabond®, VOCO y Scotchbond®, 3M) con el objetivo de comparar la fuerza de unión a largo plazo de estos materiales aplicados a la dentina. Se pudo verificar que la composición de los sistemas adhesivos influye más que el protocolo de aplicación, sea la aplicación convencional o el autograbado. Sin embargo, el CICMR puede mejorar la longevidad de la adhesión de estos sistemas adhesivos ($p<0.05$). El sexto estudio consiste en una revisión narrativa de la literatura que presenta los materiales liberadores de iones disponibles en el mercado, sus mecanismos de acción y sus indicaciones clínicas. Con esto, se puede concluir que la mayoría de los estudios sobre

materiales bioactivos aún se limitan a experimentos *in vitro* y que, a pesar de mostrar resultados positivos con relación a su bioactividad para recuperar densidad y promover la precipitación mineral, estos resultados aún no son extrapolables clínicamente. En cuanto a las técnicas de eliminación selectiva de caries, quizás sea más importante conocer los conceptos biológicos, fisiológicos y químicos de los procesos que la técnica elegida. Esto también sirve para el uso de materiales bioactivos disponibles en el mercado, que, según su composición, la indicación clínica puede variar.

Palabras clave: Biomateriales, Caries Dental, Dentina, Odontología mínimamente invasiva, Remineralización Dental.

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LISTA DE ABREVIATURAS E SIGLAS

ΔZ	Delta Z
µA	Microampere
µL	Microlitro
µm	Micrómetro
3D	Três Dimensões
AS	Saliva Artificial
ATCC	American Type Culture Collection
ATR	Reflexão Total Atenuada
AM	Amazonas
BBO	Bibliografia Brasileira de Odontologia
BHI	Brain Heart Infusion
BR	Brasil
pH	Potencial hidogeniônico
C	Celcius
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CECT	Colección Española de Cultivos Tipo (Coleção Espanhola de Tipos de Cultivo)
CFU	Colony Forming Units (Unidade Formadora de Colônia)
CIV	Cimento de Ionômero de Vidro
CLSM	Confocal Laser Scanning Microscopy
cm	Centímetro
CPZ	Cimento de Policarboxilato de Zinco
Er:YAG	Acrônimo do inglês Érbio - Itrio-Alumínio-Granada

EUA	Estados Unidos da América
FAPERJ	Fundação de Amparo à Pesquisa do Rio de Janeiro
FEG-SEM	Field Emission Gun – Scanning Electron Microscope (Microscópio Eletrônico de Varredura)
FTIR	Espectroscopia no infravermelho por transformada de Fourier
FO	Faculdade de Odontologia
g/cm^3	Gramas por centímetros cúbicos
h	Horas
Hz	Hertz
HUCFF	Hospital Universitário Clementino Fraga Filho
IL	Ilinois
Kv	Kilovolts
LED	Light Emitting Diode (Diodo Emissor de Luz)
LILACS	Literatura Latino-americana e do Caribe em Ciências da Saúde
Medline	Medical Literature Analysis and Retrieval System Online
MeSH	Medical Subject Headings
Micro-CT	Microtomografia Computadorizada
MPa	Megapascal
ml	Mililitro
ml/min	Mililitro por minuto
mm	Milímetro
N	Newton
PhD	Philosophiæ Doctor (Doutor da Filosofia)
PubMed	PubMed Unique Identifier
RJ	Rio de Janeiro

s	Segundos
SBF	Simulated Body Fluid (Fluído Corporal Simulado)
SP	São Paulo
SPSS	Statistical Package for the Social Sciences
SJR	SCImago Journal Rank
UFF	Universidade Federal Fluminense
UFRJ	Universidade Federal do Rio de Janeiro
UK	United Kingdom (Reino Unido)
USA	United States of America
UV	Ultra-violeta

LISTA DE SÍMBOLOS

®	Marca Registrada
α	Alfa
%	Porcento
X	Vezes
±	Mais ou Menos
=	Igual
Δ	Variação
<	Menor que
>	Maior que
°	Graus

SUMÁRIO

1. INTRODUÇÃO

As pesquisas na área da odontologia restauradora vêm avançando cada vez mais em diversos níveis e, com isso, estimulando o desenvolvimento de novos materiais somado a novas técnicas para empregá-los. Ao analisar cada procedimento de maneira particularizada e aplicar os conhecimentos sobre a doença cárie de forma mais abrangente, modificações das condutas clínicas são possíveis, principalmente as que vão de encontro com a filosofia de mínima intervenção que visam a máxima conservação das estruturas dentárias (SCHWENDICKE et al., 2016; TORRES et al., 2021).

Nesta mudança de paradigma, as alternativas conservadoras sobrepõem-se às técnicas invasivas, buscando sempre respeitar as características biológicas de cada um dos tecidos que compõem o órgão dental (BANERJEE et al., 2013; ALAM et al., 2021). É importante ressaltar que, apesar de todo avanço tecnológico, atualmente nenhum material restaurador substitui, em condições de igualdade a estrutura dental, e que todas as restaurações sofrem alterações no ambiente bucal, nas quais os aspectos físicos e químicos se deterioram ao longo dos anos (CARRILHO & D'ALPINO, 2018; GIANNINI & SAURO, 2021).

A cárie dentária é uma doença que acomete grande parcela da população mundial, sendo uma das enfermidades crônicas mais comuns (SELWITZ et al., 2007). De acordo com os conceitos atuais, a cárie dentária advém do desequilíbrio da homeostase bucal causado pelos próprios microrganismos residentes da microbiota bucal (SIMÓN-SORO et al., 2015). A perda mineral dos tecidos dentais duros promovido pela doença cárie é causada pela produção de ácidos orgânicos decorrentes da fermentação de carboidratos da dieta do indivíduo, e que favorece a proliferação desses microrganismos cariogênicos específicos (ERICSON et al., 2003). Além disso, a cárie dentária também é modulada por diversos fatores, tais como a susceptibilidade do hospedeiro, acúmulo de biofilme, microbiota cariogênica e substrato apropriado, que interagem entre si e favorecem o desenvolvimento das lesões (GRIGALAUSKIENĖ et al., 2015; MATHUR et al., 2018).

Pesquisas mostram que, seguido da desmineralização dos tecidos duros, metaloproteinases presentes na própria dentina e saliva apresentam um papel crucial na progressão das lesões cariosas, promovendo a degradação do colágeno presente na matriz orgânica dentinária (TJÄDERHANE et al., 2015; FEMIANO et al., 2016; ASHWINI et al., 2020; BAFAIL et al., 2020). Porém, por ser uma matriz orgânica calcificada altamente organizada, constituída por cristais de hidroxiapatita e uma rede de colágeno e proteínas, a dentina apresenta uma grande capacidade de responder a estímulos específicos de recuperação (NIU et al., 2014; HE et al., 2019; YU et al. 2021).

Avançando para o tratamento de lesões de cárie em dentina há um respaldo científico para o emprego da remoção seletiva do tecido cariado, sendo que a terminologia “dentina infectada” e “dentina afetada” são usadas para facilitar e ajudar a melhorar a compreensão e a comunicação entre os pesquisadores, educadores e os clínicos (FELDENS & KRAMER, 2013; GUEDES-PINTO, 2012; INNES et al., 2016). Isso porque essas duas zonas são distintas do ponto de vista morfológico, bioquímico, bacteriológico e fisiológico (BJØRNDAL et al., 2019). A dentina infectada é a mais externa e que apresenta intensa atividade bacteriana, apresentando tecido necrótico e irreversivelmente desnaturado, não passível de remineralização e assim, deve ser completamente removida durante o procedimento restaurador. O contrário acontece com a dentina afetada, presente em uma camada mais profunda que se mostra reversivelmente desnaturada porém passível de remineralização, devendo ser preservada. O principal critério utilizado para diferenciá-las é a dureza do tecido e sua resistência à remoção com instrumentos cortantes manuais (BANERJEE et al., 2017; RICKETTS et al., 2018; SCHWENDICKE et al., 2018).

O ponto importante para o sucesso da remoção seletiva é bom o selamento marginal, que deve ser mantido através do uso de materiais restauradores adesivos. Ou seja, se não houver micro-infiltração, haverá a possibilidade de paralisação do processo carioso na dentina subjacente e até mesmo um aumento do seu conteúdo mineral (SCHWENDICKE et al., 2016; BANERJEE et al., 2020).

Com os avanços da odontologia minimamente invasiva, além dos novos protocolos clínicos, novas técnicas também estão sendo propostas para a

remoção do tecido cariado. Já foi demonstrado que instrumentos manuais, como as curetas, parecem ser os melhores instrumentos para remover a dentina cariada, levando-se em consideração o tempo clínico e a quantidade de tecido removido (SCHWENDICKE et al. 2016). Junto a isso, observa-se menores níveis de dor e desconforto relatados por pacientes, associados a remoção do tecido cariado utilizando somente instrumentos manuais. A utilização de produtos com ação químico-mecânica (ALI et al., 2020) na técnica de remoção parcial de cárie, também são alvos de investigação e apresentam-se como ferramentas conservadoras e promissoras a serem utilizadas.

As brocas de aço inoxidável acopladas ao motor de baixa rotação são os instrumentos mais utilizados, o que proporciona a remoção do tecido cariado com maior velocidade, porém, por não ser seletivo, pode resultar na remoção de tecido sadio (BANERJEE et al. 2000), comprometendo a resistência do dente (MANHART et al., 2004). No caso dos produtos para remoção químico-mecânica compostos de papaína, a seletividade ocorre porque o colágeno sadio possui em sua composição anti-tripsina, uma antiprotease plasmática, responsável por impedir a ação proteolítica (MARTINS et al., 2009; HAMAMA et al., 2013), que não está presente na dentina cariada, permitindo assim a remoção somente da dentina infectada e necrótica (SANTOS et al., 2020; DESAI et al., 2021). Estes agentes apresentam também atividade bactericida, bacteriostática e anti-inflamatória, além de outras vantagens, como a ausência de necessidade do uso de anestésicos ou instrumentos rotatórios, que podem gerar um maior desconforto ao paciente (BUSSADORI et al., 2005).

Quando se fala em técnicas minimamente invasivas, em que se leva em consideração a resposta biológica dos tecidos afetados, o material restaurador de escolha também deve apresentar interações que promovam o reestabelecimento dos tecidos afetados à sua condição original (ou mais próxima à ela) (GIANINI & SAURO, 2021). Novos materiais estão sendo desenvolvidos, além do aperfeiçoamento de materiais já existentes, pois eles são utilizados em contato íntimo com tecidos biológicos que apresentam diferentes composições, seja ele totalmente sadio, afetado ou infectado. Portanto, a indicação de uso deve ocorrer após uma minuciosa avaliação do caso e da perspectiva da resposta desejada, levando em consideração critérios clínicos e éticos. Sabe-se

que existe uma relação que deve ser mantida em equilíbrio, bem como a do risco-benefício do tratamento, sendo necessário o conhecimento das características e propriedades dos materiais e do substrato em contato com eles (BANERJEE, 2013).

Nesse quesito, o desenvolvimento de biomateriais sintéticos visa a reparação ou substituição dos tecidos, órgãos ou funções do organismo, com o objetivo de manter ou melhorar a qualidade de vida do paciente (RATNER et al., 2004). Este desenvolvimento se dá em duas principais linhas conceituais, que representam a evolução das propriedades e da interação material/tecido biológico, sendo elas: 1) materiais bioinertes, cujo foco é o de não provocar reação de corpo estranho no organismo e; 2) materiais bioativos, capazes de promover uma resposta fisiológica induzida. Alguns materiais também são responsáveis por estimular respostas celulares em níveis moleculares (biomimética e engenharia tecidual) (BHADURI et al., 2009).

A incorporação de moléculas biologicamente ativas em materiais odontológicos pode possibilitar tratamentos que induzam o reparo ou a regeneração de tecidos de interesse, como a formação de dentina terciária, a esclerose dentinária (mineralização intratubular), o controle do processo inflamatório, ação antimicrobiana, inibição de metaloproteinases e a formação de tecido mineralizado (BAPAT et al., 2019).

Alguns materiais já são comumente utilizados para as estratégias odontológicas preventivas, como é o caso dos cimentos de ionômero de vidro (CIV). Ele possui um papel importante e ampla aplicação clínica, devido à sua característica de liberação de íons fluoreto, capaz de manter ao seu redor um ambiente propício à remineralização (SIDHU et al., 2016), sendo considerado um material bioativo com adesão química ao dente (KHOROUSHI et al., 2013; PIRES et al., 2018). Os cimentos de policarboxilato de zinco (CPZ) também apresentam adesão à estrutura dentária, porém, caíram em desuso com o advento do CIV pois não possuem boa estética e maior solubilidade que este. Entretanto, apresentam em sua composição zinco e precursores de remineralização (PAUL et al., 2015), como o ácido poliacrílico (pAA), considerado um análogo capaz de estimular a nucleação de apatitas no interior de tecidos de colágeno (QI et al., 2012).

Outros materiais considerados bioativos com grande destaque nas pesquisas científicas e de ampla utilização na odontologia minimamente invasiva são os cimentos a base de silicato de cálcio, como o MTA (PRATI et al., 2015; DAWOOD et al., 2017; ZAFAR et al., 2020). Eles não apresentam adesão à estrutura dentária, entretanto, tomam presa em contato com a água, sangue ou outros fluidos (fluídos dentinários), formando hidróxido de cálcio. Isso faz com que o pH do meio se eleve, ativando a fosfatase alcalina, responsável por iniciar o processo de mineralização (PRATI et al., 2015). Quando em contato com os fluidos teciduais, o hidróxido de cálcio também se dissocia em hidroxila e íons cálcio, produzindo uma área de necrose cáustica, responsável por liberar dióxido de carbono, e isso faz com que cristais de calcita (carbonato de cálcio) sejam formados, servindo como núcleo de calcificação e deposição de minerais (ATMEH et al., 2015). A alcalinidade do meio produzida também estimula a secreção de fibronectina, uma glicoproteína, que conjuntamente com os cristais de calcita, estimulam a formação de colágeno tipo I, presente na constituição da dentina (ATMEH et al., 2012).

Apesar desses cimentos serem capazes de mimetizar as propriedades físicas da dentina e serem considerados bons materiais substitutos, eles ainda não apresentam resistência ao desgaste e propriedades mecânicas que os tornam adequados ao longo prazo (WATSON et al., 2014). Devido a isso, estudos visam modificar a composição desses cimentos, buscando interação com outras formulações com o intuito de melhorar suas propriedades biológicas e físico-químicas (ZAFAR et al., 2020; ALAOHALI et al., 2021). O mesmo se dá com as novas formulações de materiais odontológicos considerados inertes, como o caso dos sistemas adesivos e compósitos resinosos, nos quais adições de moléculas biologicamente ativas vêm sendo testadas para estimular uma resposta fisiológica na interface e promover bioatividade (ZHANG et al., 2017; BASTOS et al., 2021).

A diversidade de aplicações dos biomateriais, assim como suas diferenças químicas, físicas, biológicas e morfológicas, faz da pesquisa nesta área do conhecimento um trabalho com características complexas e interdisciplinares. Visando contemplar esses aspectos, essa pesquisa tem desenvolvimento em seis seguimentos, sendo composta por duas revisões (uma

bibliométrica e outra narrativa, de caráter crítico) e quatro estudos laboratoriais que comparam diferentes técnicas de remoção de cárie e materiais restauradores bioativos aplicados sobre diferentes tipos de substrato dentinário.

2. PROPOSIÇÃO

2.1 Objetivo geral

Avaliar o uso e a aplicação de materiais “inteligentes” na odontologia minimamente invasiva, no que engloba a remoção da dentina cariada, bem como o potencial de recuperação tecidual provocado por esses materiais e suas indicações clínicas como materiais restauradores.

2.2 Objetivos específicos

- Descrever, através de uma revisão bibliométrica da literatura atual, os materiais bioativos com aplicação em dentina mais estudados e realizar uma mineração de dados sobre o tema;
- Testar *in vitro* a eficácia de diferentes métodos de remoção de tecido cariado e comparar a capacidade de recuperação mineral de cimentos polialqueanoatos nesse substrato dentinário remanescente;
- Analisar *in vitro* a rugosidade e a morfologia da dentina sadia após a aplicação de agentes químico-mecânicos de remoção de cárie à base de papaína;
- Avaliar *in vitro* a interação da interface dentinária com diferentes materiais liberadores de íons (compósito resinoso, ionômero de vidro, silicato de cálcio e silicato de cálcio modificado por resina);
- Comparar *in vitro* a força de união a longo prazo de sistemas adesivos universais simplificados, aplicados com ou sem condicionamento ácido prévio, utilizando materiais liberadores de íons para restauração (compósito resinoso e ionômeros de vidro modificados por resina);
- Elucidar, através de uma revisão de literatura narrativa, o mecanismo de interação com a dentina de materiais liberadores de íons indicados para dentina presentes atualmente no mercado e suas indicações clínicas.

3. DELINEAMENTO DA PESQUISA

3.1 Tipo de estudo

A presente tese é composta por seis pesquisas com desenhos metodológicos distintos, a fim de responder a cada um dos objetivos específicos propostos. Nesta composição, foram incluídas 2 revisões, sendo uma bibliométrica e outra narrativa, e 4 estudos laboratoriais de caráter experimental, longitudinal, quantitativo e prospectivo, realizado no Departamento de Odontopediatria e Ortodontia da Faculdade de Odontologia da Universidade Federal do Rio de Janeiro (FO/UFRJ) em conjunto com o Laboratório de Biomateriais Dentários e Odontologia Minimamente Invasiva do Departamento de Odontologia da Universidade Cardenal Herrera-CEU na Espanha.

3.2 Considerações Éticas

O estudo foi submetido à aprovação pelo Comitê de Ética em Pesquisa do Hospital Universitário Clementino Fraga Filho (HUCFF–UFRJ) e aprovado sob o protocolo nº 54941416.9.0000.5257 (Anexo 1), com a justificativa de se utilizar um biorrepositório de dentes. O termo de doação de dentes também se encontra em anexo (Anexo 2). O biorepositório representa uma coleção organizada de material biológico humano coletado com finalidade da realização de uma determinada pesquisa científica, sendo descartado totalmente ao final dela, conforme definido pela Resolução CNS Nº 441 de 2011 (Art. 1º) e Portaria MS Nº 2.201 de 2011 (Art. 3º).

3.3 Delineamento Metodológico

3.3.1 Estudo 1

Uma pesquisa bibliométrica foi realizada nas bases de dados MEDLINE, Scopus, Web of Science, Cochrane Library, Lilacs/BBO e Embase com o objetivo de realizar um levantamento bibliométrico sobre os tipos de biomateriais

restauradores utilizados em dentina. Os descritores utilizados foram “dentin” e “bioactive” ou “ion releasing” ou “smart materials” ou “biomimetic materials” ou “smart dentin replacement” e suas variantes linguísticas, sem restrições de idioma ou ano de publicação. Os termos de busca foram adaptados para cada base de dados e operadores booleanos (OR, AND) foram usados de acordo com a estratégia de busca.

Foram incluídos estudos laboratoriais, estudos clínicos e séries ou relatos de casos sobre o tema. Revisões, resumos, monografias, capítulos de livros, artigos de opinião ou cartas ao editor, além dos estudos relacionados à hipersensibilidade, tratamento endodôntico e pré-tratamento da dentina foram excluídos. Os títulos e resumos foram coletados por dois pesquisadores independentes, que verificaram as palavras-chave e as informações relacionadas aos critérios de inclusão antes de prosseguir com a leitura do texto completo. Os estudos com dados insuficientes nessas seções foram lidos na íntegra e, caso as informações necessárias para decidir sobre a elegibilidade não estivessem disponíveis, o estudo era excluído. Após a leitura detalhada, os estudos que atenderam a todos os critérios de seleção foram incluídos para extração dos dados e posterior análise. Nos casos de dúvidas, um terceiro pesquisador foi consultado, a fim de chegar a um consenso.

A extração dos dados de interesse de todos os estudos incluídos foi processada por dois softwares: VantagePoint® e Microsoft Excel®. Os seguintes dados foram extraídos: título dos artigos, autores, países de afiliações dos autores correspondentes, periódicos e anos de publicação. Os estudos também foram categorizados de acordo com o desenho do estudo, tipo de material, substrato testado, testes realizados e potencial de bioatividade.

Para a métrica de autores, foram considerados os principais autores com 5 ou mais estudos publicados e feita uma autocorrelação entre eles e o tipo de material testado. Um mapa-múndi com o número de estudos em cada país foi gerado a partir de dados referentes ao país de afiliação do autor correspondente, considerando todos os estudos incluídos. Com relação aos periódicos, foram considerados os que possuíam 3 ou mais estudos publicados. Além disso, foram analisados os 10 artigos mais citados neste tópico, incluindo os autores, ano de publicação e tipo de material testado. As palavras-chave mais utilizadas pelo

autor foram identificadas nos artigos e representadas em uma nuvem de palavras.

O crescimento da produção científica ao longo dos anos de acordo com o tipo de material foi representado em um gráfico de bolhas. Também foi avaliada a relação entre tipo de material e desenho do estudo, bem como entre tipo de material e substrato dentinário testado, incluindo também a análise quanto ao processo de desmineralização quando classificado como tal. O tipo de análise da interface e o potencial de bioatividade foram avaliadas de acordo com cada tipo de material.

3.3.2 Estudo 2

O segundo estudo constou de uma avaliação experimental *in vitro* que teve como objetivo comparar as mudanças na interface dentinária induzidas por cimentos de polialquenoato liberadores de íons quando aplicados após diferentes técnicas de remoção de cárie.

Preparação das amostras

O cálculo amostral foi feito através do programa Bioestat 5.3 (Instituto Mamirauá, Tefé, AM, Brasil) e usou como base um estudo anterior realizado pelo mesmo grupo de pesquisa (PIRES et al. 2018). Foi utilizado o teste t para calcular o número mínimo de amostras necessárias para avaliação dos três parâmetros avaliados no estudo: profundidade da lesão, conteúdo mineral relativo e ΔZ. O nível de significância utilizado foi de 0,01% e o poder do teste foi de 0,95. Com isso, um total de dez terceiros molares humanos hígidos extraídos foram utilizados para esse estudo.

No total, 50 cavidades foram preparadas após a completa remoção do esmalte oclusal e dois terços apicais da raiz do dente. Para isso, foi utilizado um disco de diamante de 300 μm de espessura em uma máquina de corte (Isomet, Buehler, Lake Bluff, IL, EUA). Usando uma peça de mão dental de alta velocidade acoplada a um dispositivo (Figura 1A, LABiom-R, UFF, Niterói, RJ, Brasil), cinco cavidades padronizadas foram preparadas em cada dente (Figura 1B) com uma broca esférica de diamante (1013, KG Sorensen). Posteriormente, a superfície dentinária foi recoberta com verniz ácido-resistente (Figura 1C),

deixando apenas as cavidades expostas para a realização do desafio cariogênico (Figura 1D).

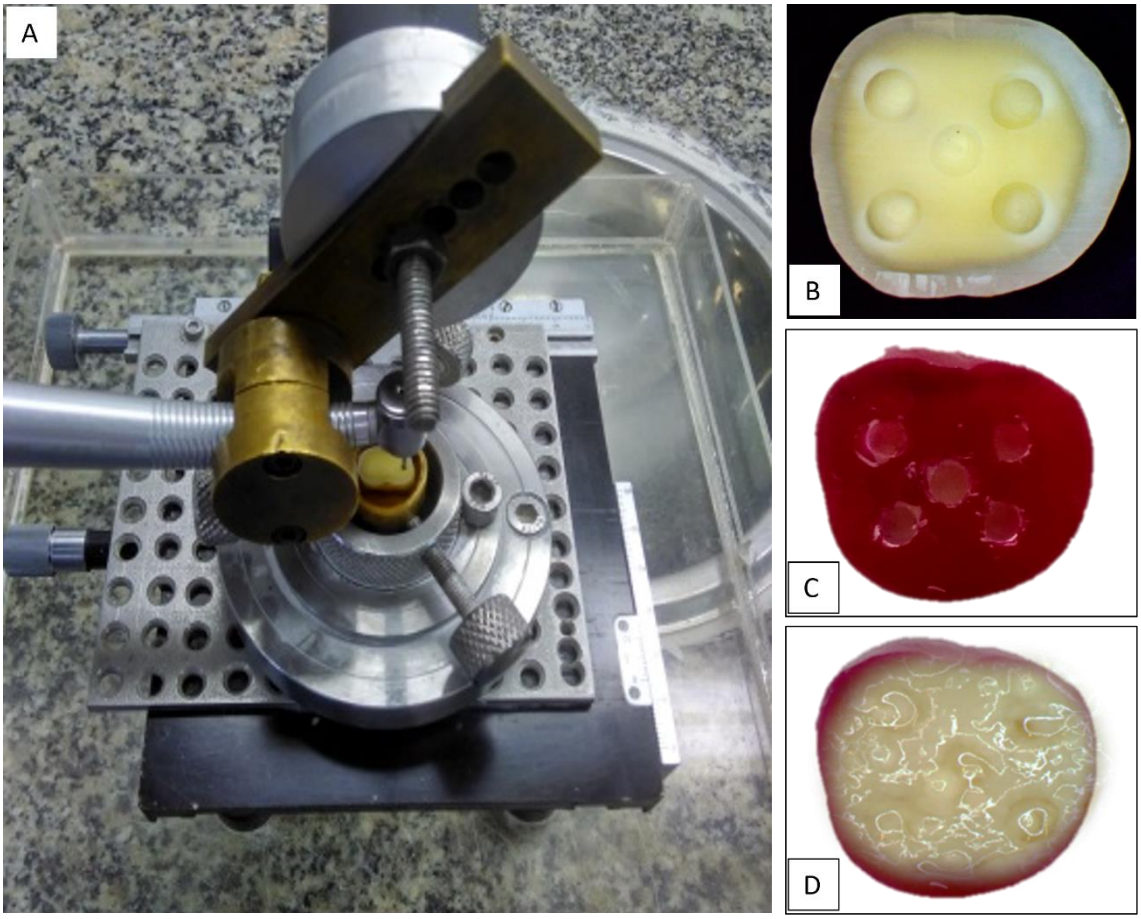

Figura 1: Confecção das cavidades padronizadas. **A)** Dispositivo utilizado para acoplar a peça de mão. **B)** Cavidades produzidas. **C)** Dentina recoberta com verniz ácido-resistente. **D)** Desafio cariogênico microbiológico.

Formação de biofilme

Os espécimes foram fixados em placas de poliestireno de 24 poços para cultura (TPP, Zellkultur Testplatte 24F, Trasadingen, Suíça) e esterilizadas sob luz ultravioleta por 40 minutos antes de receber o inóculo microbiano composto por cepas ATCC (American Type Culture Collection): *Streptococcus mutans* (ATCC 25175), *Streptococcus sanguinis* (ATCC 20556), *Streptococcus salivarius* (ATCC 7073) e *Lactobacillus casei* (ATCC 431). Cada poço contendo uma única amostra de dente recebeu 20 µL do inóculo e foi preenchido com meio de crescimento Brain-Heart Infusion (BHI) com a adição de 5 % de sacarose. O sistema foi incubado em microaerofilia por 10 dias a 37° C e renovado a cada 24

horas. Ao final do período de incubação, os espécimes foram limpos em banho ultrassônico.

Remoção de tecido cariado

Todos os métodos de escavação foram testados aleatoriamente em cada espécime, sendo um em cada cavidade (n=10) e realizados por um único operador. Desta maneira, houve uma padronização das amostras quanto aos métodos de escavação testados, sendo todos realizados em um mesmo substrato. Os métodos utilizados foram: instrumento rotatório em baixa velocidade, escavador manual de dentina e dois agentes químico-mecânicos à base de papaína: Papacárie Duo Gel® (Fórmula & Ação, São Paulo, SP, Brasil) e Brix3000® (Brix Medical Science, Carcarañá, Santa Fe, Argentina). Além disso, uma cavidade foi designada como controle onde não houve remoção de tecido cariado.

Em relação ao método de remoção com o instrumento rotatório, o tecido dentinário cariado foi removido mecanicamente com uma broca redonda de tungstênio (FG2, KG Sorensen, Cotia, SP, Brasil) montada em um contra-ângulo de baixa rotação (KaVo Dental, Biberach, Alemanha). A escavação manual foi realizada com uma cureta para remoção de dentina (Millennium N5, Golgran, São Caetano do Sul, SP, Brasil) em movimentos circulares até atingir um tecido de dentina firme. Em relação aos métodos químico-mecânicos, foi aplicada na cavidade cariada o Papacarie Duo Gel® por 40 s ou com Brix 3000® gel por 2 minutos, de acordo com as instruções do respectivo fabricante, e a dentina cariada foi escavada com uma colher de dentina não afiada sem exercer pressão, para remover o tecido cariado amolecido pacificamente. Por fim, os géis foram limpos e uma segunda porção de gel foi aplicada repetindo as mesmas instruções. O limiar para remoção de cárie foi, em todas as técnicas, dado pela capacidade do operador em remover qualquer tecido extra amolecido. Todos os procedimentos de escavação foram realizados por um único operador, previamente treinado quanto ao uso de tais métodos de escavação.

Tratamento com cimentos de polialquenoato

Após os métodos de remoção, as amostras foram subdivididas em dois grupos de acordo com o material restaurador (n=25), que foi aplicado seguindo

as normas do fabricante. Os materiais testados foram: cimento de policarboxilato de zinco (CPZ; Poly Zinc®, Prevest DenPro, Jammu, Índia) ou cimento de ionômero de vidro (CIV; Ketac Molar®, 3M ESPE, Seefeld, Alemanha). As amostras foram então mantidas sob pressão intrapulpar simulada (20 cm H_2O) usando fluido corporal simulado (SBF), pH 7,4 em temperatura ambiente e 100% de umidade relativa durante 45 dias.

Análises laboratoriais

Todas as amostras foram escaneadas em um micro-CT de alta energia (Skyscan 1173, Bruker, Kontich, Bélgica) com os seguintes parâmetros de aquisição: 70kV, corrente de fonte de 114µA, tamanho de pixel de 14,25µm, sensor com 2240x2240 pixels, filtro de alumínio de 1mm de espessura, passo de rotação de 0,5° sobre 360°, média de quadros (5) e movimentos aleatórios (40) e analisadas em três diferentes tempos: período inicial, após a remoção de cárie e após os 45 dias de armazenamento em simulação de pressão intra-pulpar. As imagens foram analisadas em softwares apropriados para se obter o volume e a densidade mineral de cada cavidade em cada período. Para calcular os volumes da cavidade escavada entre as técnicas de remoção de cárie utilizadas neste estudo, foram extraídos os volumes de interesse (VOIs) de cada cavidade, os valores de cinza foram normalizados e um limiar fixo foi escolhido (40) e aplicado nas imagens das amostras iniciais e escavadas para subtrair os valores para se obter os volumes (mm^3).

Já para obter os valores de densidade na camada residual de cárie imediatamente após a remoção e após o tratamento com os cimentos, os volumes cavitários obtidos anteriormente foram duplicados, alargados com 5 interações, subtraídos do volume original da cavidade e o VOI resultante foi usado como uma máscara para calcular os valores de densidade (em escala de cinza de 8 bits) ao redor da cavidade da superfície interna (dentina cariada, dentina residual e dentina afetada por cárie após a restauração). Após isso, os espécimes foram cortados transversalmente ao longo do centro das cavidades, pulverizados com ouro e analisados em um microscópio eletrônico de varredura (6460LV, JEOL, Tóquio, Japão).

Um mapeamento químico foi realizado usando o sistema microespectroscópico FTIR (Espectroscopia por infravermelho através de transformada de Fourier – “Spectrum Spotlight FTIR”, Perkin Elmer, Waltham, Massachusetts, USA), antes e após 45 dias das amostras serem armazenadas em saliva artificial. Devido à sensibilidade da técnica, novas amostras foram preparadas para isso, sendo confeccionadas 8 superfícies planas de dentina (0,3 mm). Para os grupos CPZ, CIV e dentina completamente desmineralizada, essas amostras foram desmineralizadas em ácido fosfórico a 10% durante 48 h. Já para o grupo controle, de dentina hígida, não houve desmineralização. As superfícies planas de dentina foram colocadas em contato direto com os cimentos e deixados inalterados até que o CPZ e CIV estivessem totalmente fixados. Os picos da composição química foram analisados após a subtração e normalização da linha de base usando o software Spectrum 10™ (Perkin Elmer), a fim de identificar os compostos inorgânicos mais característicos nos espécimes.

Análises estatísticas

O teste de Shapiro-Wilk foi empregado para verificar a normalidade dos dados, seguido pela análise de variância (ANOVA) e teste t para a comparação do volume e da densidade mineral entre os grupos nos diferentes tempos. O programa utilizado foi o Bioestat v.5.3 (Instituto Mamirauá, Manaus, AM, Brazil), com nível de significância de 5%.

3.3.3 Estudo 3

O terceiro estudo está publicado no periódico “Dentistry 3000” e constou de um experimento *in vitro,* com o objetivo de avaliar se os géis a base de papaína possuem alguma ação em dentina hígida. Para isto, a morfologia e as rugosidades linear e volumétrica da dentina após a aplicação desses géis em substrato hígido foi comparada com o efeito de ácidos pré-condicionantes de dentina.

Preparação das amostras

O tamanho da amostra foi estimado com base nos resultados de um estudo similar que comparou as diferenças de rugosidade na dentina sadia após

a aplicação de métodos experimentais de remoção de cárie quimio-mecânica à base de hipoclorito (WENNERBERG et al., 1999). A diferença mínima entre as médias dos grupos (0,002) e o desvio padrão (0,001) foram inseridas no software (BioEstat v.5.3, Instituto Mamirauá, Manaus, AM, Brasil) considerando um valor alfa de 0,05 e um poder do teste de 0,9. O resultado foi um mínimo de 8 espécimes por grupo, sendo utilizados para esse estudo 12 espécimes.

Quarenta e oito terceiros molares provenientes do biorepositório tiveram suas coroas cortadas com o auxílio de um disco de diamante montado em uma máquina de corte de baixa velocidade (Isomet, Buehler, Lake Bluff, IL, EUA) para a obtenção de blocos de dentina de aproximadamente 4 X 4 X 2 mm. Esses espécimes foram colados em aparatos de poliestireno para realização do lixamento seriado, seguido da aplicação dos géis de acordo com cada grupo (n=12).

Tratamento com géis

Os grupos foram divididos em quatro métodos:

1) Papacárie Duo Gel® (Fórmula & Ação, São Paulo, SP, Brasil);

2) Brix3000® (Brix Medical Science, Carcarañá, Santa Fe, Argentina);

3) ácido poliacrílico 11,5% (Vitro condicionador, DFL, Rio de Janeiro, RJ, Brasil);

4) ácido fosfórico a 37% (Condac, FGM, Joinville, Santa Catarina, Brasil).

O tempo de aplicação para todos os produtos foi padronizado em 30 s. Após cada tempo de aplicação, os espécimes foram lavados em água destilada por 60 s e armazenados em umidade 100% até posterior análise.

Análises laboratoriais

Dez espécimes em cada grupo foram analisados em um perfilômetro confocal ótico 3D (Nanovea PS50 Optical, Nanovea Inc., Irvine, Califórnia, Estados Unidos) antes e após o tratamento com os géis. A rugosidade linear foi obtida pela média de três leituras lineares de cada amostra, enquanto a rugosidade volumétrica foi obtida a partir de uma leitura volumétrica de cada amostra. Os dois blocos restantes por grupo foram usados para análise

topográfica de superfície em um microscópio eletrônico de varredura (6460LV, JEOL, Tóquio, Japão).

Análises estatísticas

O teste de Shapiro-Wilk foi empregado para verificar a normalidade dos dados. Por resultarem em dados não-paramétricos o teste de Kruskal-Wallis foi utilizado para comparar as rugosidades, seguido pelo teste de Dunn. O nível de significância foi de 5%. O programa estatístico utilizado foi o software BioEstat v.5.3 (Instituto Mamirauá, Manaus, AM, Brasil).

3.3.4 Estudo 4

O quarto estudo teve como objetivo investigar *in vitro* o potencial remineralizador de diferentes biomateriais liberadores de íons e analisar sua interação com a dentina artificialmente desmineralizada.

Preparação das amostras

No total, setenta e três molares humanos hígidos extraídos foram utilizados para esse estudo. Sessenta cavidades classe I foram preparadas (4 mm de comprimento × 3 mm de largura x 4 mm profundidade) usando uma peça de mão de alta velocidade e uma broca diamantada cilíndrica (3146, Komet, Alemanha) por um mesmo operador. As raízes foram removidas 1 mm abaixo da junção cemento-esmalte, usando um disco de diamante (XL 12205; Benetec, Londres, Reino Unido) montada em um micrótomo de baixa velocidade (Remet evolução, REMET, Bolonha, Itália) sob refrigeração com água. A superfície dos dentes foi recoberta com verniz ácido-resistente, deixando apenas a dentina da superfície cavidade exposta para ser submetida ao protocolo cariogênico.

As raízes dos dentes remanescentes (n=13) também foram removidas conforme descrito anteriormente e um segundo corte paralelo foi feito para remover o esmalte oclusal e expor a dentina médio-coronal, com o intuito de confeccionar superfícies planas de dentina (0,3 mm).

Formação de biofilme

Os espécimes com as cavidades foram fixados em placas de cultura de poliestireno de 24 poços (TPP, Zellkultur Testplatte 24F, Trasadingen, Suíça) e

esterilizados sob luz ultravioleta por 40 minutos antes de receber o inóculo microbiano composto por cepas CECT (Colección Española de Cultivos Tipo): *Streptococcus mutans* (CECT 479T), *Streptococcus gordoni* (CECT 804), *Streptococcus salivarius* (CECT 805T) and *Lactobacillus casei* (CECT 475T). Cada poço recebeu 20 µL do inóculo e foi preenchido com meio de crescimento Brain-Heart Infusion (BHI) com a adição de 5% de sacarose. O sistema foi incubado em microaerofilia por 28 dias a 37° C e renovado a cada 48 horas. Após o período de incubação, os espécimes foram limpos em ultrassom.

Tratamento restaurador

Quatro grupos experimentais (n=15 / grupo) foram aleatoriamente restaurados com os materiais testados, sendo que cada cavidade foi preenchida com um dos materiais, de acordo com as instruções do fabricante. Os grupos de materiais restauradores utilizados foram:

• Controle: restaurados com um adesivo universal (ZipBond®, SDI, Austrália) usado no modo autocondicionante e um compósito resinoso fluido (Aura®, SDI, Austrália), aplicado em três camadas com incremento de 2 mm cada;

• CIV: restaurados com Fuji IX® (GC Corporation, Tokio, Japão) sem nenhum pré-condicionamento da dentina;

• MTA: restaurados com Endo-Pass® (DEI Italia, Italy);

• RMTA: restaurados com TheraCal LC® (Bisco Inc, USA), colocado em camadas incrementais de 1 mm sem nenhum tratamento prévio de dentina.

Todas as amostras foram incubadas a 37˚C em condição de umidade controlada, por 24 horas antes dos processamentos específicos para cada análise laboratorial.

Análises laboratoriais

Dez amostras por grupos foram cortadas em fatias (1,5 mm) e preparadas para a análise de microdureza. Três séries de endentações paralelas foram realizadas a uma distância de 50 µm da interface, seguidas de outras três endentações realizadas abaixo de cada uma delas a intervalos de 50 µm (carga de 25 gf; tempo de permanência de 30 s). As amostras foram testadas

imediatamente após o período de formação do biofilme e após 45 e 60 dias de armazenamento em saliva artificial.

Para o escaneamento no micro-CT (Skyscan 1176, Bruker, Kontich, Belgium), três amostras por grupo foram utilizadas e analisadas antes e após 30 e 90 dias do tratamento com os materiais restauradores. Os parâmetros de aquisição foram: 80 kV, corrente de fonte de 300 µA, tamanho de pixel isotrópico de 8,92 µm, filtro de 0,5 mm Al + 0,38 mm Cu, passo de rotação de 0,5° em 360° com média de quadro de 5. A reconstrução foi realizada usando software (NRecon, Bruker) com parâmetros padronizados de correção de endurecimento de feixe (30%), suavização = 7, kernel de suavização = 2 (Gaussiano), compensação de artefato de anel = 20 e limites de contraste ideais com base nos testes iniciais de varredura e reconstrução. Os dados de imagem foram então alinhados e recortados usando outro software (Dataviewer v1.5.4.6, Bruker). Em seguida, o volume (mm^3) da dentina desmineralizada entre a restauração e o tecido hígido em cada espécime e para cada tempo de escaneamento foi obtido dentro da plataforma ImageJ (v1.8.0_112) por um único operador, cego quanto ao teste experimental. O operador definiu manualmente a região visual de interesse (ROI) para mensuração.

Já para a análise em microscopia confocal (CLSM - Olympus FV1000, Olympus Corp., Tokyo, Japan), duas amostras por grupos foram seccionadas (1,5 mm) e coradas em uma solução de 0,5 % de xilenol. Cinco imagens foram aleatoriamente capturadas e gravadas para investigação da interface da dentina. Amostras de superfícies planas de dentina (n=12) foram desmineralizadas em ácido fosfórico 10 % por 48 h e restauradas de acordo com cada grupo designado, sendo utilizadas 2 amostras por grupo. Uma amostra foi armazenada como controle sem sofrer desmineralização. A análise química dessas amostras foi realizada por um sistema microespectroscópico FTIR-ATR (Spectrum Two UATR; Perkin Elmer). Logo após, as amostras foram preparadas e analisadas através de microscopia eletrônica de varredura (FEG-SEM S-4100; Hitachi, Wokingham, UK), a fim de observar a presença de precipitação mineral após o período de armazenamento.

Análises estatísticas

Os dados de microdureza (ΔKHN%) foram primeiramente analisados usando o modelo ANOVA de três vias complementado com o teste Sidak considerando o tipo de material, as profundidades de endentação e os tempos de armazenamento como fatores fixos (α=5%). Em seguida, os testes de Kruskal-Wallis/Dunn foram usados para comparar a microdureza dos materiais dentro do mesmo tempo e o teste de Friedman foi utilizado para comparar o mesmo material ao longo dos diferentes tempos de armazenamento (α=5%). Essas análises foram realizadas separadamente para cada profundidade de endentação ou utilizando a média geral das profundidades considerando a interação entre o tipo de material e as profundidades de endentação ($p<0,001$). Em relação aos dados de micro-TC, a homogeneidade das variâncias foi avaliada preliminarmente pelo teste de Bartlett ($p<0,05$) seguido pela análise ANOVA. O teste t foi usado em consideração ao pequeno número de espécimes para avaliar possíveis diferenças significativas entre os grupos. Os dados foram analisados utilizando o software SPSS V16 para Windows (SPSS Inc., Chicago, IL, EUA).

3.3.5 Estudo 5

O quinto estudo está publicado no periódico “Materials (Basel)” e constou de uma investigação *in vitro* sobre a resistência de união a longo prazo, de sistemas adesivos universais aplicados com ou sem condicionamento ácido, quando utilizados previamente a cimentos de ionômero de vidro modificados por resina.

Preparação das amostras

No total, duzentos e dezesseis molares hígidos foram utilizados para esse estudo. As raízes foram seccionadas 1 mm abaixo da junção cemento-esmalte e o esmalte oclusal foi completamente removido usando um disco de diamante (XL 12205; Benetec, Londres, Reino Unido) montado em um micrótomo de baixa velocidade (Remet evolução, REMET, Bolonha, Itália) sob refrigeração com água.

Tratamento restaurador

As superfícies planas de dentina foram divididas em três grupos (n 72 / grupo) de acordo com os materiais restauradores utilizados, sendo eles:

1) Compósito resinoso; Aura® (SDI, Australia): aplicado em 3 incrementos de 2 mm cada;
2) Cimento ionômero de vidro modificado por resina; Ionolux® (VOCO, Alemanha): duas cápsulas foram misturadas por 10 s em uma trituradora apropriada e utilizadas em um único incremento cada;
3) Cimento ionômero de vidro modificado por resina; ACTIVA® (Pulpdent, USA): aplicado em 3 incrementos de 2 mm cada.

Além disso, uma subdivisão em cada grupo restaurador (n=18 / grupo) foi estabelecida de acordo com dois protocolos de adesão (convencional ou autocondicionante) e sistema adesivo utilizado, sendo eles Scotchbond Universal® (3M Oral Care, USA) e Futurabond M+® (VOCO, Alemanha).

Nos grupos onde foi realizada a aplicação do sistema adesivo convencional, o condicionamento prévio da dentina foi realizado com ácido ortofosfórico a 37% por 15s e enxague abundante com água destilada por 15s, seguido da aplicação do sistema adesivo selecionado e fotoativação durante 10 s. Já para os grupos onde foi utilizado o sistema autocondicionante, o adesivo foi aplicado com microbrush por 20s, seguido por 5s de jatos de ar para evaporação dos solventes e fotoativados durante 10s. Os espécimes foram finalmente restaurados com os materiais restauradores selecionados conforme mencionado nos grupos principais. Todos os procedimentos de fotoativação foram realizados usando um emissor de luz LED (> 1000 mW/cm2; Radii plus, SDI Ltd., Bayswater Victoria, Austrália).

Ciclagem mecânica

Cada subgrupo dos protocolos de aplicação de cada sistema adesivo, foi novamente subdividido em três métodos de envelhecimento (n= 6/grupo), sendo eles:

- Controle: armazenados por 24 h em água destilada;
- Ciclagem: 350.000 ciclos em saliva artificial (AS);

- Ciclagem + AS: 350.000 ciclos, seguido de 8 meses de armazenamento em saliva artificial.

Para os grupos com ciclagem, os espécimes foram montados em anéis de resina acrílica para facilitar o teste. Uma carga compressiva (3 Hz; 70 N) foi aplicada à superfície plana de dentina usando um êmbolo esférico de aço inoxidável de 5 mm de diâmetro conectado a uma máquina de carga cíclica (S-MMT-250NB; Shimadzu, Tóquio, Japão).

Análises laboratoriais

Cortes seriados com 0,9 mm de espessura foram realizados em cada dente (Remet evolução, REMET, Bolonha, Itália), iniciando-se junto a uma das faces proximais até a face oposta. Após rotação do dente em 90º, nova série de cortes foi feita, também com 0,9 mm de espessura. Ao final, obteve-se espécimes em forma de palitos com área transversal de secção de aproximadamente 0,81 $mm^{2.}$

Todos os espécimes foram inspecionados em lupa estereoscópica com aproximadamente 30 vezes de aumento. Aqueles que apresentaram defeitos na interface, presença de esmalte, bolhas ou irregularidades nas proximidades da união resina-dentina, foram excluídos da amostra. Os espécimes selecionados tiveram sua área adesiva individualmente mensurada com paquímetro digital (Mod. 500- 144b, Mytutoyo Sul Americana Ltda., SP, BR) com resolução de 0,01 mm.

Os espécimes foram individualmente fixados a um dispositivo metálico com adesivo de cianoacrilato e o ensaio mecânico de microtração foi realizado usando um dispositivo com comprimento de curso de 50 mm, com célula de carga com capacidade máxima de 500 N e um deslocamento de 0,5 mm. Os modos de fratura foram avaliados usando um estereoscópico e classificados quanto a fratura adesiva (A), mista (M) ou coesiva (C).

Cinco espécimes fraturados representativos de cada subgrupo foram processados e analisados em um microscópio eletrônico de varredura (FE-SEM S-4100; Hitachi, Wokingham, Reino Unido).

Análises estatísticas

Os valores de força de união em MPa foram avaliados inicialmente quanto a normalidade e homogeneidade usando os testes de Kolmogorov – Smirnov e Levene, respectivamente. Os dados foram então analisados usando uma Análise de Variância de três fatores (ANOVA: material restaurador, adesivo e protocolo de envelhecimento), além do teste de comparação múltipla de Newman-Keuls (α = 0,05). Os dados foram analisados utilizando o software SPSS V16 para Windows (SPSS Inc., Chicago, IL, EUA).

3.3.6 Estudo 6

O sexto estudo, publicado no periódico “British Dental Journal”, consistiu em uma revisão narrativa contemplando os mecanismos de interação de materiais bioativos atualmente disponíveis no mercado com a interface dentinária.

4. DESENVOLVIMENTO DA PESQUISA

Artigo 1: Bioactively modified restorative material applied over coronary dentine – A bibliometric and critical review.

Status: Em processo de submissão.

Artigo 2: Dentine mineral changes induced by polyalkenoate cements after different selective caries removal techniques: An in vitro study.

Status: Submetido.

Revista: Caries Research.

Artigo 3: Tridimensional roughness and morphology of sound dentin surfaces after papain-gel treatment.

Status: Publicado.

Revista: Dentistry 3000.

Artigo 4: Assessment of the remineralisation induced by contemporary ion-releasing materials in mineral-depleted dentine

Status: Submetido.

Revista: Clinical Oral Investigations.

Artigo 5: Effects of ions-releasing restorative materials on the dentine bonding longevity of modern universal adhesives after load-cycle and prolonged artificial saliva aging.

Status: Publicado.

Revista: Materials (Basel).

Artigo 6: Contemporary restorative ion-releasing materials: Current status, interfacial properties and operative approaches.

Status: Publicado.

Revista: British Dental Journal.

4.1 Artigo 1: Bioactively modified restorative material applied over coronary dentine – A bibliometric and critical review.

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Running Title: Bioactively material applied over dentine – A bibliometric review.

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Bioactively modified restorative material applied over coronary dentine – A bibliometric and critical review.

Abstract

The purpose was to analyse the scientific literature on biomodified restorative materials over dentine. Searches were conducted in MEDLINE, Scopus, Web of Science, Cochrane Library, Lilacs/BBO and Embase. Studies employing the terms “dentin” and “bioactive” or “ion releasing” or “smart materials” or “biomimetic materials” or “smart dentin replacement” were included. The following data were extracted: title, authors, year, journal and corresponding author's affiliation country. Studies were categorized according to study design, type of material, substrate, analytical method and bioactivity. A total of 7161 records were recovered and 159 were included for data extraction. Most of the publications were *in vitro* studies (n=149), testing different types of materials in sound dentine (n=115). Most studies were published in Dental Materials (n=29) and an increase in the publications could be observed after the year 2000. Most of the articles were from USA (n=34), followed by Brazil (n=28). Xu HH (n=20) and Weir MD (n = 19) were the authors with the highest publication number. Interfacial analysis was most investigated (n=105), followed by bond strength (n=86). Bioactivity potential was demonstrated for most tested materials (n=148). This review presents insights into the current trends of bioactive materials development, clearly showing a lack of clinical studies.

Keywords: Bibliometrics; Dental materials; Dentin; Dentistry; Smart materials.

1. Introduction

Many restorative materials are available for filling dental cavities, and in fact, restorations are the most frequently performed dental treatments (Schwendicke et al., 2016). Dental caries reduces the mineral content (e.g. carbonate and magnesium) and crystal orientation of the inorganic phase of dentine (Di Foggia et al., 2019). The mineral depletion exposes collagen fibres, leading to a rapid destruction of the dentinal tissue, with consequent risk for pulp exposure (Sauro et al., 2016). Although there are some most widely used dental materials for reconstruction of teeth such as adhesion-based composites, glass polyalkenoate cements and ceramics (Schwendicke et al., 2016), unfortunately, there are currently no clinically approved restorative material that promotes and regulates a specific biological response to produce reactionary dentine (Alaohali et al., 2021).

It is desirable that restorative materials exhibit bioactivity, which would allegedly improve the mechanical properties and bond strength of the tooth-material interface. These are dependent on the dissolution behaviour of the released ions and interactions triggering toughening mechanisms of crack deflection and dentine bridging microstructure morphology (Khan et al., 2019; Schumacher et al., 2007). Bioactivity of dental materials relates to their potential to induce specific and intentionally desired mineral attachment to the dentine substrate, whether carious or not (Vallittu et al., 2018).

The ultimate goal for restoration of tooth structure depends upon the use of durable, adhesive and aesthetically acceptable materials (Ferracane et al., 2014). It would be highly beneficial to develop a new generation of bioactive and therapeutic dental materials with functionalities to suppress demineralisation and promote remineralisation (Giannini & Sauro 2021). It is likely that scientific developments will improve bioactive materials formulations by incorporating specific compounds, e.g. bioactive glasses, that can rapidly release specific ions to improve longevity of dental restorations, and/or heal dental hard tissues (Maas et al., 2017; Pires et al., 2020). Other compounds, such as antimicrobial agents, calcium compounds, polymers, peptides are also under investigation (Vallittu et al., 2018).

An extensive number of published studies with bioactive materials are presented in searches databases. However, due to the lack of agreement on the matter, mapping the published scientific evidence due to the bioactivity of this materials through a bibliometric analysis could be important to identify global tendencies in research and the existence of knowledge gaps in the topic. Thus, this bibliometric review aims to identify trends in main research topics and groups, as well as to critically explore the development of scientific literature among material types, in order to guide future research in the area.

2. Materials and Methods

2.1. Information sources and search strategy

An advanced search was performed until Octuber 2021 in the following databases: MEDLINE (PubMed), Scopus (Elsevier), Web of Science Core Collection (Web of Science), The Cochrane Library (Wiley), LILACS (Virtual Health Library), BBO (Virtual Health Library) and Embase (Elsevier). This electronic bibliographic search was carried out using MeSH terms, synonyms, and entry terms for PubMed and then, adapted according to the syntax rules of each database (Table 1). No filters or limits or language restrictions were applied at this stage. Alerts were created in the databases to indicate new searches, including articles published later than Octuber 2021.

2.2. Eligibility criteria

Laboratory studies, randomized and non-randomized clinical trials, case series/ case reports, along with any other type of studies about biomodified restorative materials applied in class I cavities or flat coronary dentine were included. These studies must refer to the topic of this review, by employing specific standardized terms for search strategy, such as “dentin” and “bioactive” or “ion releasing” or “smart materials” or “biomimetic materials” or “smart dentin replacement” and its linguistic variations in the title, abstract or keywords.

Studies for hypersensitivity, endodontic treatments, dentine pre-treatment and materials without biomodification were excluded. In addition, dissertations, thesis or monographs, books, book chapters, letters to the editor, recommendations, systematic or narrative reviews, editorial and errata were also excluded.

2.3. Screening and selection of articles

All included records were imported to a text mining and bibliometric data analysis software (VantagePoint®, version 13.0, Search Technology, Inc.) and the duplicates were removed, first automatically and then manually (Figure 1).

Titles and abstracts were collected by two independent researchers (PMP and TCR), which would check the keywords and the information related to the inclusion criteria before proceeding with the reading in full text. Studies with insufficient data in these sections were read in full and, if the information needed to decide upon eligibility was not available, the study was excluded. Disagreements were resolved by consensus meeting with others two experienced researchers (ANN and LCM), when necessary. After reading in detail, the studies that met all the selection criteria were included to have data extracted and further analysed.

2.4. Data items and collection process for study characteristics

Extracted data from selected articles was manually collected by one researcher (PMP) and checked by another (TCR). Disagreements between individual judgments were resolved in a consensus meeting with a third researcher (AAN). Each study was classified according to study design, type of material tested, substrate, analytical method and bioactivity. The same study could be classified into more than one category or subcategory, reason why the final count may not match with the total number of included articles

In the “study design” category, the subcategories were: *in vitro*, *in vivo* and *ex vivo*. For “type of material tested”, the study could be classified according to the biomodified material tested: adhesive, cement, or composite. The “substrate” category was subdivided into sound or demineralised dentine. If the dentine was classified as demineralised, the study was then, classified according to demineralisation process: chemical, microbiological, or naturally. For “analytical method”, the subcategories were: ions releasing capability, bond strength, hardness, antibacterial effect, interfacial analysis, chemical characterization, and

others. Finally, studies were subdivided if they presented bioactivity as: yes, no or no difference.

2.5. Data items and collection process for bibliometric analysis

For the metric analyses, the following variables were considered: “title”; “keywords”; “authors”; “year of publication”; “journal” and “corresponding author’s affiliation country”. The recovery rate was over 90%. The bibliometric evaluation of the extracted data was performed using VantagePoint® and Microsoft Excel®.

For author metrics, those with 5 or more studies published were considered, together with the autocorrelation between main authors. Co-occurrence between main authors and the type of material tested were also analysed. In addition, a world map with the number of studies undertaken in each country was generated from data referring to the corresponding author's affiliation country, considering all included studies. Regarding main published journals, 3 or more studies published were considered and the SCImago Journal Rank (SJR) was verified for each journal at https://www.scimagojr.com, considering the year 2021. Furthermore, the top 10 most cited articles in this topic were analysed, including the authors, year and the type of material tested. The most frequently author’s keyword used were identified from articles and represented in a word cloud.

The growth of the scientific articles published over the years according to type of material was represented in a bubble chart. Also, the co-occurrence between type of material and study design were evaluated, as well as the co-occurrence between type of material and the substrate, including the type of dentine demineralisation process, when available. The analytical methods used and the bioactivity were evaluated due to the co-occurrence between the type of material.

3. Results

A total of 7161 records were recovered on the topic of bioactive restorative materials for dentine. After 3468 duplicated records were excluded, 3693 articles remained for a first reading of the title/abstracts/keywords. Subsequently, 605 articles were eliminated for not complying with the eligibility criteria and thus, 764 articles were included for full-text reading. After this, 159 studies have been included. The flowchart of the search selection procedures is shown in Figure 1.

For “study design” category, 149 articles were classified as *in vitro,* 11 as *in vivo* and 2 as *ex vivo*. For the *ex vivo* subcategory, only one “type of material tested” was evaluated (cement), while for *in vitro* and *in vivo*, all types of materials were tested (adhesive, composite and cement). However, for *in vitro* studies, the most tested material were adhesives while for *in vivo* studies; cements were mostly used (Figure 2).

According to the “publication year”, the first article published on the topic of biomodified restorative materials for dentine was in 1994 using a silicate-based cement biomodified with hydroxyapatite. After 2000, there was a rapid increase in the number of publications on the topic, including different types of materials being studied, as shown in Figure 3. Also, the distribution of journals with more than three articles published on the topic are identified (Figure 4A). *Dental Materials* was the most published journal (n=29; SJR: 5.304), followed by *Journal of Dentistry* (n=14; SJR: 4.379) and *Clinical Oral Investigations* (n=7; SJR: 3.573).

Regarding the number of publications by authors, only those with more than five articles published were identified (Figure 4B), being Xu HH the author with the highest publication number (n=20), followed by Weir MD (n = 19). Both of them tested only 2 types of materials: adhesives and composites (Figure 5). On the other hand, Sauro S with 15 publications and Osório R and Toledano M, both with 13 publications, tested all types of materials: adhesives, composites and cements. Figure 3 shows distribution of materials studied.

The affiliation country of the corresponding author in each study revealed a total of 27 countries, represented in Figure 6. USA was the country with more published articles (n=34), followed by Brazil (n=28), China (n=17) and Spain (n=14). The collaboration network by authors is shown in Figure 7, encompassing USA, China, Brazil, Spain and UK. The ten most cited articles (October 2021) in the Scopus database are shown in Table 2. Half of these were published in Dental Materials and the USA was the affiliation country of most of them (n=4). This include Melo, MA affiliation, which is the author of 2 most cited articles, both published in 2013, about biomodified adhesives.

According to substrate category, most of the studies were conducted on sound dentine (n=115), being adhesives the most tested material (Figure 8). For demineralised dentine (n=61), cements were the most tested material. Regarding

the demineralisation process, chemical models were the first choice (n=38), followed by natural caries (n=22) and microbial models (n = 5), being cements the most tested material of all of them. All type of materials were analysed in “analytical method” subcategory (Figure 9). Interface analysis was the most studied (n=105), followed by bond strength (n=86), chemical characterization (n= 60), ions releasing (n=57), hardness (n=32) and antibacterial effect (n=22). The majority of the studies included bioactivity (yes=148, no=6, no difference=5), as shown in Figure 10. The 100 most cited keywords by the authors are presented in Figure 11, being “dentin” (n=161), “materials testing” (n=97) and “dental bonding” (n=69) the most frequently observed.

4. Discussion

The growing public demand for better dental restorations has led to continuous evolution of restorative materials. The benefits of preserving natural and intact tooth tissues have gained many advocates and has shifted the paradigm of contemporary dentistry towards minimally invasive approaches (Banerjee et al., 2017) and has prompted development of new materials (Schmalz & Galler, 2017). The science of biomaterials for restorative dentistry is derived from materials science but now, interdisciplinary approaches are being adopted by researchers to expand the horizon of biomaterials and maximize their clinical benefits (Iftikhar et al., 2021). This is understandable because biomaterials science crossroads with other biological sciences. However, it might be responsible for some controversial definitions and classification, such as “biomimetic”; “bioactive”; “ion-releasing”; “smart materials”; used according to the different interaction between the materials and the tooth substrate (Owens et al., 2018; Vallittu et al., 2018). There seems to be confusion within the dental profession (scientists, practitioners and corporate members) regarding to what extent materials can be appropriately termed.

Within these classes, the materials that can leach ions, with a potential role in biomineralisation are glass polyalkenoate and calcium silicates cements (Atmeh et al., 2012; Watson et al., 2014). In this study, they were classified according to the “type of material” as “cements”. Dental composites are made of relatively biostable thermoset resin matrix and particulate glass or ceramic fillers that are not intended to leach ions or interact in some way with the interface (Maas et al.,

2017). That is why new formulations of resin composites and adhesive systems are being developed by incorporating specific compounds to improve the interaction with the dental tissues (Tahmasebi et al., 2020).

A significant rise has been observed in modification of inert materials to induce specific and intentional interactions at material / tissue interface (Schwendicke et al., 2016). The results of this study showed that the number of publications on this field has grown over the years (Figure 2), probably due to the application of nanotechnology in dentistry and dental tissue engineering (Yazdanian et al., 2021). Furthermore, the knowledge of dental caries histopathology in accordance with minimal invasive dentistry concepts might contribute to this improvement (Kidd et al., 2004; Schwendicke et al., 2019a).

The shift in the way practitioners manage diseased or damaged hard dental tissues has been very clear, however most of the current advancements in “smart dental materials” are clearly based on “*in vitro*” studies (Figure 2). For *in vivo* and *ex vivo* studies, cements were the most tested type of material, probably because they are not classified as inert material, being classified as bioactive, even when small modifications at the formulation are made (Atmeh et al., 2015; Iftikhar et al., 2021).

Sound dentine was the most tested substrate (Figure 8), where mainly adhesive materials were tested probably because they require a stable bond at interface, which is not the case when caries-affected dentine is used (Erhardt et al., 2008; Meraji et al., 2018; Nakajima et al., 2020). On the other hand, for demineralised dentine, cements were the most tested type of material. Regarding the demineralisation process, chemical models were the first choice, followed by natural caries lesion and microbial models (Figure 8C). An established chemical model for caries lesion can be found at the literature for *in vitro* studies (Moron et al., 2013; Skucha-Nowak et al., 2015). As most of the studies included in this review were *in vitro*, this choice can be explained. In these models, dentine caries has been simulated by partial demineralisation of sound dentine using pH cycling and, in some studies, total demineralisation. Microbial models contemplate the aetiology of caries lesion, but unfortunately, no specific protocol is well established (Maske et al., 2016; Pires et al., 2018; Schwendicke et al., 2019b).

We identified 3 articles with 100 or more citations in this review (Table 2), which can be classified as a “classic article” (Andersen et al., 2006). All were published at *Dental Materials*, the journal with highest number of publications on the topic (Figure 4) with an impact factor of 5.304, ranking it 8 out of 91 in Dentistry, Oral Surgery & Medicine Journals. For instance, the number of citations a publication receives could indicate other researchers’ interest in using the information for their own research (Bornmann & Daniel, 2008). The geographic distribution of publications reflects the concern and the worldwide efforts among researchers to understand and develop new formulations for this type of restorative materials (Figure 6). In the present study, the presence of 4 main clusters of research groups worldwide has been shown: United States, Brazil, China, and Spain (Figure 7). It can be regarded as impactful because of the large numbers of articles (Figure 6).

Publications mainly focused on interfacial analysis and bond strength, followed by chemical characterization, ions releasing ability, hardness recover and at least, antimicrobial activity (Figure 9). As bioactivity can be represented by a material which can improve the mechanical properties and bond strength, depending upon the dissolution behaviour of ions from the surface, these analyses were employed to evaluate the performance of these restorative materials (Schumacher et al., 2017; Khan et al., 2019). For the cements, dissolution of calcium aluminofluoro silicate glass particles promote interaction with hydroxyl apatite of dentine and the process can be considered as bioactivity (Giacaman et al., 2016; Vallittu et al., 2018). The significant use of various bioceramics in dentistry is due to their potential of controlled release of supersaturated ions of calcium and phosphate (Eliaz et al., 2017; Iftikhar et al., 2021). This may enhance the longevity of restorations, reduce the chance of bacterial growth, and increase the cell proliferation at the tissue-material interface (Sauro et al., 2016). To improve the properties of adhesives and resin composites, certain functional additives have been added to the traditional formulations, e.g., addition of bioactive components such as hydroxyapatite and bioactive glasses (Khvostenko et al., 2016; Tezvergil-Mutluay et al., 2017). In fact, one of the limitations of this review was to not explore these additives, being a recent trend of dental biomaterials research.

The final category was about the bioactivity, which almost all studies showed some potential (Figure 10). However, none of the biomodified restorative materials can be regarded as ideal for clinical indications due to the lack of *in vivo* studies. This is a large current gap in dental materials research in university laboratories, dental industry and dental practices and should be overcome in the next years.

Thus, the overall purpose of this study was to provide a comprehensive bibliometric review of the research published on biomodified restorative materials applied to coronary dentine. Most of the publications with the highest citations were mainly from the United States and published in *Dental Materials*. However, trends showed that multiple authors worked together and published papers on this topic, but there is a clear shortage of clinical studies on this field. This review will help researchers in evaluating the needs of specific materials and their properties to design future research topics. Furthermore, it might contribute to the development of new modified materials and minimize the gap between laboratories research and dental practice.

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Conflicts of interest

The authors state no conflicts of interest.

Author contributions

Conceptualization: Paula Maciel Pires, Aline de Almeida Neves. **Methodology:** Paula Maciel Pires, Lucianne Cople Maia, Salvatore Sauro, Aline de Almeida Neves. **Software:** Thamirys da Costa Rosa, Mariana Batista Ribeiro-Lage, Lucianne Cople Maia. **Validation:** Lucianne Cople Maia, Salvatore Sauro, Aline de Almeida Neves. **Formal analysis:** Salvatore Sauro, Aline de Almeida Neves.

Investigation: Paula Maciel Pires, Thamirys da Costa Rosa, Mariana Batista Ribeiro-Lage, Maysa Lannes Duarte. **Resources:** Lucianne Cople Maia, Aline de Almeida Neves. **Data Curation:** Paula Maciel Pires, Mariana Batista Ribeiro-Lage. **Writing – original draft preparation:** Paula Maciel Pires. **Writing – review and editing:** Lucianne Cople Maia, Salvatore Sauro, Aline de Almeida Neves. **Visualization:** All authors. **Supervision:** Lucianne Cople Maia, Salvatore Sauro, Aline de Almeida Neves. **Project administration:** Lucianne Cople Maia, Salvatore Sauro, Aline de Almeida Neves. **Funding acquisition: :** Lucianne Couple, Aline de Almeida Neves.

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Figures

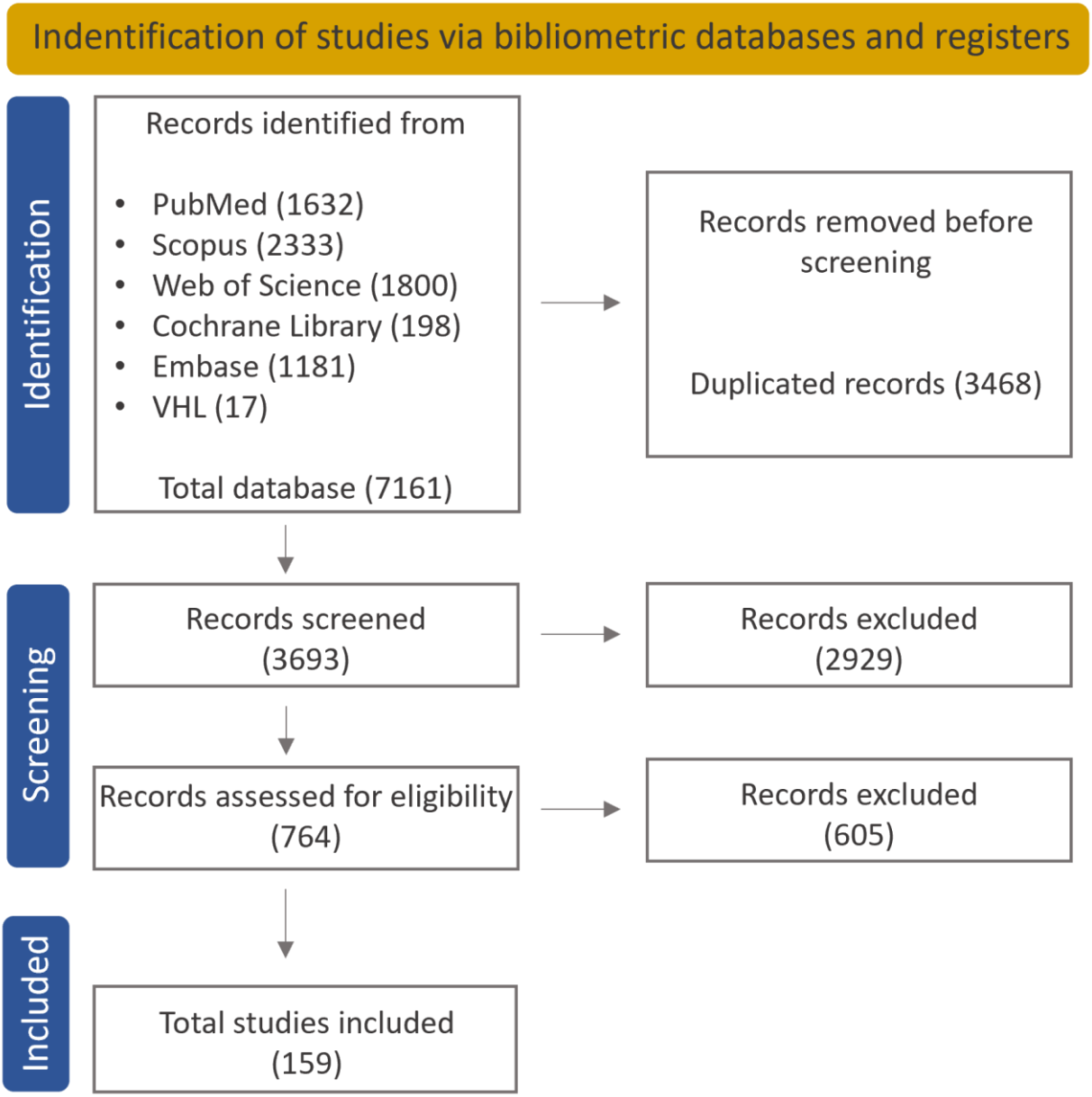


Figure 1: Diagram showing the studies included in the critical review.

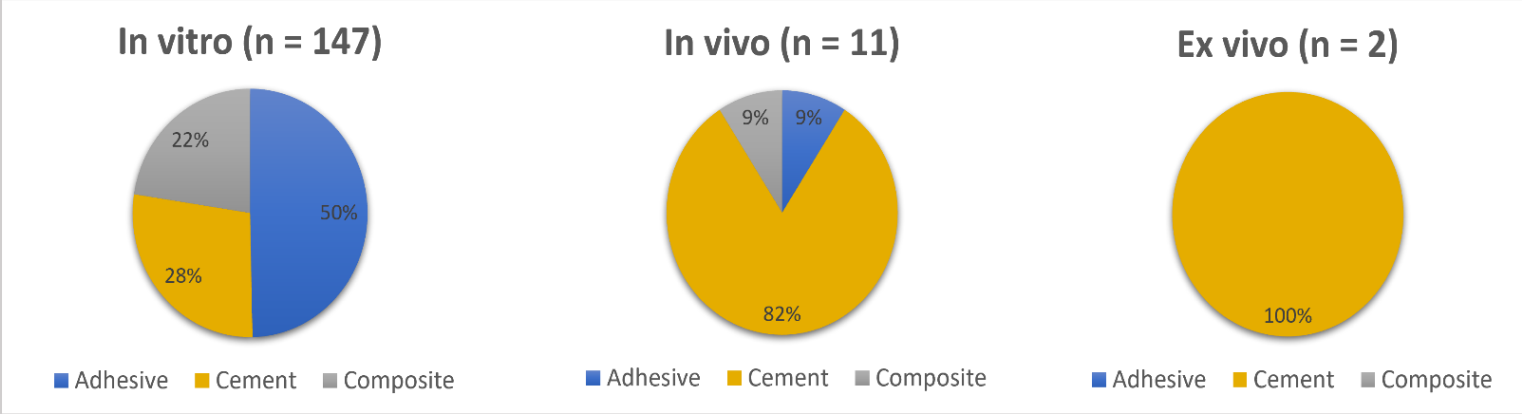


Figure 2: Distribution of type of material tested according to the study design.

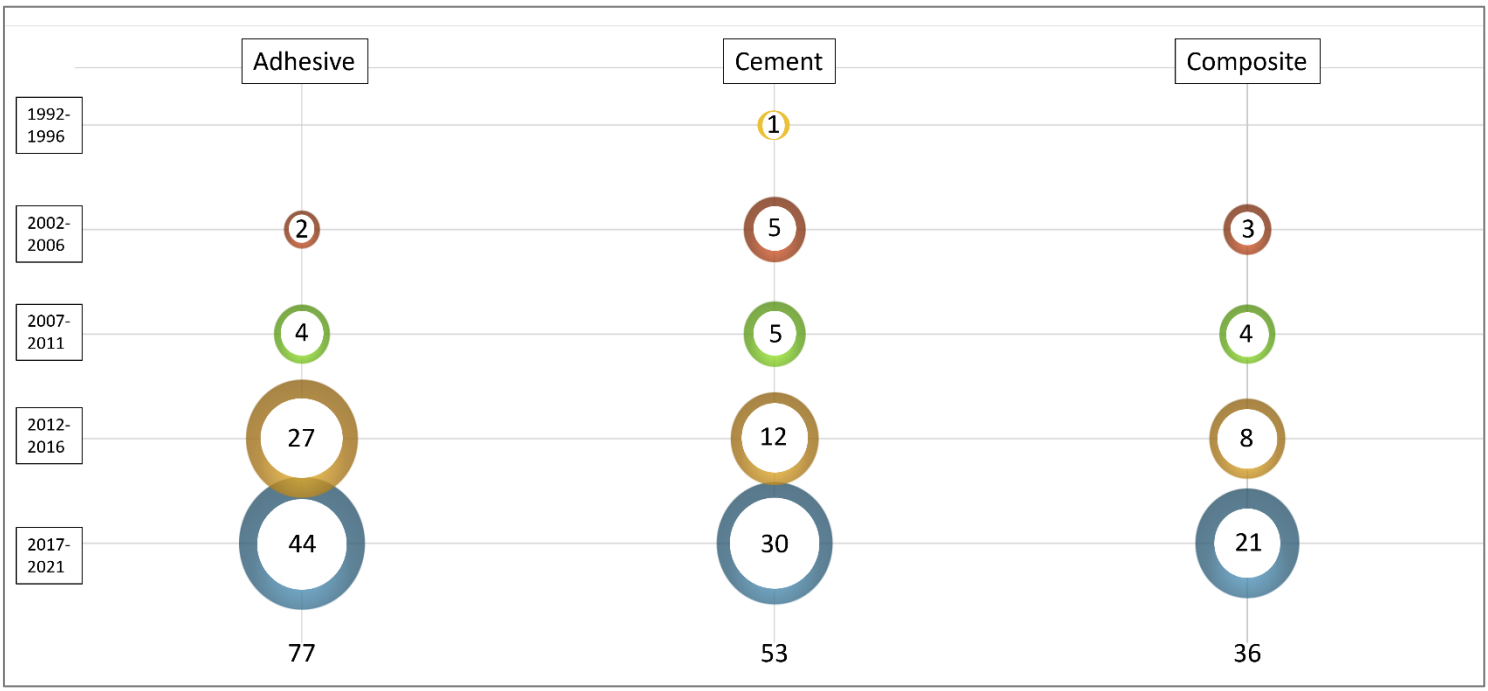


Figure 3: Growth of scientific publications over the years for type of materials tested. The larger the bubble, the higher the number of studies published in the period analysed.

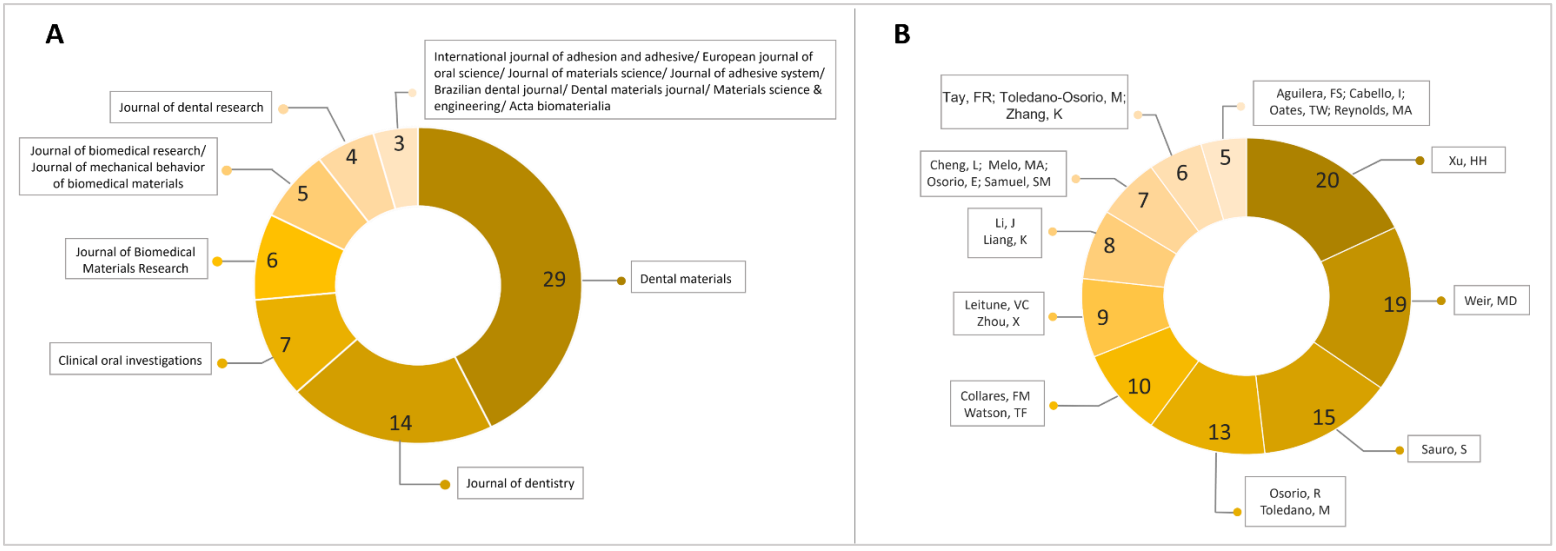


Figure 4: Publication analysis. **A)** Distribution of publication among the journals. **B)** Number of publications according to authors.

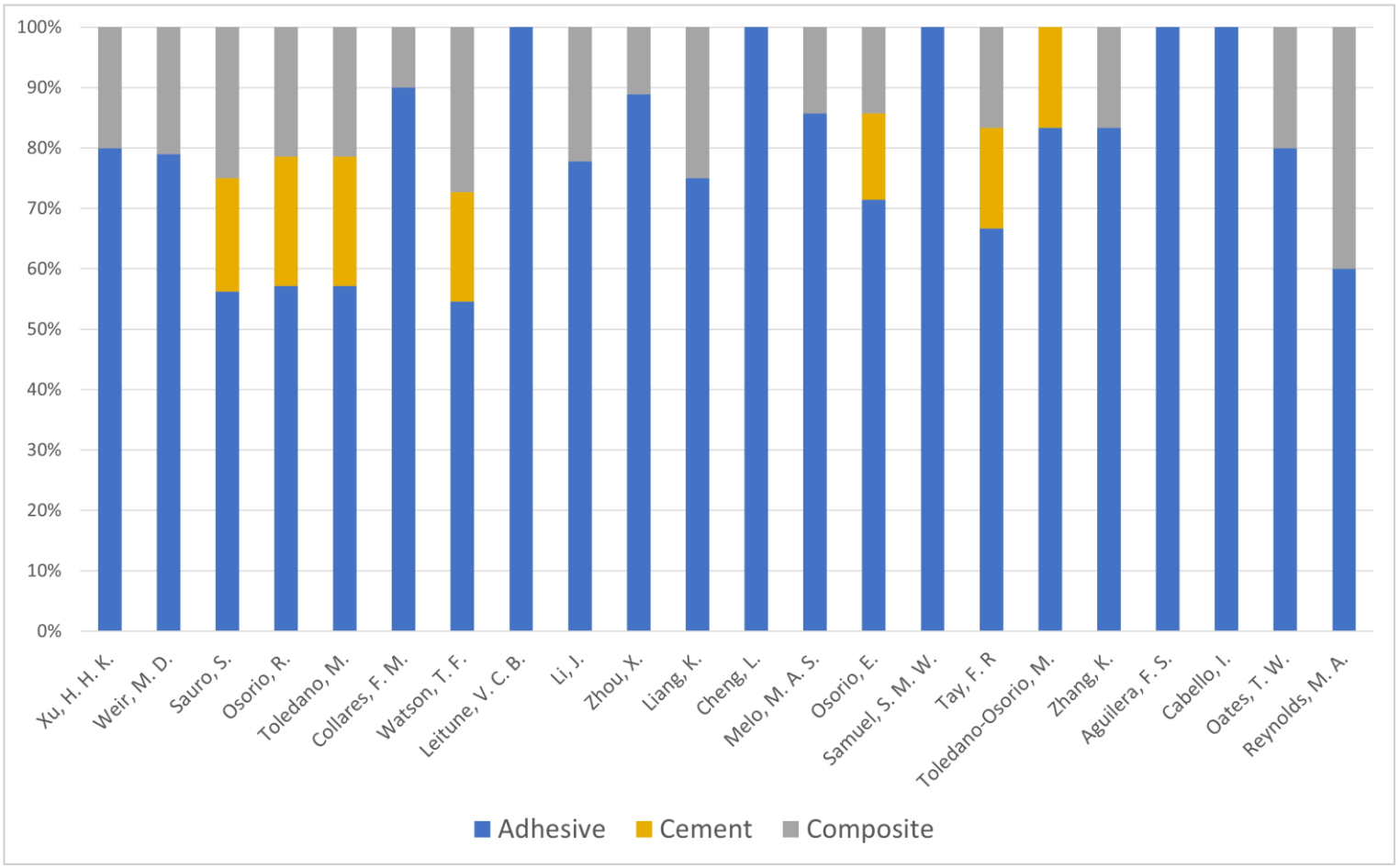
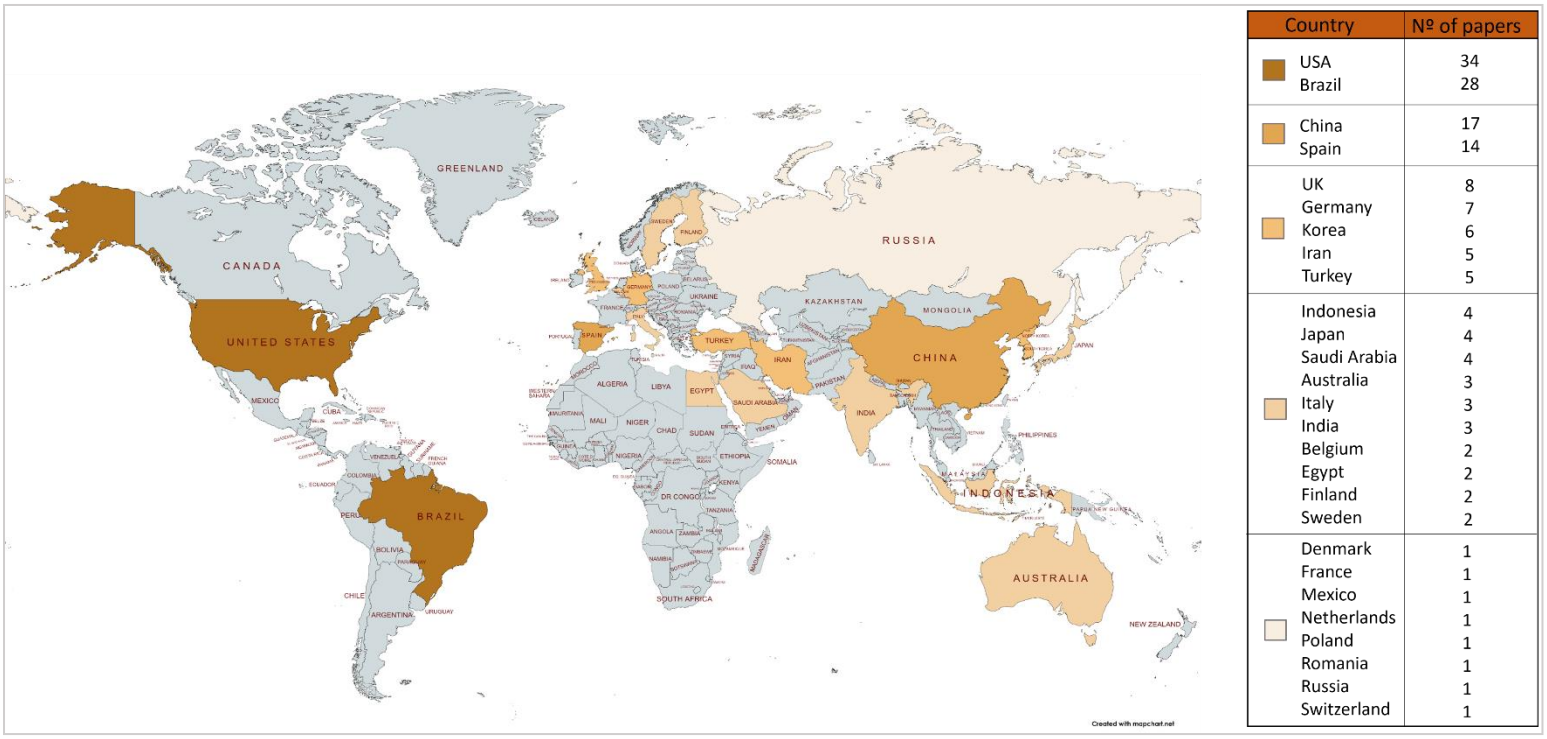


Figure 5: Distribution of type of materials tested for each author.



Country	№ of papers
USA	34
Brazil	28
China	17
Spain	14
UK	8
Germany	7
Korea	6
Iran	5
Turkey	5
Indonesia	4
Japan	4
Saudi Arabia	4
Australia	3
Italy	3
India	3
Belgium	2
Egypt	2
Finland	2
Sweden	2
Denmark	1
France	1
Mexico	1
Netherlands	1
Poland	1
Romania	1
Russia	1
Switzerland	1

Figure 6: World map with the number of studies by country. Colors represent the number of studies in each country; grey color represent countries without any publication on the subject.

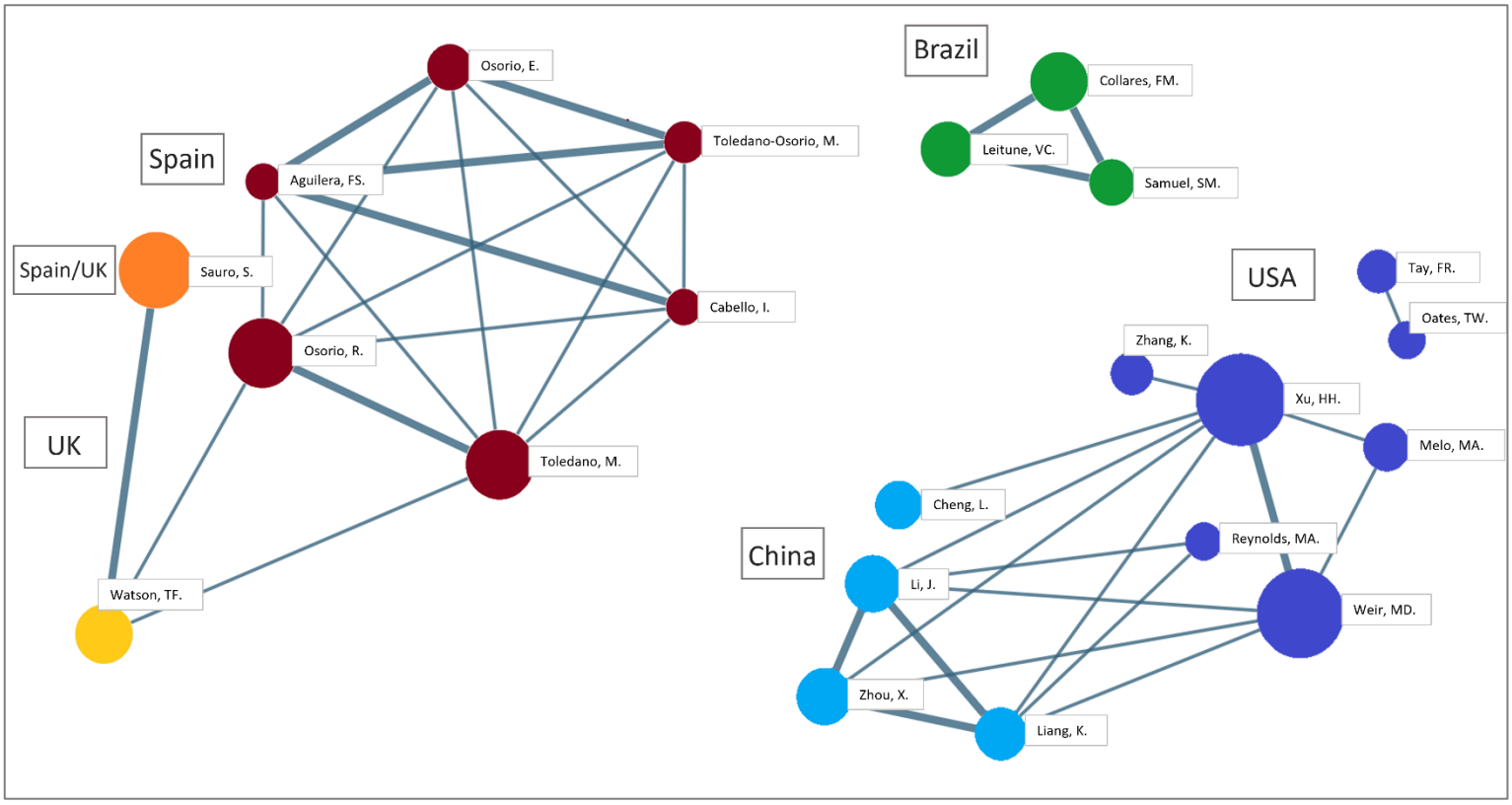


Figure 7: Network of authors collaboration.

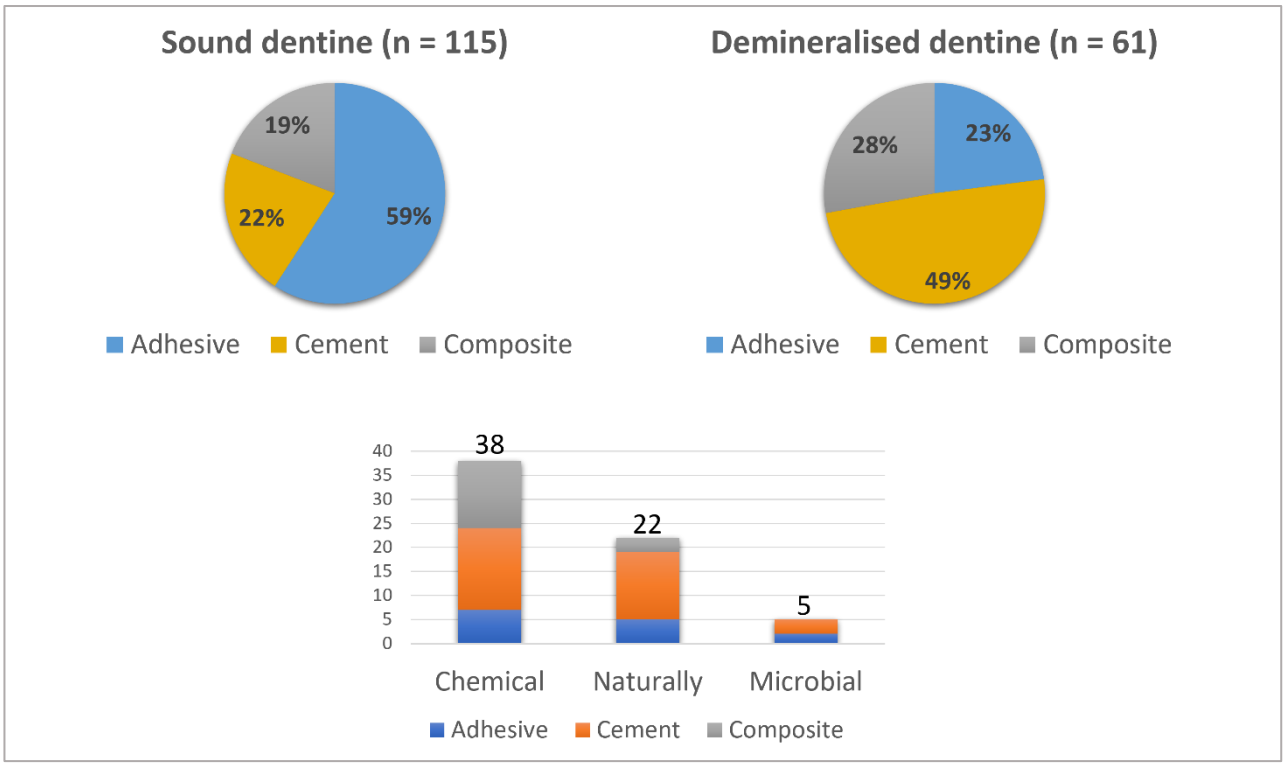


Figure 8: Distribution of substrate according to the type of material tested. **A)** Sound dentine. **B)** Demineralised dentine. **C)** Demineralisation process according to the type of material tested.

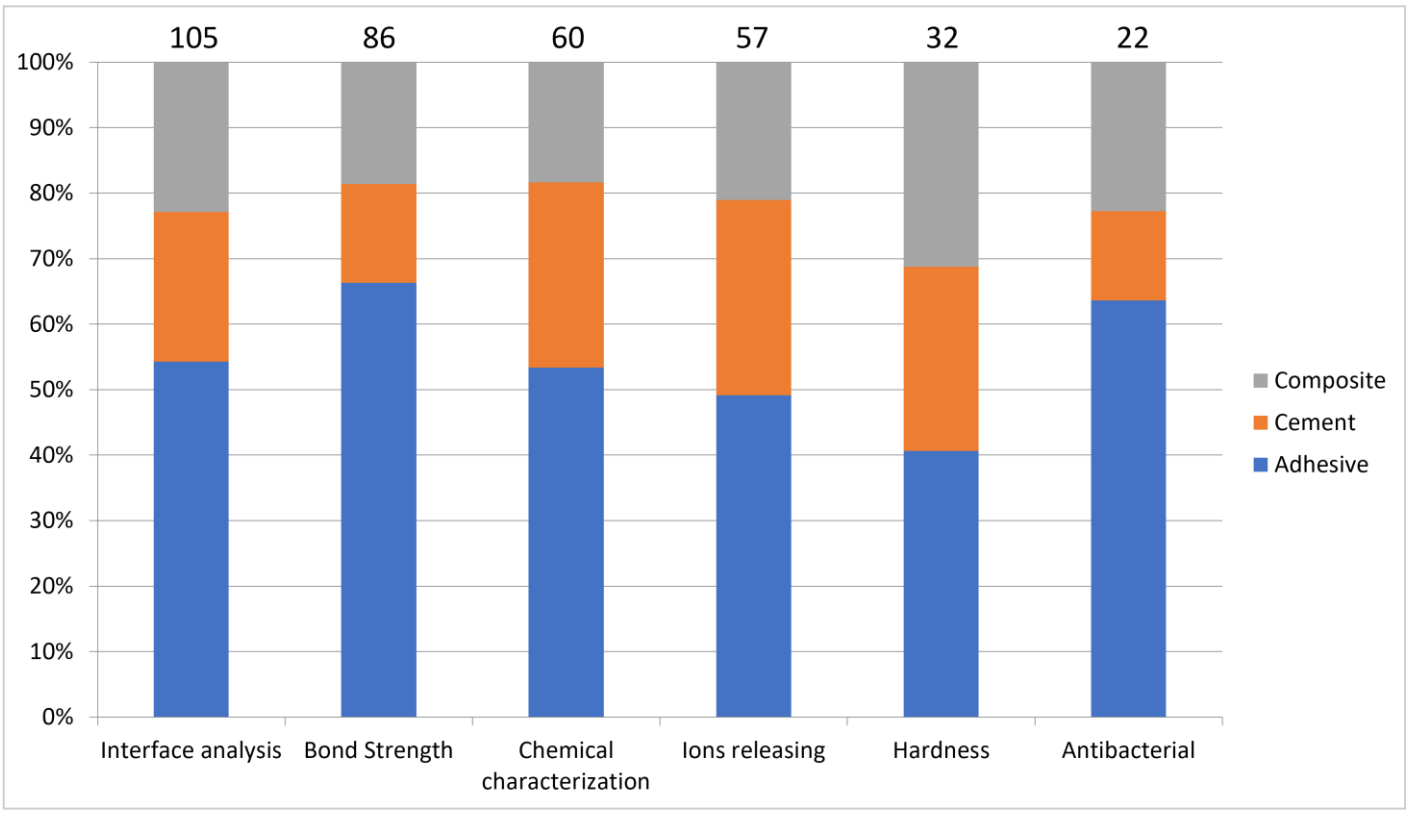


Figure 9: Distribution of analytical methods used according to the type of material tested.

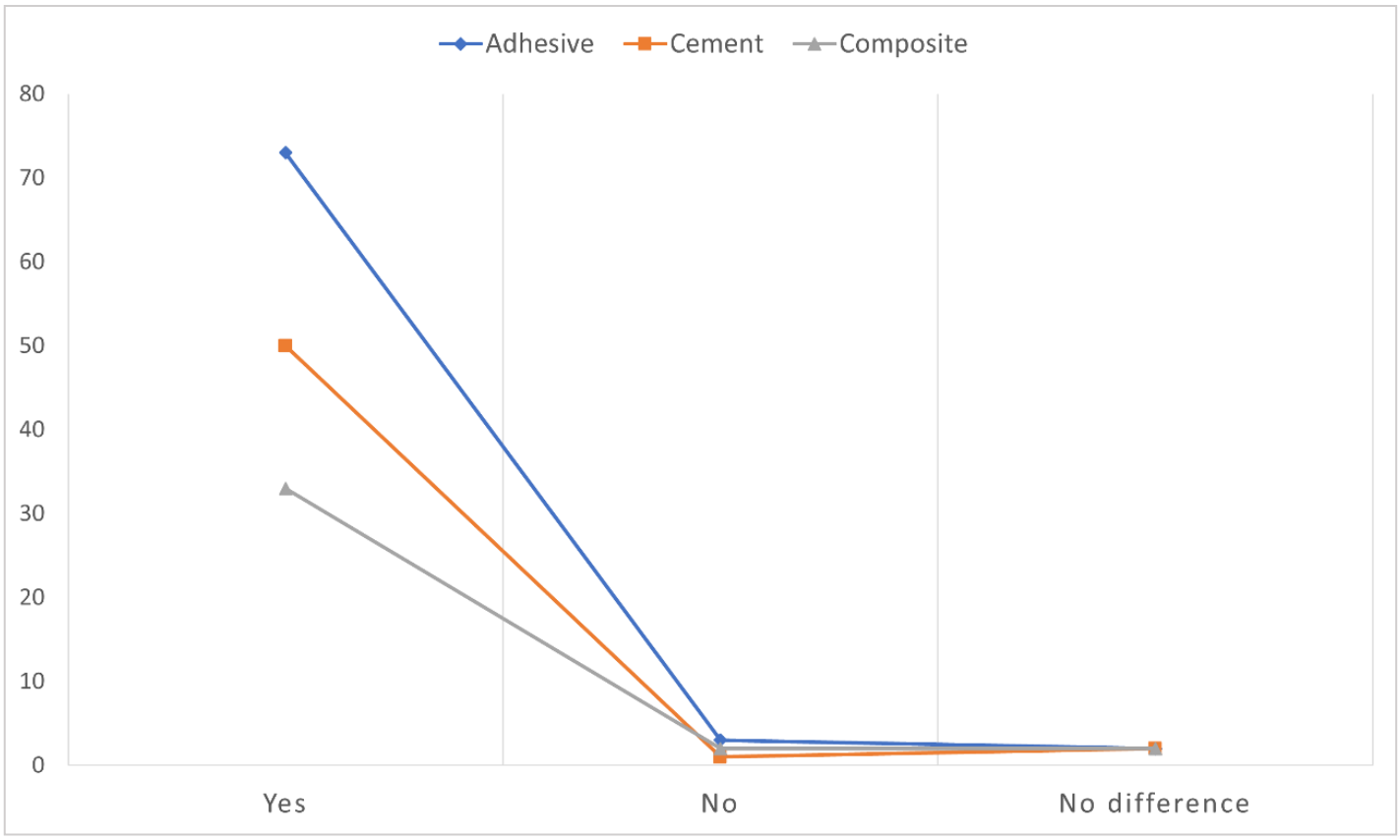


Figure 10: Presence, absence or not statiscally significant bioactivity in the materials (adhesives, cements and composites) tested by the surveyed studies.

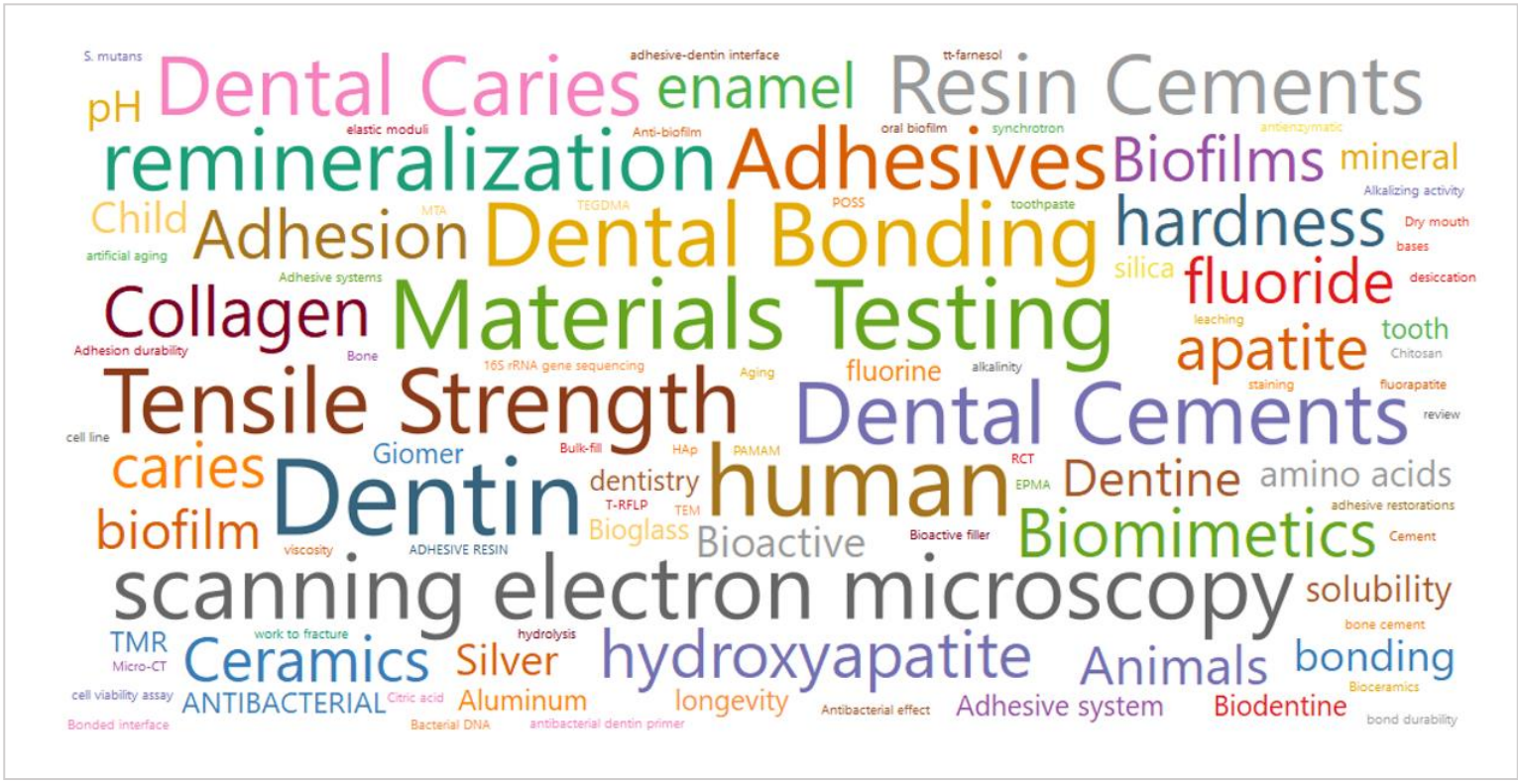


Figure 11: Network visualization per author keywords.

Tables

Table 1: Search strategy according to the different databases used in this study.

Database	Strategy
PubMed	((dentin[MeSH Terms]) OR (dentin*[Title/Abstract])) AND ((((((((((((((bioactiv*[Title/Abstract]) OR (biomater*[Title/Abstract])) OR (“ion releasing”[Title/Abstract])) OR (“ions releasing”[Title/Abstract])) OR (ion releasing material[Title/Abstract])) OR (ions releasing mater*[Title/Abstract])) OR ("Smart Materials"[Mesh])) OR ("Smart Materials"[Title/Abstract])) OR ("Smart Material"[Title/Abstract])) OR ("Biomimetic Materials"[Mesh])) OR ("biomimetic mater*"[Title/Abstract])) OR (Biomimetics[Mesh])) OR (Biomimetic*[tiab])) OR ("Smart Dentin Replacement"[Supplementary Concept])) OR ("Smart Dentin Replacement"[tiab])) OR (Dentin* Replacement[Title/Abstract]))
Scopus	(TITLE-ABS-KEY (dentin) OR TITLE-ABS-KEY (dentin*) AND TITLE-ABS-KEY (bioactiv*) OR TITLE-ABS-KEY (biomater*) OR TITLE-ABS-KEY ("ion releasing") OR TITLE-ABS-KEY ("ions releasing") OR TITLE-ABS-KEY ("ion releasing material") OR TITLE-ABS-KEY (ions AND releasing AND mater*) OR TITLE-ABS-KEY ("smart materials") OR TITLE-ABS-KEY ("smart material") OR TITLE-ABS-KEY ("biomimetic materials") OR TITLE-ABS-KEY (biomimetic AND mater*) OR TITLE-ABS-KEY (biomimetics) OR TITLE-ABS-KEY (biomimetic*) OR TITLE-ABS-KEY ("smart dentin replacement") OR TITLE-ABS-KEY (dentin*replacement))
Web of Science	TS=(dentin) OR TS=(dentin*) AND TS=(bioactiv*) OR TS=(biomater*) OR TS=(“ion relasing”) OR TS=(“ions releasing”) OR TS=(“ion releasing material”) OR TS=(ions releasing mater*) OR TS=(“smart materials”) OR TS=(“smart material”) OR TS=(“biomimetic materials”) OR TS=(biomimetic mater*) OR TS=(biomimetics) OR TS=(biomimetic*) OR TS=(“smart dentin replacement”) OR TS=(dentin*replacement)
Cochrane Library	ID Search Hits #1 MeSH descriptor: [Dentin] explode all trees 1260 #2 (dentin*):ti,ab,kw 4468 #3 (bioactiv*):ti,ab,kw 2166 #4 (biomater*):ti,ab,kw 573 #5 ("ion releasing"):ti,ab,kw 10 #6 ("ions releasing"):ti,ab,kw 0 #7 ("ion releasing material"):ti,ab,kw 2 #8 (ions releasing mater*):ti,ab,kw 5 #9 MeSH descriptor: [Smart Materials] explode all trees 1 #10 ("Smart Material"):ti,ab,kw 1 #11 MeSH descriptor: [Biomimetic Materials] explode all trees 1340 #12 (biomimetic mater*):ti,ab,kw 54 #13 MeSH descriptor: [Biomimetics] explode all trees 11 #14 (Biomimetic*):ti,ab,kw 124 #15 ("Smart Dentin Replacement"):ti,ab,kw 7 #16 (Dentin* Replacement):ti,ab,kw 71 #17 #1 OR #2 4468 #18 #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 4208 #19 #17 AND #18 198
VHL (BBO / LILACS)	(mh: dentin OR dentin*) AND (bioactive* OR biomater* OR “ion releasing” OR “ions releasing” OR “ion releasing material” OR ions releasing mater* OR mh: “smart materials” OR “smart material” OR mh: “biomimetic materials” OR biomimetric mater* OR mh: biomimetics OR biomimetic* OR “smart dentin replacement” OR dentin*replacement) AND (db:("LILACS"))
EMBASE	dentin:ti,ab,kw OR dentin*:ti,ab,kw AND bioactiv*:ti,ab,kw OR biomaterial:ti,ab,kw OR biomater*:ti,ab,kw OR 'ion releasing':ti,ab,kw OR 'ions releasing':ti,ab,kw OR 'ion releasing material':ti,ab,kw OR 'ions releasing mater*':ti,ab,kw OR 'smart materials':ti,ab,kw OR 'smart material':ti,ab,kw OR 'biomimetic materials':ti,ab,kw OR 'biomimetic material':ti,ab,kw OR biomimetics:ti,ab,kw OR biomimetic*:ti,ab,kw OR 'smart dentin replacement':ti,ab,kw OR 'dentin* replacement':ti,ab,kw

Table 2: Ten most cited articles published, their affiliation country, first author, publication year, journal, type of material tested, and times cited.

Study	Country	First Author	Year	Journal	Material	Times cited
Hydroxyapatite nanorods as novel fillers for improving the properties of dental adhesives: Synthesis and application	Iran	Sadat-Shojai, M	2010	Dent Mater	Adhesive	181
Mechanical properties and biochemical activity of remineralizing resin-based Ca-PO4 cements	USA	Dickens, S.H	2003	Dent Mater	Cement	155
Novel dental adhesives containing nanoparticles of silver and amorphous calcium phosphate	USA	Melo, MA	2013	Dent Mater	Adhesive	132
Toughness, bonding and fluoride-release properties of hydroxyapatite-added glass ionomer cement	Japan	Lucas, ME	2003	Biomater	Cement	99
Novel dental adhesive containing antibacterial agents and calcium phosphate nanoparticles	USA	Melo, MA	2013	J Biomed Mater Res B Appl Biomater	Adhesive	90
Therapeutic effects of novel resin bonding systems containing bioactive glasses on mineral-depleted areas within the bonded-dentine interface	UK	Sauro, S	2012	J Mater Sci Mater Med	Composite	90
Biomimetic remineralization of human dentin using promising innovative calcium-silicate hybrid "smart" materials	Italy	Gandolfi, MG	2011	Dent Mater	Composite	84
Anti-biofilm dentin primer with quaternary ammonium and silver nanoparticles	USA	Cheng, L	2012	J Dent Res	Adhesive	80
Antibacterial activity and ion release of bonding agent containing amorphous calcium phosphate nanoparticles	China	Chen, C	2014	Dent Mater	Adhesive	76
Remineralization of artificial dentinal caries lesions by biomimetically modified mineral trioxide aggregate	China	Qi, YP	2012	Acta Biomater	Cement	75

4.2 Artigo 2: Dentine mineral changes induced by polyalkenoate cements after different caries removal techniques: An *in vitro* study.

Short Title: Dentine mineralisation of polyalkenoate cements after caries removal

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Declarations of interest:

None

Abstract

Objective: To evaluate the self-limiting properties of caries-removal techniques and mineral changes induced by two polyalkenoate cements in dentine.

Materials and Methods: Five dentine cavities were prepared in 10 sound molars (n=50). Carious lesions were induced using a microbial protocol and excavation was performed through different approaches: 1) bur; 2) hand excavator; 3) Papacárie Duo®; 4) Brix3000®. In a control group, carious dentine was untreated. Half cavities (n=5) were restored using a zinc polycarboxylate cement (ZPC) and the other with a glass ionomer cement (GIC). Specimens were stored under simulated pulpal pressure and immersed in PBS for 45 days. Scanning with a micro-CT was performed after caries formation, excavation and restoration. Cavity volumes (mm^3) and % changes in density were calculated. Statistical analysis was performed with a 5% significance. Further, specimens were prepared for SEM and FTIR (n=8).

Results: Baseline caries and cavity volume increase after caries-removal was similar for all excavation groups. A significant interaction was seen between the material and dentin density, with ZPC resulting in higher values than GIC (33.6% X 6%, $p<0.01$). SEM revealed that the chemo-mechanical technique produced a smoother dentine. FTIR showed silicon-based minerals in GIC-treated dentine while ZPC-treated presented mineral deposits, suggestive of amorphous calcium phosphate. In both cases, carbonate was detected.

Conclusions: All caries-removal techniques allowed minimally invasive excavation. ZPC showed higher density increase within caries-affected dentine via deposition of calcium-phosphates, compared to GIC.

Clinical Significance: Polyalkenoate cements are suitable materials to induce mineral recovery of dentine after minimally invasive caries removal.

Keywords: Dental Caries; Dentine; Papain; Polyalkenoate Cements; Tooth Remineralisation.

1. Introduction

The current knowledge and understanding of dental caries aetiology have encouraged the management of minimal intervention concepts to handle this disease to the point that such concepts are nowadays supported by the WHO (World Health Organization, 2017). Regarding the most appropriate method for caries removal, there is insufficient evidence to recommend any specific method (Schwendicke et al., 2018), but a consensus among clinicians and researchers indicates that only infected and irreversibly destroyed dentine should be removed, leaving residual caries-affected dentine to be repaired (Schwendicke et al., 2019).

Several clinical techniques are currently available for removal of caries lesions, but the most common and widespread methods seem to be based on the use of dental burs and hand excavators (Banerjee et al., 2000a). Unfortunately, such methods are not self-limiting in the removal of caries-infected dentine; these still involve active discriminatory action from the operator (Banerjee et al., 2000b). Chemo-mechanical caries removal agents have been demonstrated to be more self-selective, and therefore, more accurate in removing infected/irreversibly destroyed tissue, preserving the caries-affected dentine (Neves et al., 2011a; Ali et al., 2018). Papain-based agents are currently popular in some parts of the world and among these, two commercial products are currently available on the market: Papacárie Duo Gel® (Fórmula & Ação, São Paulo, SP, Brazil) and Brix3000® (Brix Medical Science, Carcarañá, Santa Fe, Argentina).

The residual carious dentine at the bottom of the cavity prepared through minimally invasive approaches is characterised by a low mineral content and partially demineralised dentine collagen fibrils (Banerjee et al., 2000c; Neves et

al., 2011b). Therefore, the use of ion-releasing materials has been advocated to remineralise such mineral-depleted caries-affected substrate (Pires et al., 2020). Moreover, the use of ion-releasing restorative materials, capable of bonding to this structurally altered tissue, may also prevent hydrolytic or enzymatic degradation, and increase the longevity of the restoration (Tjaderhane et al., 2013; Sauro et al., 2016a; Tezvergil-Mutluay et al., 2017). It is well-known that polyacrylic acid (pAA) has a predisposition to bind cations and stabilise amorphous calcium phosphate nano-precursors (Hoppe et al., 2011; Liu et al., 2011a; Qi et al. 2012; Ma et al., 2014). It is interesting to consider that polyalkenoate cements such as zinc polycarboxylate (ZPC) and conventional glass-ionomers (GIC), are constituted by a liquid phase of polycarboxylic acids such as polyacrylic acid. These materials are also advocated for pulp protection (Pires et al., 2018; Pires et al., 2020; Neves *et al.,* 2011b), but there is still little information on their remineralising capability when used for dentine replacement after selective carious tissue removal.

Thus, the aim of this study was to evaluate the self-limiting properties of different excavation techniques in simulated dentine caries lesions, as well as the ability of different polyalkenoate cements to induce dentine remineralisation. The first hypothesis was that all excavation methods would be equally able to remove the dentine carious tissue. The second one was that both ZPC and conventional GICs would have the same ability to promote density and mineral changes in demineralised dentine.

2. Materials and Methods

2.1. Sample preparation

Sound human molars were extracted for periodontal reasons under the protocol of the present study that was approved by the Institution's Ethical Committee at government databases (n.54941416.9.0000.5257) and after obtaining the patient's written consent. All extracted teeth were stored in distilled water at 5˚C for no longer than 3 months.

Complete removal of occlusal enamel was performed using a 300 µm thick diamond disk mounted on a slow speed cutting machine (Isomet, Buehler, Lake Bluff, IL, USA) in order to obtain a flat occlusal dentine surface. A second parallel cut was performed 2 mm underneath the enamel-cementum junction to expose the pulpal chamber. Sample size determination was based on a previous study (Pires et al., 2018) and five occlusal standardised dentine cavities (1.5 mm diameter and 1 mm depth) were created in 10 teeth (total cavities number = 50) with a round diamond bur under copious water-cooling, using a dental turbine mounted on a device to perform standardised dental cavities (LABiom-R, UFF, Niterói, RJ, Brazil). Subsequently, the dentine surface was covered with an acid-resistant varnish, leaving only the cavities exposed to the cariogenic challenge.

2.2. Artificial caries formation by multispecies ATCC biofilm model

The protocol for artificial caries formation used in this study was in accordance with that previously described by Antonio et al., (2011) and Pires et al., (2018). In brief, the specimens were fixed in 24-well polystyrene plates (TPP, Zellkultur Testplatte 24F, Trasadingen, Switzerland) and sterilised under UV light for 40 minutes before receiving the microbial inoculum. The following bacterial samples

were used to prepare the inoculum: *Streptococcus mutans* (ATCC 25175); *Streptococcus sanguis* (ATCC 20556), *Streptococcus salivarius* (ATCC 7073) and *Lactobacillus casei* (ATCC 431). Isolated bacterial colonies were selected and transferred to BHI solution until reaching an optical density between 0.08 to 0.13 in a spectrophotometer. Each well containing a single tooth specimen received 20 µL of the inoculum and was filled with Brain-Heart Infusion (BHI) growth media with the addition of 5% sucrose. The system was incubated in microaerophilia for 10 days at 37°C to allow biofilm growth. Every 24 hours, the growth media in each well was refreshed and the pH was monitored. All procedures were performed inside a laminar air-flow chamber, under an aseptic environment. At the end of the incubation period, the specimens were cleaned from the biofilm formed on the dental surface via immersion in an ultrasonic bath.

2.3. Excavation procedures

After the microbial cariogenic challenge, the carious cavities in each tooth specimen were randomly treated with a different excavation method (10 per group): low-speed handpiece, hand excavator or the two papain-based chemo-mechanical agents, Papacárie Duo Gel® (Fórmula & Ação, São Paulo, SP, Brazil) and Brix3000® (Brix Medical Science, Carcarañá, Santa Fe, Argentina). Moreover, one carious cavity in each specimen was assigned to the negative control group and was left unexcavated. Regarding the handpiece method, the carious dentine tissue was mechanically removed using a round tungsten-carbide bur (FG2, KG Sorensen, Cotia, SP, Brazil) mounted in a contra-angle slow-speed handpiece (KaVo Dental, Biberach, Germany). Hand excavation was accomplished using a spoon excavator (Millennium N5, Golgran, São Caetano do Sul, SP, Brazil) in circular scratching movements, as previously described

(Banerjee et al., 2017; Dorri et al., 2017; Tedesco et al., 2018) until a firm dentine tissue was reached. Regarding chemo-mechanical caries removal methods, the carious cavity was covered with Papacarie Duo Gel® for 40 seconds or with Brix 3000® gel for 2 minutes, according to respective manufacturer's instructions, and the carious dentine was excavated with an unsharpened spoon excavator without pressure, to remove the softened carious tissue (Santos et al., 2020). Finally, the gels were cleaned, and a second portion of gel was then applied repeating the same instructions. The threshold for caries removal was, in all techniques, given by the inability of the excavator in removing any extra soft tissue (Banerjee et al., 2017). All excavation procedures were performed by a single operator who was previously well trained on the use of such excavation methods.

2.4. Restoration procedures with polyalkenoate cements

Each excavated cavity was restored using two different polyalkenoate cements (25 cavities each group): 1) zinc polycarboxylate cement (Poly Zinc, Prevest DenPro, Jammu, India); 2) glass ionomer cement (Ketac Molar, 3M ESPE, Seefeld, Germany). Both materials were hand mixed according to the manufacturer's instructions and directly applied to the cavities. Subsequently, the specimens were kept under simulated intrapulpal pressure (20 cm H_2O) using a simulated body fluid solution (pH 7.4) at room temperature and 100% relative humidity for 45 days, according to a previously described methodology (Scheffel et al., 2014).

2.5. Micro-CT analysis of cavity volume and density changes

All the specimens were scanned after the cariogenic challenge (baseline stacks) and after caries removal (excavated stacks) using a high energy micro-CT

(Skyscan 1173, Bruker, Kontich, Belgium) with the following acquisition parameters: 70kV, 114µA source current, 14.25µm pixel size, sensor with 2240x2240 pixels, 1mm-thick Al filter, 0.5° rotation step over 360°, frame averaging (5) and random movements (40). A third acquisition was performed (restored stacks) after 45 days of SBF storage in all the specimens restored with the tested materials.

Three image stacks were obtained from each specimen (baseline, excavated and restored) and spatially recorded with an affine algorithm and 10 degrees of freedom using the 3D Slicer 4.10.2 software interface, as previously described (Pires *et al.,* 2018). To calculate the excavated cavity volumes among the caries removal techniques used in this study, volumes of interest (VOIs) of each cavity were extracted, Gray values were normalised, and a fixed threshold was chosen (40) and applied in the baseline and excavated stacks in order to obtain volumes (mm^3) (Figure 1).

To obtain density values at the residual caries layer immediately after caries removal (excavated stacks) and after treatment with the cements (restored stacks), the previously obtained cavity volumes were duplicated, widened with 5 interactions, subtracted from each original cavity volume stacks and the resulting VOI was used as a mask to calculate density values (in 8-bit grayscale) around the internal surface cavity (carious, residual dentine and caries-affected dentine after restoration) in each stack (Figure 2), as described in previous study (Pires *et al.,* 2018). The percentage of these density changes in residual and caries-affected dentine were calculated based on baseline values. These analyses were performed by a single operator that was blinded regarding excavation technique or treatment groups (AAN). After all micro-CT scanning procedures, the

specimens were transversally cut along the centre of the cavities, gold-sputtered and imaged using a field-emission scanning electron microscope (6460LV, JEOL, Tokyo, Japan) to analyse the ultra-morphology on the material-dentine interface.

2.6. SEM and FTIR-ATR analysis

Flat sound dentine surfaces were specifically prepared for this analysis (n = 8 dentine discs, thickness 2 mm), and two were assigned to each experimental condition tested in this study: sound dentine, demineralised dentine, ZPC and GIC. The dentine specimens were demineralised in phosphoric acid 10% during 48 h, except for those in the control group (sound dentine), which were wrapped in a wet paper (deionised water) and stored at 4ºC until SEM and FTIR analysis. For ZPC and GIC groups, the dentine discs were placed in direct contact with the cements and left undisturbed until the GIC and ZPC were totally set. The chemical mapping was performed using a FTIR system (Spectrum Spotlight FTIR-ATR imaging system; Perkin Elmer) with a resolution of 4 cm^{-1} before and after 45 days in artificial saliva (AS) storage (Sauro et al., 2016b). The peaks were analysed subsequent to baseline subtraction and normalization using the Spectrum 10™ software (Perkin Elmer), in order to identify the most characteristic inorganic compounds in the specimens. After the FTIR analysis, the specimens were gold-sputtered and imaged using a field-emission scanning electron microscope (6460LV, JEOL, Tokyo, Japan) to image the presence and the ultramorphology of the demineralised dentine after storage.

2.8. Statistical analysis

Shapiro–Wilk test was used to test the normality of the obtained data followed by one-way ANOVA and paired t-tests to compare the cavity volume and density

changes at different time-set. A significance level of 0.05 was used throughout the analysis that was carried out using Bioestat v.5.3 (Instituto Mamirauá, Manaus, AM, Brazil).

3. Results

The results obtained through the micro-CT analysis are presented in Table 1. Overall, the baseline values for cavity volume attained in each experimental caries removal group were similar ($p>0.01$). For all selective caries excavation procedures, mean cavity volume significantly increased for all techniques ($p<0.01$), but there was no significant difference in cavity volume among the tested techniques ($p>0.01$). Regarding density values in residual dentine (Table 2), these increased after caries excavation, without any significant difference between the caries removal techniques ($p>0.01$). SEM images of experimental surfaces after caries excavation (Figure 3) showed that caries removal with round burs mounted on a slow handpiece (Figure 3A), as well as those obtained with hand excavation (Figure 3B) resulted in a rougher dentine surface compared to the specimens treated using the chemo-mechanical methods (Figure 3C and 3D).

Regarding the increase in density after treatment using the tested ion-releasing cements (Table 3), ZPC showed higher increase in density values at the residual dentine cavity compared to GIC ($p<0.01$). SEM images of the control group cavities (without caries removal) after the SBF storage period in contact with the restorative materials showed a high contrast region in proximity of the ZPC surface layer (Figure 4A, white arrow), resembling an increase of mineral density; this was not observed in the control group restored with GIC (Figure 4B). A graphic representation of mean pooled density values distribution of ZPC

compared to GIC at baseline, excavated and restored specimens is shown in Figure 5.

FTIR analysis (Figure 6) confirmed the ability of the tested cements to deposit minerals after AS storage. Indeed, a clear deposition of silicon-based substance (Si-O:1060 cm^{-1}) was seen in the specimens treated with GIC, while those treated with ZPC showed a likely deposition of amorphous calcium-phosphates (stretching absorption band at 1,200-900 cm^{-1}), along with the presence of carbonates, which was observed in both GIC and ZPC groups. Moreover, peaks at 870 cm^{-1}, 1408 cm^{-1}, 1445 cm^{-1} indicating bands of carbonate (CO_3^{2-}) substitution in apatite were detected especially in sound dentine; this latter was also characterised by P-O vibrational peaks at 970 cm^{-1}, 1016 cm^{-1} and 1100 cm^{-1}. Amide I, II and III in dentine collagen were represented by absorption peaks at 1645 cm^{-1}, 1450 cm^{-1} and 1245 cm^{-1}, respectively. SEM images confirmed the presence of minerals on the dentine surfaces, which completely covered the dentine tubules (Figure 6A and 6D). Conversely, the demineralised dentine was characterised by patent dentinal tubules with no presence of mineral clusters on the dentine surface (Figure 6C). Sound dentine showed a consistent smear layer on the surface produced during cutting and polishing procedures (Figure 6B).

4. Discussion

The results of the present study indicate that the different selective caries removal techniques may have similar outcomes in dentine, therefore the first hypothesis must be accepted. Conversely, the second hypothesis was rejected, as there was a significant difference in density values between the different tested polyalkenoate cements.

Although this was an *in vitro* study, the microbial protocol employed to generate artificial dentine caries in dentine was able to mimic a clinical scenario (Simon-Soro *et al.,* 2015). Although pH-cycling models are widely used in *in vitro* studies (Arnold et al., 2007; Yu et al., 2017), the microbiological method seems to be more appropriate to simulate natural dentine caries lesions (Marquezan et al., 2009; Enrich-Essvein et al., 2021). This was corroborated in the present study, as the cariogenic challenge resulted in homogeneous demineralisation layer in all specimens (Table 1 and 2), that could be excavated similarly to a typical clinical scenario (Figure 3).

Optimal standardisation of the specimens when using a non-destructive micro-CT method involves spatial registration. This is an important methodological aspect for this type of *in vitro* experimental studies. In this case, exact in loco measurement of density changes between baseline, excavated and treated specimens were obtained. Therefore, each specimen served as own control, as all excavation methods were accomplished in the same substrate, reducing the need of a large sample size to account for variability in mineral density concentration. Regarding the calculation of the sample size, a previous study (Pires *et al.,* 2018), where mineral density change data (Liu *et al.* 2011b) was used to calculate sample size, estimated at 9 specimens per group. To round up this number, the current study was performed by using 10 teeth per group, resulting in 50 cavities totally.

Typically, caries excavation is performed using handpieces and burs or hand excavators (Banerjee *et al.,* 2013). The most used caries removal threshold used by dental practitioners to perform a clinical excavation of the infected dentine is the hardness/texture of the tissue. Unfortunately, this approach lacks

standardisation as it may be quite subjective and it may vary among individuals (Celiberti et al. 2006). Such variations may have clinical implications, including differences in the size of the cavities, as well as the mechanical properties of the remaining tooth structure (Schwendicke *et al.,* 2018).

In the present study, all the tested methods were able to remove similar amounts of carious dentine (Table 1 and 2). According to Schwendicke *et al.,* 2018, although mechanical rotatory techniques are not self-discriminatory, dentists can be highly trained at using conventional removal methods, justifying that an adept operator can effectively practice minimally invasive operative caries management strategy using these instruments. Alternative techniques, such as chemo-mechanical caries removing agents (Banerjee *et al.* 2000b; Neves *et al*. 2011a) may represent a relatively more standardised method for selective caries removal, as in agreement with the modern concepts of minimally invasive dentistry (Neves *et al.,* 2015).

In the case of papain-based agents, a greater selectivity in caries removal was associated to the specific enzymatic action of papain (Bussadori *et al.,* 2014). Indeed, degraded collagen in caries-infected dentine lacks the inhibitor α1-anti-trypsin, and this allows papain to break down denatured dentine collagen in caries-infected dentine, enabling its removal with hand instruments (Bussadori *et al.,* 2008). Papacárie Duo Gel®, used in this study, is a gel containing 3% papain, 0.5% chloramine and toluidine blue. Conversely, Brix3000® contains 10% of papain in a buffer emulsion technology, which keeps the gel at an ideal pH, so that papain is released only when collagen proteolysis occurs (Alkhouli et al., 2020).

The SEM morphology of the dentine surface revealed important differences in residual tissue (Figure 3). In a selective minimal invasive approach, partial removal of caries preserves more dental tissues, reduces the incidence of pulpal exposure in deep cavities and may favour the formation of tertiary dentine after restoration (Banerjee et al., 2013). The remaining partially demineralised collagen within the caries-affected dentine may play an essential role in terms of remineralisation, since it may act as an active template for crystal deposition upon ion releasing from remineralising restorative materials (Gower, 2016 and Sauro et al., 2016a). Conventional methods for caries removal are well established in literature and clinical practice (Banerjee et al., 2000c) and several studies demonstrated that, similar to the conventional methods od caries removal, papain-based gel treatments do not interfere with the bond strength of adhesive restorative materials applied to sound and/or demineralised dentine (Gianini *et al*., 2010; Botelho *et al.,* 2011).

Preparation of minimally invasive cavities should be ideally supported by therapeutic materials able to offer protection to the tissue interface and induce mineralisation. Both cements used in this study induced an increase in micro-CT density values after the experimental period, although ZPC showed greater values (33.66%) compared to GIC (6.03%). The nature and the process of the mineral formation in this tissue is still unknown, but it seems that an important role was played by ions released from the tested materials, which may have bonded and reinforced the dental tissue (Giannini & Sauro, 2021 and Cascales et al., 2022).

Polyalkenoate cements set by acid–base reaction (including ZPC and GICs) are dental materials that demonstrate chemical adhesion to mineralised tooth

structures, such as dentine and enamel (Anusavice *et al.,* 2012). The bonding process occurs between the calcium in hydroxyapatite within the tooth structures and the acidic functional carboxylic acid groups present in the anionic polymer polyacrylic acid (pAA). The final pH of the set cement (Nicholson et al., 2020) and its biologic properties are excellent; zinc may prevent enzymatic degradation in dentine (Osorio et al., 2014) and enhance the deposition of minerals (Hoppe et al., 2011; Ma *et al.,* 2014; Pires *et al.,* 2018). This material is typically employed as liner in deep cavities after selective removal (Pires at al., 2020).

GIC is currently used as remineralising fluoride-releasing material. Nevertheless, it has been demonstrated that GIC-based materials fail to completely remineralise apatite-depleted dentine due to a lack of nucleation of new apatite (Gu *et al.,* 2010; Kim *et al.* 2010). However, the effect of such dentine-replacement materials is clinically relevant, and they may provide feasible means to extend the longevity of material-dentine interface and less incidence of secondary caries (Peumans et al. 2014).

The results of the current study revealed that ZPC has outperformed GIC regarding density recovery in caries-affected dentine. Although the residual dentine layer left was morphologically different among the excavation methods (Figure 3), it did not seem to influence the density recovery (Table 3), with similar patterns for all caries excavation techniques, for ZPC or GIC. Furthermore, SEM analysis showed a clear presence of mineral deposits on the surface of the dentine adjacent to the ZPC and GIC. Fourier-transformed infrared spectroscopy (FTIR) revealed presence of carbonates (Tartari et al., 2016) in both GIC- or ZPC-treated dentine, but the specimens treated with GIC were mainly characterised by residual silicon-based clusters (Yamakami et al., 2018), while those treated

with the ZPC presented some possible deposition of amorphous calcium-phosphates crystals (Marovic et al., 2014) over time.

Further studies should be performed to ascertain if such a mineralisation induced by GIC and ZPC may be able to recover the mechanical properties of demineralised caries-affected dentine.

5. Conclusion

All excavation methods tested in this study presented similar characteristics in terms of caries removal. The cements tested in the present study (ZPC and GIC) represent a suitable choice as dentine replacement materials after selective carious removal. However, ZPC seems to be more effective in recovering the density of residual caries-affected dentine after different excavation methods.

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Figures

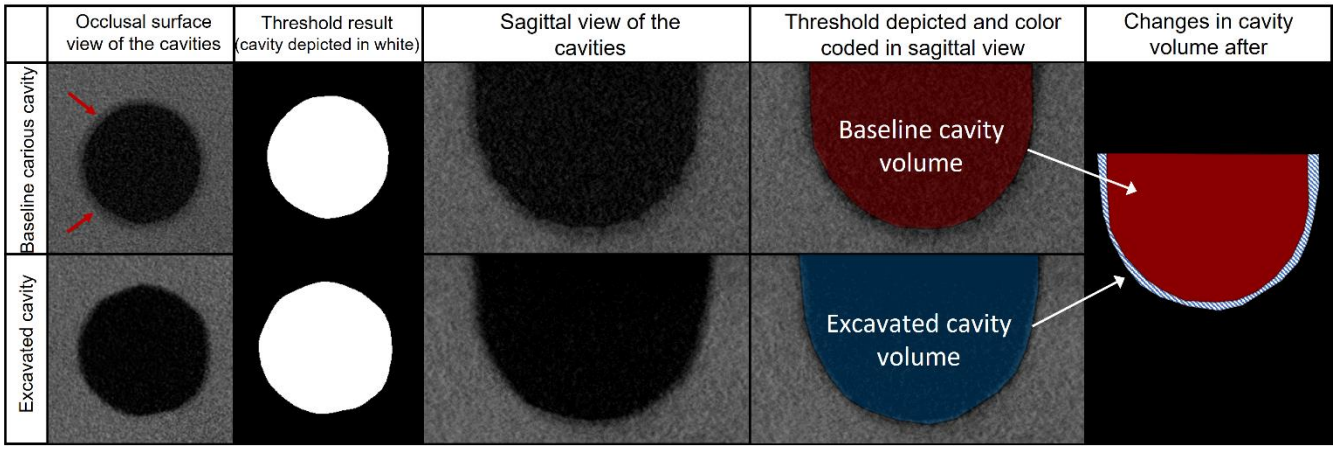

Figure 1. Digital image processing steps for obtaining changes in cavity volume (mm^3) after caries excavation. Red arrows point to the baseline carious dentine layer.

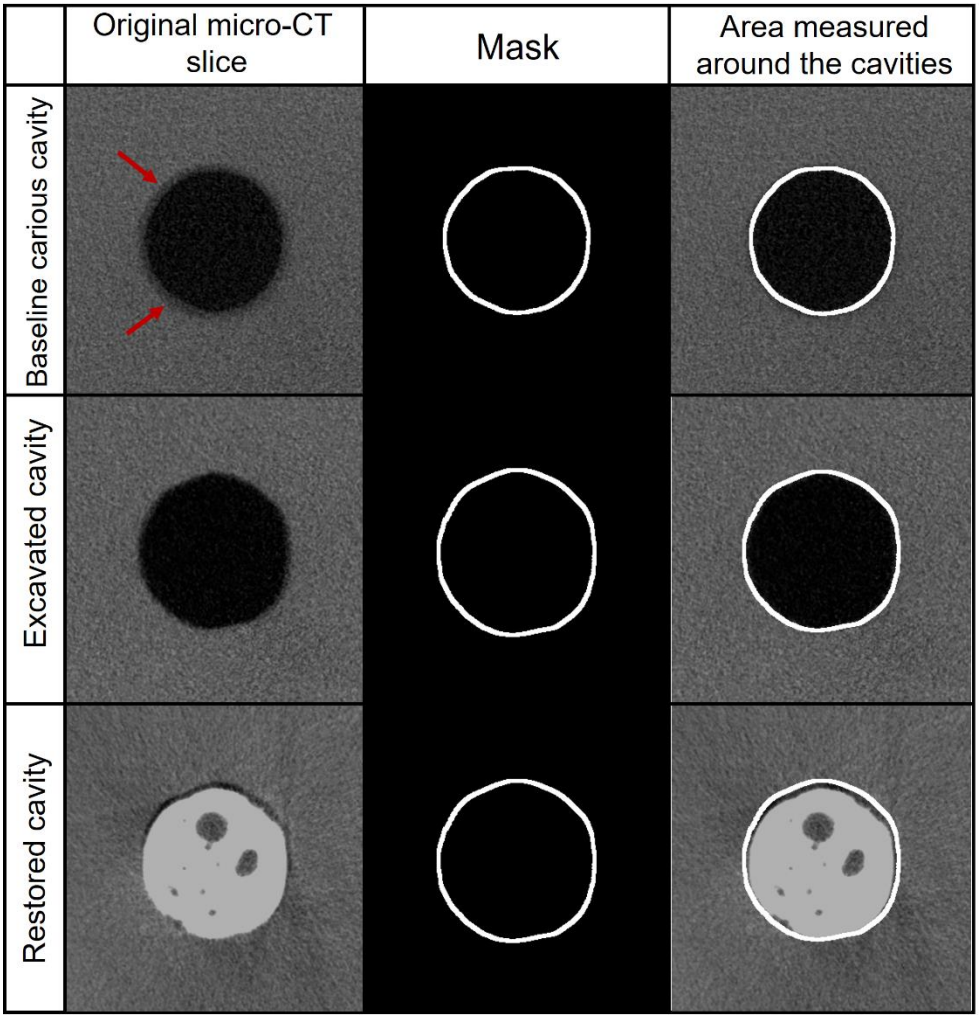

Figure 2. Calculation of density values along the carious cavity walls (baseline carious cavity) or residual dentine left (excavated and restored cavity). Red arrows point to the baseline carious dentine layer.

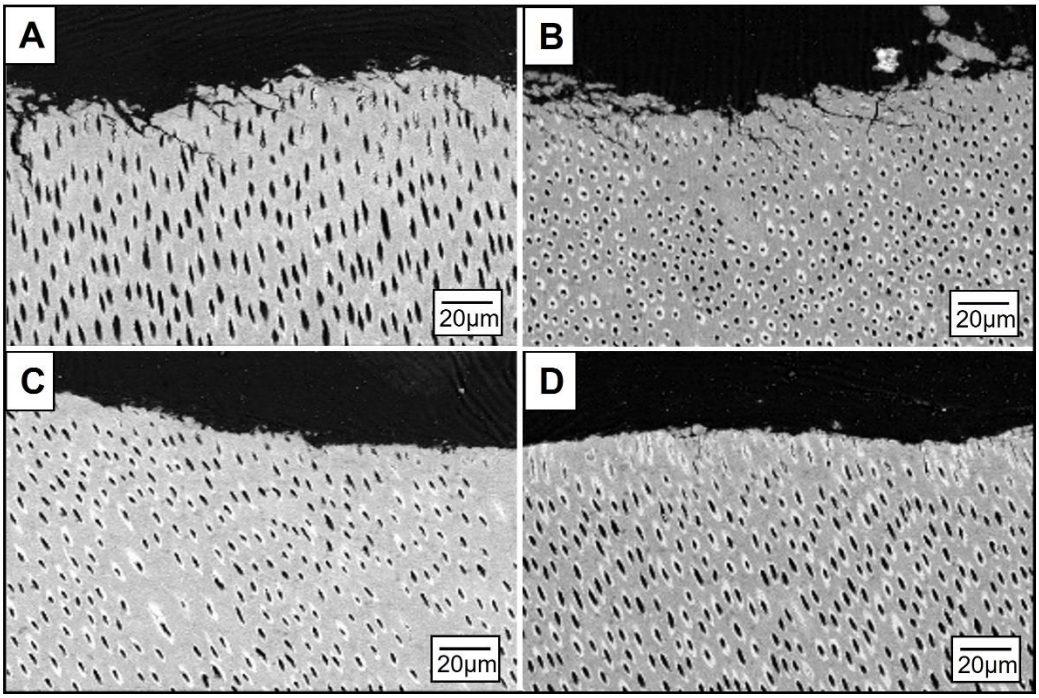

Figure 3. SEM of carious dentine after excavation. **A)** Drill method. **B)** Hand method. **C)** Papacarie Duo Gel®. **D)** Brix3000®.

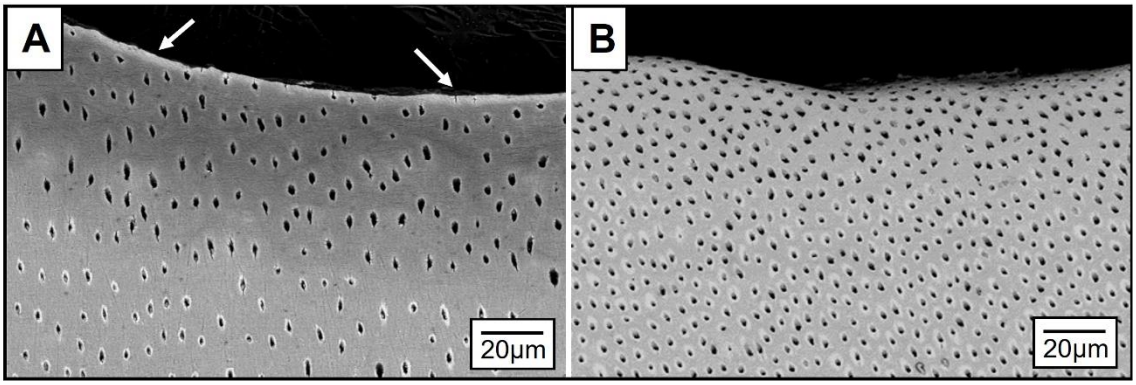

Figure 4. SEM of control group. **A)** Control restored with ZPC. White arrow indicated a high contrast outer layer formed. **B**) Control restored with GIC.

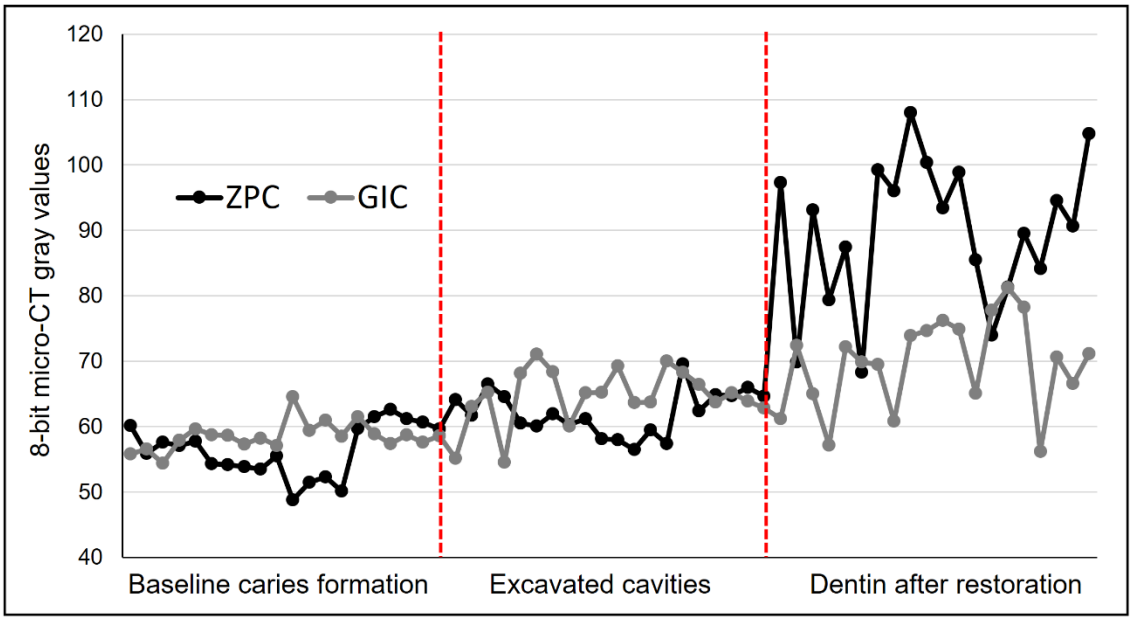

Figure 5. Distribution of density values for all cavities (pooled data) according to the restorative material (ZPC or GIC) at the different experimental stages (baseline caries, after caries excavation, after restoration). ZPC had higher density recovery compared to GIC.

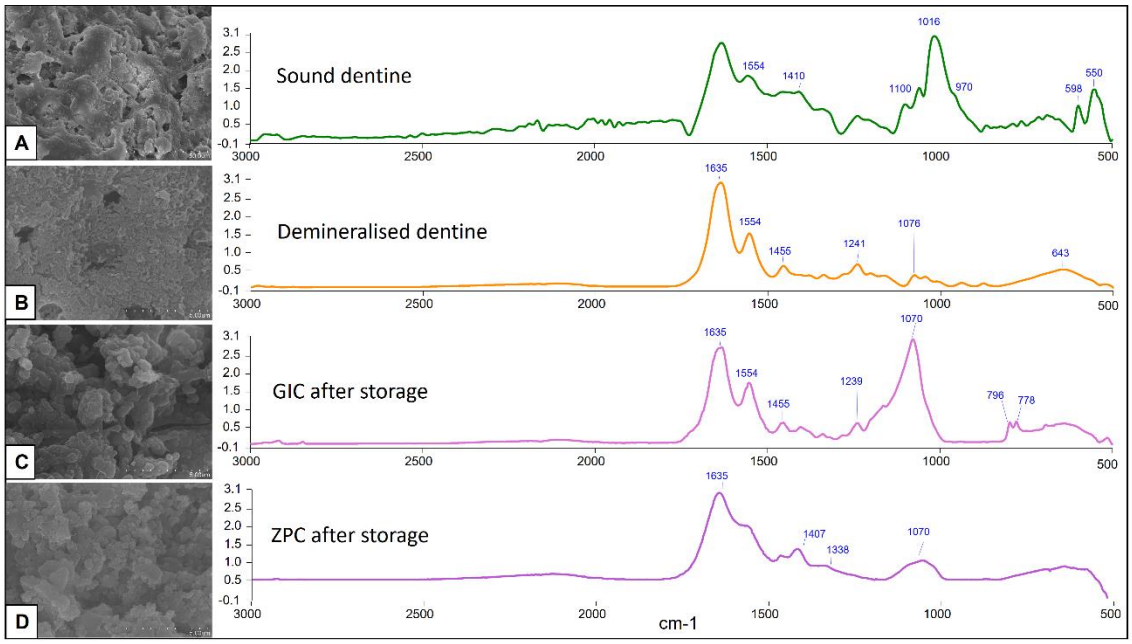

Figure 6. FTIR analysis showing a clear deposition of silicon-based substance (Si-O:1060 cm^{-1}) by GIC and possible amorphous calcium-phosphates (stretching absorption band at 1,200-900 cm^{-1}) by ZPC; carbonates are presents in both cases. Moreover, peaks at 870 cm^{-1}, 1408 cm^{-1}, 1445 cm^{-1} indicate characteristic bands of carbonate ($CO3^{2-}$) substitution in apatite, especially in sound dentine, which also presents characteristic peaks of P-O at 970 cm^{-1}, 1016 cm^{-1} and 1100 cm^{-1}. Amide I, II and III in dentine collagen are represented by absorption peaks at 1645 cm^{-1}, 1450 cm^{-1} and 1245 cm^{-1}, respectively. **A)**

Dentine restored with GIC showing the presence of some deposits on the surface. **B)** Sound dentine with smear layer. **C)** Demineralised dentine with dentine tubules exposed. **D)** Dentine restored with ZPC showing the presence of minerals deposited on the surface.

Tables

Table 1. Mean cavity volume (cm^3) for cavities before and after excavation with each caries-removal method.

Caries-removal method	***Baseline (cm^3)***	***Excavated (cm^3)***	***% increase cavity size***
Drill	1.49 ± 0.33 ^{A,a}	1.64 ± 0.34 ^{A,b}	10.51 ± 7.67 ^{A}
Hand excavator	1.45 ± 0.47 ^{A,a}	1.63 ± 0.49 ^{A,b}	11.02 ± 4.73 ^{A}
Papacárie	1.72 ± 0.43 ^{A,a}	1.92 ± 0.44 ^{A,b}	12.44 ± 4.63 ^{A}
Brix3000	1.55 ± 0.23 ^{A,a}	1.79 ± 0.27 ^{A,b}	15.44 ± 5.26 ^{A}
Mean	**1.56 ± 0.39 ^{a}**	**1.73 ± 0.42 ^{b}**	**12.28 ± 6.08**

In each column, means followed by same uppercase letters are not statistically significant (one-way ANOVA, $p<0.05$). In each row, means followed by different lowercase letters denote significant differences (paired t-test, $p>0.05$). Increase of cavity size percentage was calculated to evaluate the differences in volume between baseline and after excavation (one-way ANOVA, $p<0.05$).

Table 2. Mean density changes for cavities before and after excavation in each method.

Caries-removal method	***Baseline***	***Excavated***	***% increase mineral density***
Control	57.04 ± 2.99 ^{A,a}	56.21 ± 2.85 ^{A,a}	-0.95 ± 4.01*
Drill	56.84 ± 2.78 ^{A,a}	60.99 ± 3.43 ^{B,b}	7.43 ± 6.22 ^{A}
Hand	57.54 ± 3.46 ^{A,a}	63.01 ± 4.04 ^{B,b}	10.71 ± 4.72 ^{A}
Papacárie	58.33 ± 3.86 ^{A,a}	65.15 ± 2.98 ^{B,b}	11.94 ± 4.78 ^{A}
Brix3000	57.40 ± 2.89 ^{A,a}	64.10 ± 4.16 ^{B,b}	11.71 ± 5.33 ^{A}

In each column, means followed by same uppercase letters are not statistically significant (one-way ANOVA, $p<0.05$). In each row, means followed by different lowercase letters denote significant differences (paired t-test, $p>0.05$). Increase in mineral density was calculated for between baseline and after excavation (one-way ANOVA, $p<0.05$). *No differences were expected to be seen between baseline and excavated control cavities.

Table 3. Increase in density (%) at the caries-affected dentine layer for ZPC and GIC cement after restoration and for each caries-removal method.

Method	***Zinc Polycarboxylate***	***Glass Ionomer***
Drill	35.77 ± 17.52 ^{A,a}	7.72 ± 5.58 ^{A,b}
Hand	32.01 ± 10.03 ^{A,a}	2.80 ± 6.95 ^{A,b}
Papacárie	35.32 ± 12.57 ^{A,a}	5.22 ± 5.95 ^{A,b}
Brix3000	24.59 ± 14.28 ^{A,a}	6.84 ± 4.88 ^{A,b}
Mean	**33.66 ± 13.38** ^{a}	**6.03 ± 5.76** ^{b}

In each column, means followed by same uppercase letters are not statistically significant (one-way ANOVA, $p<0.01$). In each row, means followed by different lowercase letters denote significant differences (t-test, $p>0.01$).

4.3 Artigo 3: Tridimensional roughness and morphology of sound dentin surfaces after papain-gel treatment.

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Tridimensional roughness and morphology of sound dentin surfaces after papain-gel treatment

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Abstract

PURPOSE: To investigate the effect of chemomechanical caries removing agents (CCRAs) based on papain (Papacárie Duo Gel® and Brix3000®) over dentin surfaces compared with 37% phosphoric acid and 11.5% polyacrylic acid.

MATERIALS AND METHODS: Sound human molars were sectioned at the crown level, resulting in 48 dentin blocks, which were randomly divided into 4 groups (n=12): 1) Papacárie Duo Gel®; 2) Brix3000®; 3) 11.5% polyacrylic acid solution and 4) 37% phosphoric acid gel. All products were applied for 30s. Ten blocks per group were analyzed by a non-contact 3D profilometer before and after treatments for linear (Ra) and volumetric roughness (Sa). The superficial morphology of the remaining blocks in each group (n=2) was evaluated by scanning electron microscopy (SEM). Normality was rejected for the data (Shapiro-Wilk test) and therefore, Kruskal-Wallis test, followed by Dunn's proof or Wilcoxon signed rank test with its respective effect size calculation were used to compare the results with α=5%.

RESULTS: Ra and Sa values for specimens submitted to Papacárie Duo Gel® and Brix3000® were statistically similar to baseline values. Application of phosphoric and polyacrylic acid resulted in a statistically increase in roughness compared to the CCRAs. SEM evaluation showed that Papacárie Duo Gel® resulted in surface debris. Polyacrylic acid and Brix3000® resulted in partial opening of the tubules but dentin exposed to polyacrylic was able to remove more smear layer than Brix3000®, while phosphoric acid resulted in total opening of the dentinal tubules.

CONCLUSION: Both Papacárie Duo Gel® and Brix3000® did not result in roughness changes when applied in sound dentin.

KEYWORDS: dental caries; chemomechanical caries removal; dental materials; dentin; papain; pediatric dentistry

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Introduction

Non-treated caries lesions are still a burden to dental services around the world [1]. Chemomechanical caries removal agents (CCRAs) gained interest over the last twenty years due to many factors, including: 1) increased dental tissue preservation due to selective tissue removal; 2) ability to provide a more objective caries removal threshold; 3) reduced need for anesthesia during operative procedures and 4) possibility of undertaking operative procedures in non-dental settings [2-4].

Initially, hypochlorite-based CCRAs such as Carisolv® (RLS Global, Mölndal, Sweden) where developed, in which good effectiveness and a selective action in removal of carious dentin has been demonstrated [4]. In Brazil however, papain-based CCRAs became more commercially available and therefore, much commonly used

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(Papacárie Duo Gel®, Fórmula & Ação, São Paulo, SP, Brazil). This product is a gel containing 3% papain, 0.5% chloramine and toluidine blue and has been also found to be effective in removing infected carious dentin [5] with a user-friendly technique [6] and being more comfortable to the child dental patient than the drilling method [7]. More recently, another papain-based CCRA has been developed (Brix3000®, Brix Medical Science, Carcarañá, Santa Fe, Argentina) which allegedly contain an increased papain concentration (3.000 U/mg or 10%). According to the manufacturer, in this gel, the enzyme is protected by a buffer emulsion technology, which immobilizes and stabilizes it at an ideal pH, releasing papain only at the time of collagen proteolysis, resulting in an increase of the enzymatic activity by up to 60%. Differently from Papacárie Duo Gel®, Brix3000® does not contain chloramine in its composition [8].

From the currently available minimally invasive caries removal methods, CCRAs agents result in the best threshold for conservative caries removal [9,10]. Papain is an enzyme extracted from papaya fruit with broad proteolytic, bactericidal, bacteriostatic and anti-inflammatory activity [11]. Papain is able to breakdown infected or necrotic tissues because degraded collagen lacks α1-anti-trypsin, which normally inhibits protein digestion in healthy tissues [12,13], guaranteeing thus, the selectivity of the CCRA. The lack of anti-trypsin in infected/necrotic tissues will allow papain to further breakdown this substrate, enabling easy removal with hand instruments [14].

The concept of minimal invasive dentistry (MID) means removing only infected and irreversibly destroyed dentin and leaving, as residual substrate for further adhesive procedures, a slightly demineralized caries-affected dentin [15]. Like other active substances in CCRAs, papain is supposed to acts exclusively on the breakdown of the partially degraded collagen in carious dentin, without damaging intact collagen fibrils [16], leaving thus, sound dentin intact. Keeping with the MID approach, CCRAs are constantly used over sound dentin surfaces, especially if the enamel-dentin junction is prepared to "scratchy" dentin to ensure appropriate sealing of the restoration [17]. While hypochlorite-based CCRAs have been investigated on its topographic effects over sound dentin [18,19], the possible effects of papain-based CCRAs sound dentin surfaces have not yet been investigated.

To evaluate the effect of Papacárie Duo Gel® and Brix3000® on the superficial morphology and roughness of sound dentin compared to commonly used dentin conditioning agents (37% phosphoric acid and 11.5% polyacrylic acid), which purposely cause exposure of dentin collagen. Surface roughness and morphological features have been investigated by non-contact profilometry measurements and scanning electron microscope, respectively.

Material and Methods

Sound human third molars, extracted for clinical reasons were used after the patient has given written consent. This protocol has been approved by the institution's ethical committee and it is registered at "Plataforma Brazil" (17389213.5.0000.5257). The teeth were selected according to the following inclusion criteria: absence of caries lesions or restorations/sealing material. After sectioning, the specimens were examined under a stereomicroscope for presence of any defects or areas of sclerotic dentin, and if present, they were excluded from the sample. Excluded teeth were discarded via standardized protocol for biological material.

Sample size was estimated for comparison of differences in more than two experimental groups considering a 0.05 alpha value and a 0.9 power of the test, based on results of a previous study on the effect of a hypochlorite-based chemo-mechanical caries removing method on the roughness of sound dentin [18]. The following information was input to the software (BioEstat v.5.3, Instituto Mamirauá, Manaus, AM, Brazil): minimum difference between mean of groups = 0.002; standard deviation

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of the experimental error = 0.001, which resulted in a minimum of 8 specimens per group.

The tooth crowns were cut, with the aid of a diamond disk mounted on a low speed cutting machine (Isomet, Buehler, Lake Bluff, IL, USA) into dentin blocks (approximately 4 X 4 X 2 mm) in which the occlusal dentin was selected as the test surface.

Forty-eight dentin blocks were selected among those obtained and attached with sticky wax to polyethylene devices with the test surface exposed at the top. The specimens were further polished with 400, 800 and 1200 grit sandpaper, in this order, under water-cooling with the aid of a semi-automatic polishing machine (PLF, Fortel, São Paulo, SP, Brazil). After that, twelve specimens were randomly allocated to each four experimental groups: 1) application of Papacárie Duo Gel® (Fórmula & Ação, São Paulo, SP, Brazil); 2) application of Brix3000® (Brix Medical Science, Carcarañá, Santa Fe, Argentina); 3) application of a 11.5% polyacrylic acid solution (Vitro condicionador, DFL, Rio de Janeiro, RJ, Brazil) 4)

application of a 37% phosphoric acid gel (Condac, FGM, Joinville, Santa Catarina, Brazil). Application time for all products was standardized in 30s. After each application time, and for all groups, the specimens were washed in distilled water for 60s and stored under 100% humidity until further analysis.

Before and after the treatment regimens, 10 specimens in each group were analyzed in a 3D non-contact chromatic confocal optical profilometry (Nanovea PS50 Optical, Nanovea Inc., Irvine, California, United States). Linear roughness (Ra) was obtained by averaging three linear readings from each sample while volumetric roughness (Sa) was obtained from one volumetric reading over each specimen.

The remaining two blocks per group were used for surface topographic analysis. The blocks were fixed on stubs with double-faced carbon tape, gold-sputtered (30µm) and analyzed in a scanning electronic microscope (6460LV, JEOL, Tokyo, Japan) in secondary electrons mode after the

treatment regimens.

Shapiro-Wilk's test was used to evaluate normality of the data. As normality was rejected, Kruskall-Wallis test, followed by Dunn's proof was used to disclose statistical significance among the roughness values within the experimental time (baseline or treated) and an epsilon squared effect size was calculated [19]. Wilcoxon signed rank test was used to test differences in each experimental group between time (baseline or treated) and the effect size was calculated as the *r* estimate [19, 20]. The α-level chosen was 5% and all analysis were undertaken using BioEstat v.5.3 (Instituto Mamirauá, Manaus, AM, Brazil).

Results

Both surface roughness parameters evaluated (Ra and Sa) were similar among the groups at baseline. For the treated specimens, polyacrylic acid and phosphoric acid treated specimens showed statistically significant higher Ra and Sa values compared to both papain-treated groups, with large effect sizes (Tables 1 and 2).

Intra-group comparison of tridimensional surface roughness analysis disclosed that specimens submitted to the papain-based caries removing gels did not show significant changes in both roughness parameters tested (Ra and Sa) after the treatments (Tables 1 and 2). On the other hand, specimens treated with phosphoric acid or polyacrylic acid resulted in a statistically significant higher linear (Ra) and volumetric (Sa), with very strong or strong effect sizes.

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Table 1: Mean (±SD) of linear (Ra) roughness measurements (µm) before and after treatments in each experimental group.

Experimental Group	Ra		
	Baseline	Treated	Difference
Phosphoric acid	0.45 ± 0.11 $^{a, A}$	1.34 ± 0.48 $^{a, B}$	0.89 ± 0.53 ^{a}
Polyacrylic acid	0.48 ± 0.04 $^{a, A}$	1.84 ± 0.79 $^{a, B}$	1.37 ± 0.79 ^{a}
Papacárie Duo Gel®	0.41 ± 0.09 $^{a, A}$	0.47 ± 0.09 $^{b, A}$	0.07 ± 0.05 ^{b}
Brix3000®	0.40 ± 0.04 $^{a, A}$	0.39 ± 0.04 $^{b, A}$	0.03 ± 0.03 ^{b}

* Different lowercase superscript letters indicate statistical significance in the same column (Kruskal-Wallis followed by Dunn's test, $p<0.05$). Effect size was 0.76 (very strong) and 0.69 (very strong) for the column "treated" and "difference", respectively [19]. Different uppercase superscript letters indicate statistical significance between baseline and treated specimens in the same group (Wilcoxon signed rank test). Effect size was 0.83 (large) and 0.84 (large) for phosphoric acid and polyacrylic acid respectively [20].

Table 2: Mean (±SD) of volumetric (Sa) roughness measurements (µm) before and after treatments in each experimental group.

Experimental Group	Sa		
	Baseline	Treated	Difference
Phosphoric acid	0.61 ± 0.51 $^{a, A}$	2.12 ± 1.38 $^{a, B}$	1.51 ± 1.42 ^{a}
Polyacrylic acid	0.88 ± 0.25 $^{a, A}$	4.70 ± 3.75 $^{a, B}$	3.82 ± 3.65 ^{a}
Papacárie Duo Gel®	0.59 ± 1.79 $^{a, A}$	0.65 ± 0.16 $^{b, A}$	0.06 ± 0.17 ^{b}
Brix3000®	0.96 ± 0.62 $^{a, A}$	1.21 ± 1.69 $^{b, A}$	0.25 ± 1.30 ^{b}

* Different lowercase superscript letters indicate statistical significance in the same column (Kruskal-Wallis followed by Dunn's test, $p<0.05$). Effect size was 0.63 (strong) and 0.51 (strong) for the column "treated" and "difference", respectively [19]. Different uppercase superscript letters indicate statistical significance between baseline and treated specimens in the same group (Wilcoxon signed rank test). Effect size was 0.84 (large) and 0.81 (large) for phosphoric acid and polyacrylic acid respectively [20].

SEM analysis of the superficial morphology of the dentinal tubules revealed that phosphoric acid resulted in full opening of the dentin tubules (Figure 1A). Polyacrylic acid and Brix3000® resulted in partial opening of the dentin tubules (Figure 1B and 1D) while Papacárie Duo Gel® resulted in the presence of debris, probably resulting from the polishing procedures undertaken during specimen preparation which remained over the tubules (Figure 1C).

Discussion

The results of the present study revealed no statistically significant changes on linear (Ra) and volumetric (Sa) roughness in specimens submitted to the papain-based CCRAs before and after the treatments. Specimens treated with phosphoric acid or polyacrylic acid resulted in a statistically significant increase in both Ra and Sa. These results are in agreement with previous investigations of the effect of Carisolv® in sound dentin surfaces. Roughness parameters has also not

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been changed after Carisolv® application but increased substantially when phosphoric acid was applied in this substrate [18]. Carisolv® is a CCRA based on chloramine, while the CCRAs used in the present study are based on papain. Therefore, an investigation on the effect of these on the sound topography of dentin was warranted.

The morphology of the dentin surface as revealed by SEM showed that phosphoric acid resulted in full opening of the dentin tubules (Figure 1A). Polyacrylic acid and Brix3000® resulted in partial opening of the dentin tubules (Figures 1B and 1D) but dentin exposed to polyacrylic acid resulted in less smear layer than Brix3000®. Papacárie Duo Gel® resulted in a relatively smeared dentin surface the presence of debris (Figure 1C).

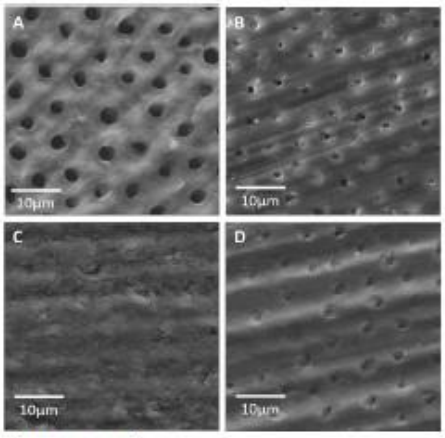

Figure 1. *SEM images of dentin surfaces after experimental treatments. A) 37% phosphoric acid treatment. B) 11.5% polyacrylic acid treatment. C) Papacárie Duo Gel® treatment. D) Brix3000® treatment.*

A previous SEM study comparing Carisolv® and other dentin conditioners over sound dentin also showed that this CCRA was unable to remove smear layer and completely open dentin tubules [21]. However, when Carisolv® has been used over carious dentin, the surface generally appeared clean, with open tubules [22]. The same is likely to be true for Papacárie, since many studies have also reported similar residual carious dentin surface morphology after it use, with open tubules and reduced smear layer [23-25]. Nonetheless, one study showed presence of smear layer and debris partially occluding the dentin tubules in residual carious primary teeth dentin after caries removal with Papacárie Duo Gel® [26].

It has been claimed that chloramine present in Papacárie Duo Gel® would be responsible for opening the dentinal tubules of the outer surface of the carious dentin, softening it chemically and facilitating its removal [27]. In the present study however, sound dentin surfaces treated with this product still showed a smear layer covered surface.

The main function of phosphoric acid 37% and polyacrylic acid 11.5%, used during conditioning of dentin surfaces prior to composite and glass ionomer restorations, is to guarantee removal of the smear layer, which is accompanied by the superficial mineral dissolution of the dentin, exposure of collagen fibers and opening of the superficial dentin tubules to allow composite tag formation [28]. The SEM observations of the present study corroborate the effect of both phosphoric and polyacrylic acid on sound dentin. Although complete removal of the smear layer is frequently seen with phosphoric acid [29], this is invariably followed by great peritubular dentin dissolution and exposure of collagen fibers [30]. Polyacrylic etched sound dentin surfaces normally retain part of the smear layer [31] and also result in calcium-enriched surfaces [29].

There have been claims that non-mineralized type I collagen fibrils would be partially degraded (without fiber rupture) by a papain-gel, as shown by atomic force evaluation of the surface of collagen fibers in sound dentin [32], what could be evidence of a detrimental effect of the gel in sound dentin. Others, however, using Fourier-transformed infrared spectroscopy claimed no collagen degradation to occur [33] and no removal of calcium of sound dentin after Papacárie application [34]. Regarding surface roughness and morphological characteristics, the results of the present study support the selective action of papain gels in carious dentin.

The restoration of cavities using a MID approach requires adhesive materials such as composite resins or glass ionomer cements, which directly bond to the dentin surfaces. Several studies have reported that papain-based gel treatment does not interfere with the bond strength of the adhesive restorative materials to

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sound and demineralized dentin [35-37]. However, these CCRAs do not promote enough surface roughness in sound dentin; thus, it's use probably does not replace the use of conditioning agents before restoration. More studies are indeed, necessary to investigate possible ultrastructural changes in sound and carious dentin after use of papain-based CCRAs in order to optimize its use in light of the MID approach.

Limitations of this study include the fact that the dentin quality among the experimental groups were not fully standardized. Factors such as age of the patient, anatomical location of the tooth or distance of the cut from the dentin-pulp junction, might result in morphological differences at the dentin level that could have influenced the results (i.e., the high variability of data obtained). However, as distribution of the dentin specimens was randomized among the groups in the present study, this effect was probably evenly distributed among the groups.

Papain-based caries removing gels did not induce significant roughness changes in sound dentin. Regarding the morphologic characteristics of the surfaces, application of Brix3000® resulted in partially opening of dentin tubules, similar to that caused by polyacrylic acid treatment but this removed more smear layer. Papacárie Duo Gel® did not remove smear layer and left dentin tubules obliterated. This study has shown that papain-based gels are harmless when applied in sound dentin surfaces.

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4.4 Artigo 4: Assessment of remineralisation induced by contemporary ion-releasing materials in mineral-depleted dentine.

Short title: Remineralisation induced by ion-releasing materials.

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ABSTRACT

Objectives: Evaluate the ion-releasing materials' ability to remineralise bacteria-driven artificial caries lesions.

Methods: Standardised class I cavities were obtained in 60 extracted human molars. Specimens underwent a microbiological cariogenic protocol (28d) to generate artificial caries lesions, then were randomly divided into four restorative groups: Adhesive+composite (negative control); Glass ionomer cement (GIC); calcium silicate cement (MTA); resin-modified calcium silicate cement (RMTA). Microhardness analysis (ΔKHN) was performed on 40 specimens (10/group, t=30d, 45d, 60d in artificial saliva, AS). Micro-CT scans were acquired (3/group, t=0, 30d, 90d in AS). Confocal microscopy was employed for interfacial ultra-morphology analysis (2/group, t=0, 60d in AS). Additional specimens (n=13) were prepared and processed for scanning electron microscopy (SEM) and FTIR (n=3/group + control) to analyse the ability of the tested materials to induce apatite formation on totally demineralised dentine discs (60d in AS). Statistical analyses were performed with a significance level of 5%.

Results: Adhesive+composite specimens showed the lowest ΔKHN values and the presence of gaps at the interface when assessed through micro-CT even after storage. Conversely, all the tested ions-releasing materials presented an increase in ΔKHN after storage ($p<0.05$), while MTA best reduced the demineralised artificial carious lesions gap at the interface. MTA and RMTA also showed apatite deposition on totally demineralised dentine surfaces (SEM/FTIR).

Conclusion: All tested ion-releasing materials expressed mineral precipitation in demineralised dentine. Additionally, calcium silicate-based materials induced apatite precipitation and hardness recovery of artificial carious dentine lesions over time.

Clinical Relevance: Overall, the use of ions-releasing materials can recover carious dentine. Thus, MTA should probably be used in deep carious lesions while RMTA followed by GIC are probably more appropriate to preserve the caries-affected collagen and bio-remineralise it.

Keywords: Dental biomaterials; Dentine replacement materials; Ions-releasing materials; Remineralisation; Restorative dentistry.

1. INTRODUCTION

Preservation of dental hard tissues (i.e. dentine and enamel) through minimally invasive clinical approaches should be prioritised in contemporary operative dentistry [1-3]. Furthermore, selective carious tissue removal should be mainly considered an operative option for cavity preparation, especially in deep lesions near the pulpal chamber [4,5]. Indeed, it is advised to remove caries-infected dentine entirely while preserving the affected dentine [6]. Such an approach reduces the risk of excessive tissue removal with consequent unnecessary pulp exposure in deep cavities [7]. It was seen that tissue preservation is indeed one of the main factors improving a restoration's longevity, provided that the restoration is able to seal the carious cavity adequately [8]. Nevertheless, conventional adhesive systems partially infiltrate this demineralised caries-affected substrate due to its unfavourable chemical-morphological characteristics [9,10]. The collagen fibrils not infiltrated by the adhesive system are the leading cause of the low bond strength of the whole composite restoration and its reduced longevity [2,11].

A reasonable approach to restoring caries-affected dentine is represented by the use of pulp-protective materials with high remineralisation properties [12,13]. However, in such a scenario, there is still a need to develop innovative restorative materials, which should possess adhesive properties and the ability to "repair" caries-affected tissues through ion-releasing remineralisation processes [2,14,15]. The research yielded several materials based on such repairing properties, each having pros and cons so that the ideal restorative material with remineralising properties is still missing. Among the materials available nowadays, glass-ionomer cements (GICs), calcium silicate-based cements, and

resin-modified types of the latter are commonly employed as restorative materials after selective caries removal [2,16]. GICs were demonstrated to exhibit chemical adhesion to dental hard tissues thanks to a well-known ion exchange process [17,18]. Moreover, such materials are responsible for the long-term fluoride release in the microenvironment [19]. The latter can prevent the formation of recurrent lesions along the margins of the restoration [8,20]. The resin-modified hydraulic calcium-silicate cement (resin-modified mineral trioxide aggregate, RMTA) showed sustained release of Ca^{2+} and OH^- ions [21], able to induce tissue remineralisation [22]. Moreover, RMTA is a photocuring material that can be relatively compatible with an adhesive restoration [23]. However, data comparing the remineralising abilities of such materials in conditions as similar as possible to the clinical ones are still scarce and inconsistent.

The purpose of this study was to investigate the remineralising effect of different ion-releasing restorative materials in mineral-depleted simulated carious dentine by means of microhardness test and micro-CT analysis. The tooth-material interface ultra-morphology was also analysed through confocal scanning microscopy (CLSM). Moreover, the ability of the tested materials to induce apatite precipitation in demineralised dentine was assessed through scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). The null hypothesis was that the tested materials would be able to remineralise the affected dentine to the same extent.

2. MATERIALS AND METHODS

2.1. Specimen preparation

Sound human molars (n=73), extracted for periodontal or orthodontic reasons, were collected according to the guidelines of the local ethics committee, under protocol number (blind for revision) and stored in distilled water at 4 ˚C for no longer than 3 months. Figure 1 shows the distribution of the specimens based on the experimental groups and methodological procedures.

Standardised class I cavities (4 mm length × 3 mm width) were obtained in 60 teeth with the floor ending in deep dentine (4 mm deep), using a high-speed handpiece with a cylindrical diamond bur (3146, Komet, Germany). Subsequently, the roots were removed 1 mm below the cement–enamel junction using a diamond-embedded blade (XL 12205; Benetec, London, UK) mounted on a low-speed microtome (Remet evolution, REMET, Bologna, Italy) under water cooling. All surfaces of the teeth were covered with an acid-resistant varnish (Deliplus®, Mercadona, Valencia, Spain), leaving only the dentine of the cavity exposed to be submitted to the cariogenic protocol.

The roots of the remaining teeth (n=13) were removed 1 mm beneath the cement–enamel junction as described previously. A second parallel cut was made to remove the occlusal enamel. Mid-coronal dentine was exposed and flattened using 320-grit SiC papers. These specimens were stored at 4˚C and 100% humidity until further use.

2.2. Artificial caries lesions formation

The Class I cavity specimens were fixed on the bottom of 24-well polystyrene plates (TPP, Zellkultur Testplatte 24F, Trasadingen, Switzerland) and sterilised under UV light for 40 minutes before receiving the microbial inoculum, according to the methodology previously described by Pires et al., [24]. The following bacterial strains were obtained from the type culture collection (CECT, Paterna,

Valencia, Spain) used to prepare the inoculum: *Streptococcus mutans* (CECT 479T), *Streptococcus gordonii* (CECT 804), *Streptococcus salivarius* (CECT 805T) and *Lactobacillus casei* (CECT 475T). A pure suspension of each microorganism was obtained in Brain Heart Infusion (BHI, used as nutrient growth substrate) after overnight incubation in a 5% supplemented CO_2 environment at 37 °C. Cells were harvested by centrifugation (2200 x g, 19°C, 5 min), washed twice with phosphate-buffered saline (PBS), and resuspended. The suspension was then subjected to low-intensity ultrasonic energy (Sonifier model B-150; Branson, Danbury, CT, USA) to disperse bacterial chains and was finally adjusted to an equivalent of 0.5 on the McFarland scale.

Twelve µL (1.5×10^8/well) of the microbial inoculum was placed in each well along with the brain-heart infusion (BHI) broth with 5 wt.% of sucrose. The plates were incubated at 37 °C in a 5% CO_2 supplemented atmosphere to allow biofilm growth. Every 24 h, the growth medium in each well was replaced with a fresh one, and the pH was monitored (Sension+ PH3, HACH LANGE, Barcelona, Spain). There was also a control plate without microorganisms to assess possible issues related to contamination. After 28 days, the specimens were gently detached from the plates and biofilm was detached by immersion in an ultrasonic bath for 5 minutes. The specimens were then immersed in ethanol (97%) for 2 min and rinsed with PBS for 1 min.

2.3. Restorative procedures

Tooth specimens with artificial caries lesions were randomly allocated to four experimental groups (n = 15 / group) based on the tested restorative materials (Table 1). According to the manufacturer's instructions, each dental cavity was filled with one of the materials.

- GIC: Specimens were rinsed with tap water (20 s) and air-dried (5 s). The GIC (Fuji IX, GC, Japan) powder was divided into 2 equal parts: the first aliquot was mixed with the GIC liquid for 10 s while the remaining one was subsequently incorporated and mixed for the following 20 s (ratio powder / liquid 1:1). The material was finally applied into the cavities without pre-conditioning the dentine. The material was left to set for 10 min.

- MTA: Specimens were rinsed with tap water (20 s) and air-dried (5 s). The cement (Endo-Pass, DEI Italia, Italy) was mixed with bi-distilled water (powder / water ratio = 2:1) to obtain a creamy consistency. The cement was placed in the cavity and left to set in contact with a wet cotton pellet for 20 min.

- RMTA: Specimens were rinsed with tap water (20 s) and gently air-dried (5 s), leaving the dentine moist. The RMTA (TheraCal LC, Bisco Inc, USA) was placed in incremental layers of 1 mm without any previous dentine treatment. Each increment was light-cured for 20 s using an LED curing unit (Radii plus, SID Ltd., Bayswater VIC, Australia, 1200 mW/cm^2).

- Negative control: Specimens were rinsed with tap water (20 s) and air-dried (5 s). Then, a universal adhesive (ZipBond, SDI, Australia) was used in self-etch mode. The adhesive was brushed inside the cavity for 10 s using a microbrush, left for 10 s, air-blown for 5 s to evaporate the solvent, then light-cured using the LED light-source (Radii plus) for 10 s. The specimens were restored with a flowable resin composite (Aura, SDI, Australia), applied in 2 mm increment layers, each light-cured for 20 s (Radii plus).

All specimens were incubated at 37 ˚C in artificial saliva for 24 h before further processing. The composition of the AS was 0.103 g L^{-1} of $CaCl_2$, 0.019 g L^{-1} of $MgCl_2$ x $6H_2O$, 0.544 g L^{-1} of KH_2PO_4, 30 g L^{-1} of KCl, and 4.77 g L^{-1} HEPES (acid) buffer, pH 7.4 [25].

2.4. Microhardness evaluation

Specimens (n = 10 / group) were prepared for microhardness analysis. These were first embedded in epoxy resin and then cut into 1.5 mm slabs perpendicular to the occlusal surface to expose the tooth-material interface. Slices not containing a tooth-material interface were discarded. On each slice, three parallel indentation series were performed. In each series, the first probing (25 gf load; 30 s dwell time) was made 50 µm away from the interface, while additional three indentations were performed below the first one (throughout the artificial caries lesion) at 50 µm intervals (Figure 1). Each specimen was tested 24 h after restoration placement (baseline) and after storage in 25 ml of AS for 30, 45, and 60 days. The AS solution was replaced with a fresh one every 7 days.

The average microhardness was measured for each slab by an operator, who was unaware of the treatment groups. The percentage difference between each storage time-point with baseline was calculated for each group at different depths (ΔKHN%).

2.5. Micro-CT analysis

A total of 12 restored specimens (n=3/group) were scanned (Skyscan 1176, Bruker, Kontich, Belgium) 24h after restoration (t0) and after 30 and 90 days of storage at 37 °C in AS (t30 and t90, respectively). The acquisition parameters were: 80 kV, 300 µA source current, 8.92 µm isotropic pixel size, 0.5 mm Al +

0.38 mm Cu filter, 0.5° rotation step over 360° with frame averaging of 5. Reconstruction was performed using proprietary software (NRecon, Bruker) using standardised parameters of beam hardening correction (30%), smoothing = 7, smoothing kernel = 2 (Gaussian), ring artefact compensation = 20, and optimal contrast limits based on the initial scanning and reconstruction tests. Image data were then aligned and resliced using another proprietary software (Dataviewer v1.5.4.6, Bruker). After that, the volume (mm^3) of the demineralised dentine extending between the restoration and the sound tissue in each specimen and for each scanning time was obtained within the ImageJ platform (v1.8.0_112) by a single operator that was blinded regarding the experimental groups. The operator manually defined a visual region of interest (ROI) on each slice of the CT stacks. The presence of CT artefacts due to materials' radiopacity and air bubbles trapped inside materials during mixing procedures (especially GIC) prevented an automatic AI-based threshold detection method to be applied.

2.6. Confocal Microscopy – Interface analysis for mineral precipitation

Eight specimens (n = 2 / group) were prepared as previously described (Par. 2.4) and submitted to confocal microscopy to investigate the interfacial characteristics of the demineralised/remineralised dentine interfaces. After sectioning, half of the specimens were stored in AS for 60 days and the other half were immediately immersed in 0.5 wt% Xylenol Orange solution (XO: FITC – Merck Life Science SLU, Spain) for 24h at 37°C (pH: 7.2). XO is a calcium-chelator fluorophore commonly used as a dye to trace the deposition of minerals within the interfaces of restored teeth due to its ability to form complexes with Ca^{2+} ions [26]. Specimens stored in AS were immersed in XO solution as the control specimens. After immersion in XO, all specimens were ultrasonicated for 2 min in an

ultrasonic bath containing distilled water to remove excess XO and then polished for 30 s each side with 1200-grit and 2400-grit SiC papers. The specimens were finally ultrasonicated in distilled water for 5 min to remove the surface smear layer and immediately submitted to confocal microscopy analysis (CLSM - Olympus FV1000, Olympus Corp, Tokyo, Japan) a 63X/1.4 NA oil immersion lens and 514 nm LED illumination. Optical transmission and fluorescence (568 nm emission filter) images were obtained with a 1-µm z-step to optically section the specimens to a depth of up to 20 µm below the surface [27]. The z-axis scan of the interface surface was pseudo-coloured arbitrarily for improved visualisation and compiled into both single and topographic projections using the CLSM image-processing software (Fluoview Viewer, Olympus). The configuration of the system was standardised and used at constant settings for the entire investigation. Three to five image stacks were randomly captured and recorded, representing the most noteworthy morphological features observed along the dentine-material interfaces [28,29].

2.7. FTIR-ATR and FEG SEM – evaluation of mineral precipitation

Flat dentine specimens (n = 13) previously obtained (Par. 2.1) were divided into four groups (n = 3 / group, Figure 1) and demineralised in a 10% phosphoric acid solution for 48 h [28] except for one sound dentine specimen that was stored at 4º C and 100% humidity. The tested materials were placed in direct contact with the demineralised specimens as previously described. Orthodontic rubber bands were used to ensure tight contact between the material and dentine specimens. The chemical analysis was performed on the surfaces of the specimens before and after 60 days of AS storage in contact with the materials using an FTIR-ATR microspectroscopic system (Spectrum Two UATR; Perkin Elmer). Specimens

were scanned (3,000 - 650 cm^{-1}) at a 4 cm^{-1} spectral resolution. For standardisation, an initial correction and normalisation were applied to all scans.

After that, the specimens were mounted on aluminium stubs, dehydrated in silica gel for 24 h, gold sputter-coated, and analysed using a field-emission gun scanning electron microscope (FEG-SEM S-4100; Hitachi, Wokingham, UK, acceleration voltage, 20 kV and 15-20 mm working distance) to observe the presence of mineral precipitation after storage in AS (t60) at different magnifications.

2.8. Statistical Analysis

Shapiro-Wilk's test was used to check the normality of distribution for all datasets (α=5%). Microhardness data (ΔKHN%) were first analysed using a three-way ANOVA model complemented with Sidak test considering the material type, the indentation depths, and the storage time-points as fixed factors (α=5%). Then, the Kruskal-Wallis/Dunn tests were used to compare the microhardness of materials within the same time-point, and Friedman tests compared the same material throughout the time-points (α=5%). These analyses were performed separately for each indentation depth or using the overall average of depths considering the interaction between the material type and the indentation depths ($p<0.001$). Regarding micro-CT data, homogeneity of variances was preliminarily assessed using Bartlett's test (α=5%) followed by two-way ANOVA analysis considering the material type and the scan time as fixed factors. Student's post-hoc t-test was used in consideration of the small number of specimens to assess significant differences between the groups (α=5%).

3. RESULTS

The results of the dentine microhardness assessment are depicted in Figure 2. When comparing the dentine microhardness using the overall average of depths, all ion-releasing materials provided a significant increase in microhardness when compared to the control group after 45 and 60 days ($p<0.05$). However, only MTA and RMTA induced an overall increase of the dentine microhardness at all the different storage time-points (30, 45, and 60 days) ($p<0.05$; Figure 2A). Also, only the effect promoted by MTA on the overall dentine microhardness increased after 60 days compared with the other time-points ($p<0.05$).

Figure 2 B-D shows the dentine microhardness compared separately for each indentation depth. At 50 μm sampling (Figure 2B), all ion-releasing materials provided a significant increase in microhardness when compared to the control group at all time-points ($p<0.05$). However, only RMTA increased the dentine hardness after 60 days compared with the other time-points ($p<0.05$). At 100 μm sampling (Figure 2C), only MTA increased microhardness at all time-points ($p<0.05$). Also, only MTA and RMTA increased the microhardness over time ($p<0.05$; Figure 2C). There was no significant difference in dentine microhardness between groups at 150 μm and 200 μm depths over time (Figure 2D and 2E).

Shapiro-Wilk's test revealed that micro-CT data did not have normal distribution. Thus, a cubic root function transformation was applied to approach normality. A highly significant influence of both considered factors on demineralised volume can be seen, while no significant interaction between the factors was highlighted. The volume analysis shows a progressive decrease of the demineralised volume during storage. This decrease was significant for GIC and MTA at t90 compared

to baseline (Figure 3). MTA produced the highest reduction in the demineralised volume, resulting in less than 50% of the carious volume after three months (t90). For RMTA, an initial, albeit non-significant reduction of volume was observed in t30, while no further reduction could be seen at t90. No volume reduction could be observed in the control group.

Confocal single projection images (Average Intensity Projection - AIP) of the specimens treated with the different materials and analysed at baseline and t60 are presented in Figure 4. At the baseline assessment, the calcium-staining dye highlighted the presence of an extended (100-120 µm) demineralised dentine layer just underneath the material-dentine interface in all tested materials (Figure 4A). At t60, the specimens restored with the adhesive composite system (negative control) were still characterised by the presence of an extended layer of demineralised dentine with no sign of remineralisation and areas of possible collagen degradation (Figure 4B). Conversely, at t60 the specimens restored with GIC showed the presence of calcium-stained minerals at 20-30 µm depth underneath the GIC-dentine interface (Figure 4C). MTA specimens at t60 showed calcium-stained minerals accumulated only very few microns underneath the interface (Figure 4D). Similar to MTA, the RMTA specimens at t60 showed the presence of calcium-stained minerals that accumulated few microns underneath the RMTA-dentine interface (Figure 4E).

The SEM analysis confirmed the presence of demineralised dentine collagen in the negative control group at t60 (Figure 5A). Then again, no exposed collagen fibrils were observed at the dentine surface restored with GIC, but the presence of minerals on the top of the surface together with partially obliterated dentine tubules (Figure 5B). MTA specimens clearly showed needle-like crystals (Figure

5C), while RMTA showed globular-like crystals (Figure 5D) on the dentine surface.

The FTIR analysis (Figure 6) confirmed the presence of remineralised dentine in MTA and RMTA-treated specimens due to apatite deposition (CaP stretch vibrations at: 559 cm^{-1}, 598 cm^{-1}, 970 cm^{-1}, 1024 cm^{-1} 1088 cm^{-1}). GIC-treated specimens showed no apatite precipitation but the preservation of collagen phosphorylation (1030-1080 cm^{-1}) plus the presence of carbonates (870 cm^{-1}) corresponding to the v_2CO_3 vibrations. The same collagen phosphorylation (1030-1080 cm^{-1}) band could be observed in the demineralised dentine at baseline.

4. DISCUSSION

Understanding the bio-interaction at the interface between the tooth and ion-releasing materials is crucial in predicting the longevity of such restorations and their physicochemical properties [14,30]. The formulation of innovative adhesive systems and composites with ion-releasing properties [31,32] may represent an alternative to reduce the degradation and repair the resin-dentine interface through remineralisation [2,33]. Since “bioactive” materials release specific ions from their fillers, the exposed collagen fibrils inside the demineralised dentine would become filled with apatite crystals, so recovering the initial stiffness [34] and fossilising the endogenous proteases [35,36]. The results obtained with the GIC material showed an increase in microhardness over time in the demineralised dentine underneath the bonding interface to a maximum depth of 100 μm. However, at 50 μm depth, there was no significant difference compared to the negative control (composite) up to 45 days of AS storage. The micro-CT analysis only showed a slight reduction in demineralised volume. In a previous

study of Pires et al. [31], the authors demonstrated through micro-CT analysis the potential of a GIC in increasing the mineral density of artificially-induced carious dentine, confirming our results.

Further confirmation of the remineralisation ability by the tested GIC was obtained using confocal microscopy. Indeed, a porous mineral accumulation was observed in demineralised dentine 30-40 µm underneath the GIC-dentine interface after 60 days of storage in AS. The SEM analysis showed the presence of mineral deposits on the demineralised dentine surface, while FTIR demonstrated that those deposits were not made by apatite. In accordance with these results, a study found that GICs may not be effective in remineralising simulated caries-affected dentine via intrafibrillar apatite deposition, even when analogues of salivary proteins were employed [37].

It is well known that GICs have the ability to undertake a dynamic ionic exchange with the surrounding microenvironment for a relatively long time [19,31,38,39]. Indeed, such materials exhibit chemical bonding to dental tissues, along with their ability to release specific ions (e.g. fluoride, strontium) at the bonded interface, being classified as bioactive ion-releasing material [2,18,40].

The MTA and RMTA produced the highest increase in KHN values over time, especially at 50 and 100 µm underneath the interface. The micro-CT analysis confirmed the ability of MTA to reduce the demineralised volume significantly. Interestingly, this reduction did not occur to the same extent as RMTA, which showed a performance comparable to GIC. Confocal microscopy observations supported the difference in mineralisation ability of MTA and RMTA. MTA specimens showed only few microns of residual calcium-stained minerals, indicating that, after 60 days of storage, most of the caries-affected dentine was

mineralised. On the contrary, RMTA-dentine interfaces were characterised by calcium-stained minerals accumulated 20-30 microns underneath the interface, indicating that such area was not yet wholly remineralised.

The results of this study agree with the literature that demonstrated dentine remineralisation in the presence of hydraulic calcium silicate cements [21,41]. Such studies demonstrated mineral precipitation with different shapes and dimensions in artificial carious dentine treated with a calcium silicate cement, but no evidence of collagen remineralisation. On the other hand, the RMTA material used in the present study is a light-cured resin-modified calcium silicate cement that can be considered a bioactive material [16,23,33], as confirmed by our results. In these MTA-based materials, an intrinsic instability and rearrangement of filler particles due to the release (or re-uptake) of ions are apparently not compatible with a material designed to be inert [39]. It can be speculated that the presence of a resin-based matrix, albeit hydrophilic, reduces the material's bioactivity once it is placed in the cavity. Therefore, since the bioactive materials tested in the present study showed different behaviour in remineralising artificial caries-affected dentine, the null hypothesis can be rejected.

When the universal adhesive and resin composite were used to restore the artificial caries-affected dentine substrate, an incomplete infiltration of the adhesive was observed together with degradation over time. Furthermore, as shown by micro-CT and microhardness analyses, no sign of remineralisation and no significant increase in hardness was observed. Indeed, it is well known that the peculiar chemical and morphological characteristics of caries-affected dentine are mainly responsible for the unsatisfactory bonding performance of adhesive/composite restorations [42,43]. Moreover, the poorly resin-infiltrated

dentine collagen fibrils underneath the resin–dentine interface are much more prone to collagen degradation due to hydrolytic and enzymatic activity [43,44].

Nevertheless, it has to be noted that the presence of a soft, caries-affected dentine layer at the bottom of a cavity does not affect a composite restoration's longevity, provided that good adhesion is obtained in the surrounding tissue to form a seal. In fact, such a condition can arrest the primary lesion progression [8].

The protocol used in this study to obtain artificial caries-affected lesions in class I cavities was suitable for creating an extended (100-120 µm) mineral-depleted dentine. Xylenol Orange solution is a calcium-chelator fluorophore commonly used to trace the deposition of minerals within the interfaces of restored teeth due to its ability to form complexes with Ca^{2+} ions. Such a dye is able to bind dental substrates where there is a substantial availability of calcium-rich substrates expressing free ions, non-complexed calcium-based minerals, partially demineralised hydroxyapatite, or in case there is a freshly formed calcium-rich complex [26,45]. This latter aspect may support the speculation that the RMTA- or GIC-dentine interfaces induced less consistent and porous mineral precipitation underneath the interface. Conversely, the reason why the MTA-dentine interface showed only a slight signal was probably a consequence of increased mineralisation of the demineralised dentine, characterised by more complex calcium based-complex (e.g. hydroxyapatite, as supported by the results in SEM and FTIR analysis). This may also be the reason why the extended 100-150 µm mineral-depleted simulated caries dentine layer was not observed after prolonged storage of the specimens treated with all the ion-releasing tested materials in AS. Moreover, due to their strongly alkaline pH, calcium silicate

cements (MTA) have a caustic effect on demineralised collagen fibrils. The space left after their degradation is replaced by calcium carbonates and apatite-like minerals, which also penetrate several microns inside the dentine tubules [46,47].

The choice of bioactive dentine replacement materials often placed over caries-affected dentine has to be considered in the light of a minimally invasive tooth-restoration concept to preserve the substrate that is still remineralisable [4, 21]. Nevertheless, the behaviour of such materials has to be assessed in terms of translatability to the clinical setting when directly applied to caries-affected dentine. The interactions with overlying restorative materials and their influence on the mechanical performances of the whole restoration also have to be addressed. In this sense*, in vivo* comparative studies are needed to confirm their performances.

5. CONCLUSION

Contemporary ion-releasing materials such as GIC, MTA and RMTA can all remineralise the artificial caries-affected dentine, but to different extents. MTA showed the highest ability to induce apatite precipitation and remineralisation of extended mineral-depleted dentine. Conversely, RMTA and GIC are probably more appropriate for preserving the demineralised collagen fibrils in the outer layer of caries-affected dentine and remineralising it.

6. COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: All authors gave their final approval and agreed to be accountable for all aspects of the work. They have no conflict of interest with respect to the authorship and/or publication of this paper.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent: For this type of study, formal consent is not required. Human molars used in this study were collected according to the guidelines of the local Ethics Committee (CEI20/098).

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FIGURES

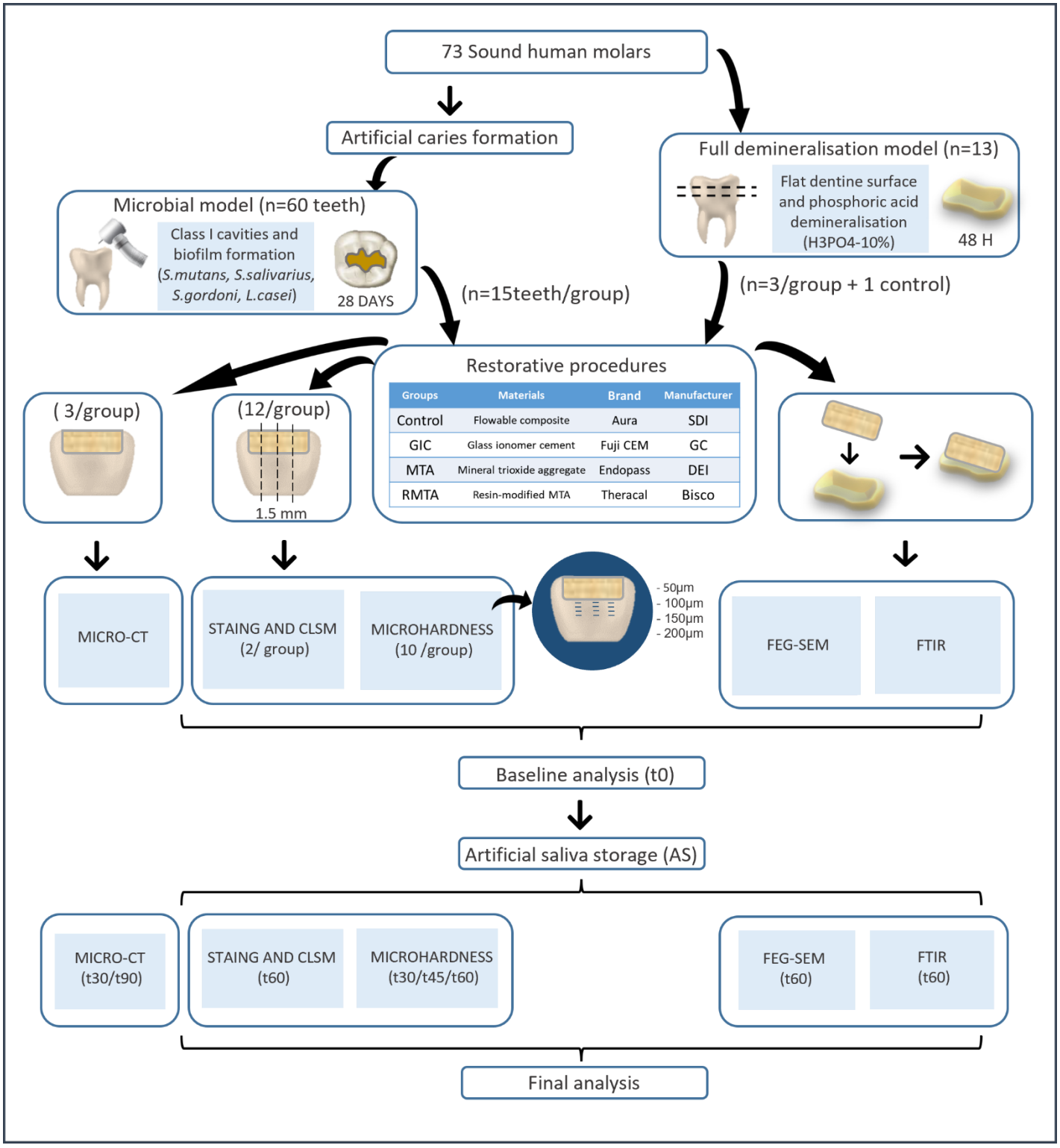

Figure 1: Distribution of specimens and methodological study design.

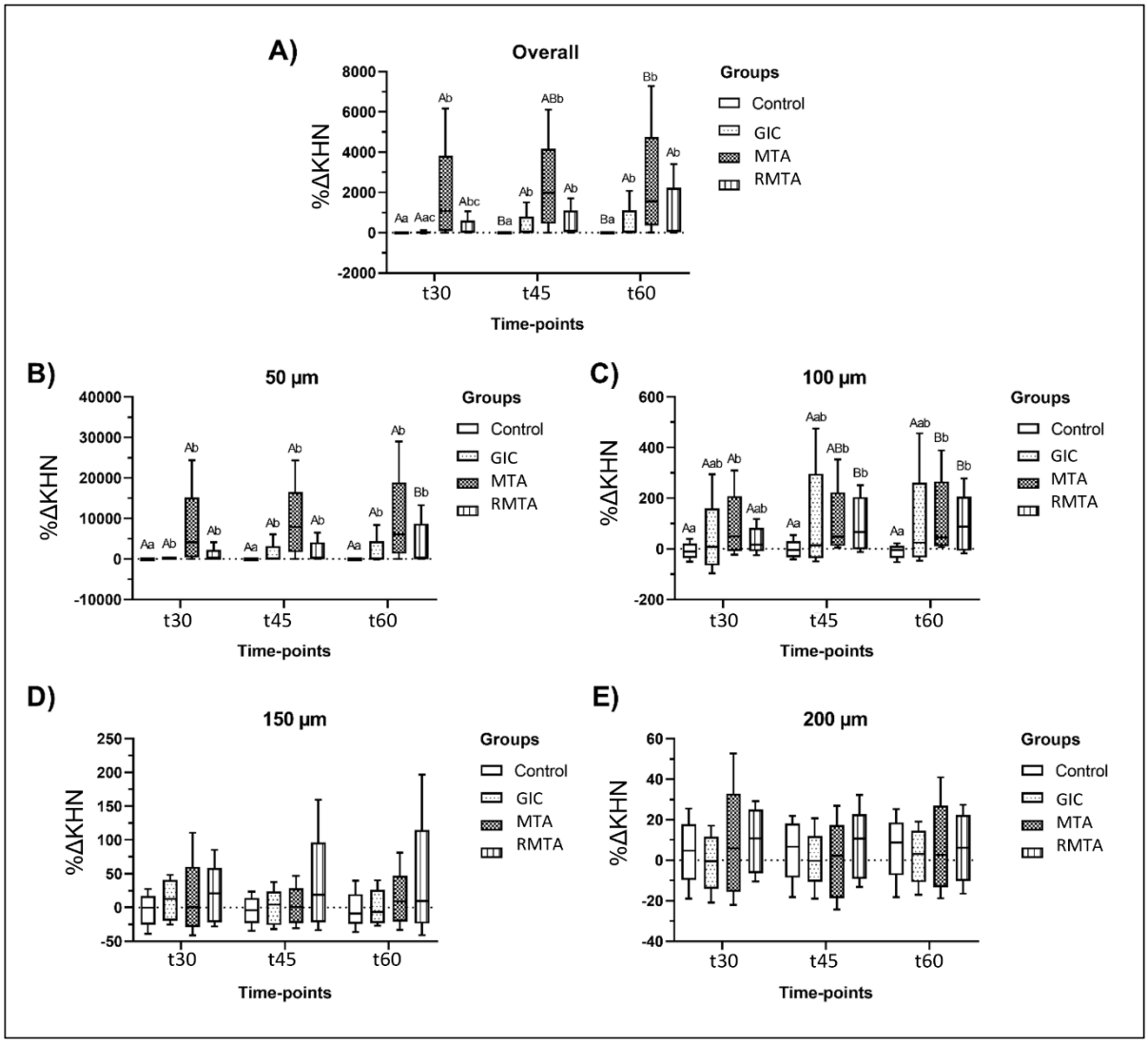

Figure 2: Microhardness assessment of tested materials at different depths and storage times. **(A)** Box plot of the overall microhardness (ΔKHN (%)) obtained at different time-points evaluation T1, T45, and T2 (average of the results of 50, 100, 150, and 200 µm). The ΔKHN (%) results obtained over time (T1, T45, and T2) at different depths are shown in figures **(B)** 50 µm, **(C)** 100 µm, **(D)** 150 µm, and **(E)** 200 µm.

Different uppercase letters indicate significant differences between the groups at different time-point evaluations (Friedman test; α=5%)

Different lowercase letters indicate a significant difference between groups at the same time-point evaluation (Kruskal-Wallis/Dunn tests; α=5%).

The absence of letters in graphs (D) and (E) indicates no differences between groups.

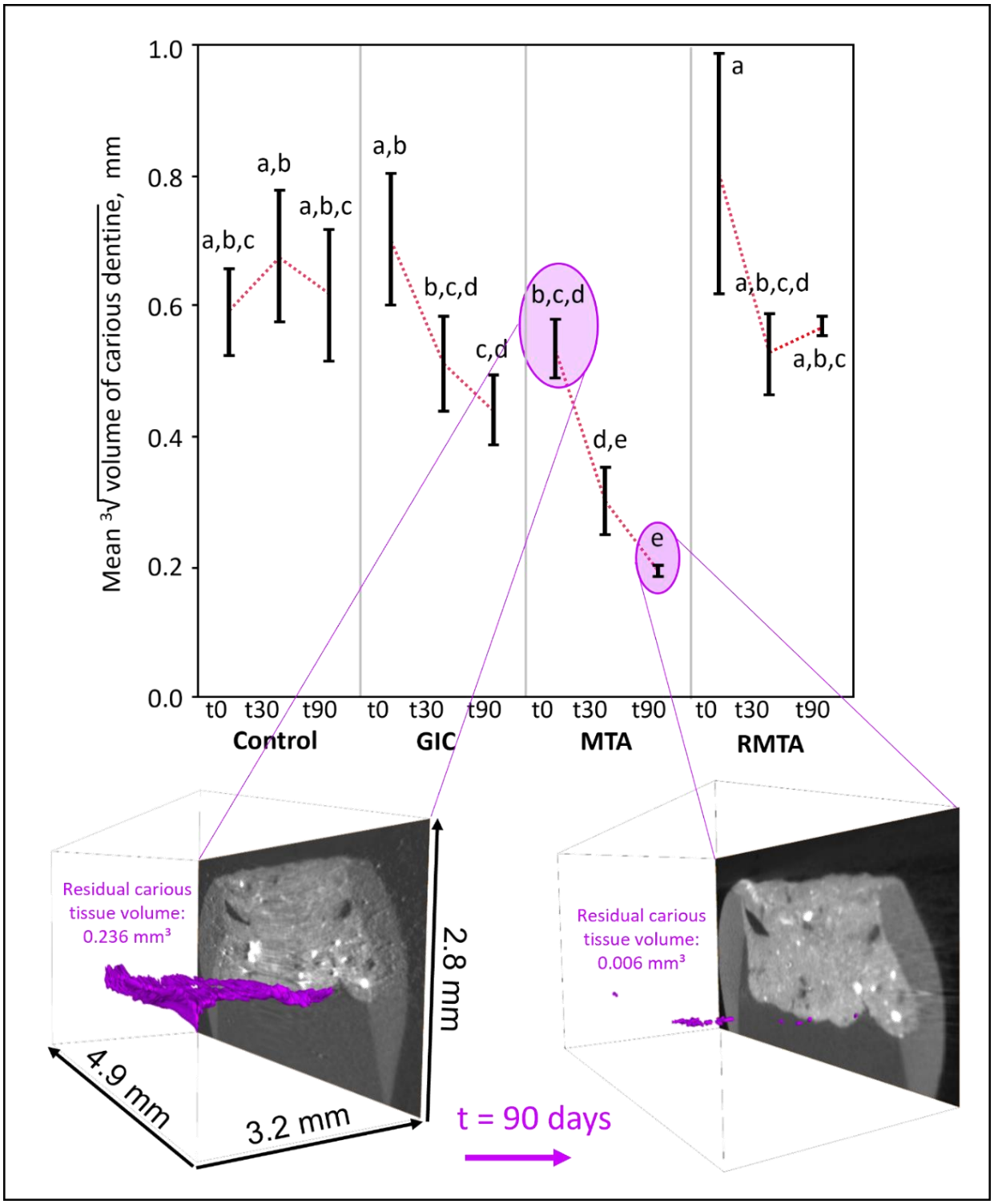

Figure 3: Micro-CT volumetric analysis of the carious dentine zone. Means and standard errors are indicated. Levels connected by different letters are statistically significant (Student's t-test; α=5%).

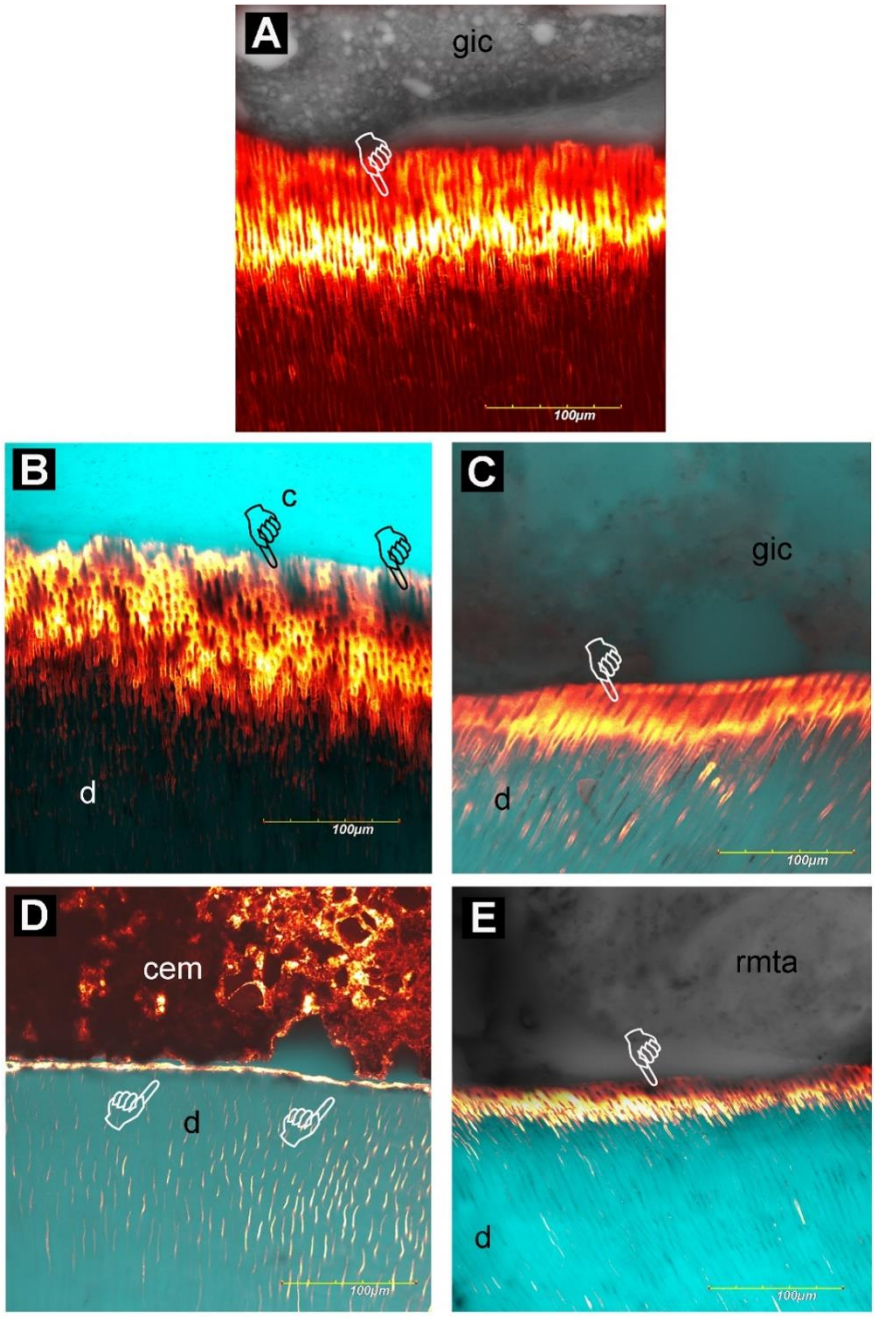

Figure 4: Confocal single projection images captured in transmission/fluorescence mode of the specimens treated with the different materials tested in this study and analysed after 60 d storage in AS. **A)** This is a comparative image in a specimen treated with the GIC at baseline (24h). An extended (100-120 µm) mineral-depleted dentine layer (pointer: simulated caries-affected dentine) just underneath the GIC-dentine (d) interface can be observed. Such a morphological scenario was comparable between all groups at the baseline. **B)** Adhesive+composite specimen showing no sign of remineralisation within the mineral-depleted dentine. Areas of degradation (pointers) occurred during the storage period. **C)** Morphological features observed in GIC-restored specimens. Most of the calcium-stained minerals accumulated only 30-40 µm underneath the GIC-dentine interface. **D)** MTA specimen showing residual calcium-stained minerals accumulated only few microns underneath the MTA-interface after prolonged storage in AS (pointer). Calcium-stained minerals are deposited also within the cement (cem). **E)** Specimen treated with RMTA. Similar to **(D)**, calcium-stained minerals accumulated only few microns (pointer) underneath the RMTA-dentine (d) interface.

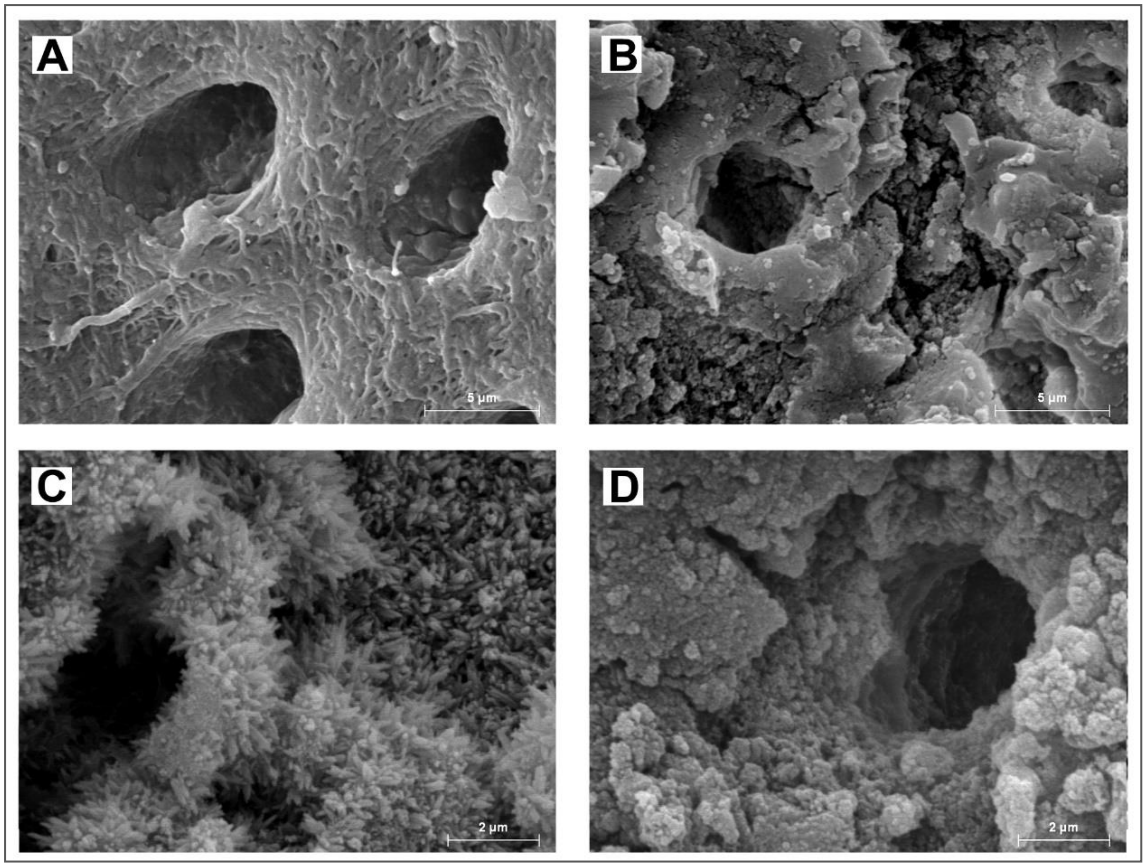

Figure 5: SEM micrographs obtained after prolonged storage (60 days) in AS. **A)** Representative specimen treated with conventional adhesive/composite materials showing the presence of demineralised dentine collagen fibrils with no presence of mineral precipitation (no signs of remineralisation). **B)** This specimen treated with GIC shows a surface with no exposed collagen fibrils, partially covered by minerals and residual material. **C)** This is a representative specimen treated with MTA where it is possible to note the clear presence of needle-like crystals deposited on the dentine surface. **D)** This specimen treated with RMTA presents globular-like crystals deposition of the dentine surface.

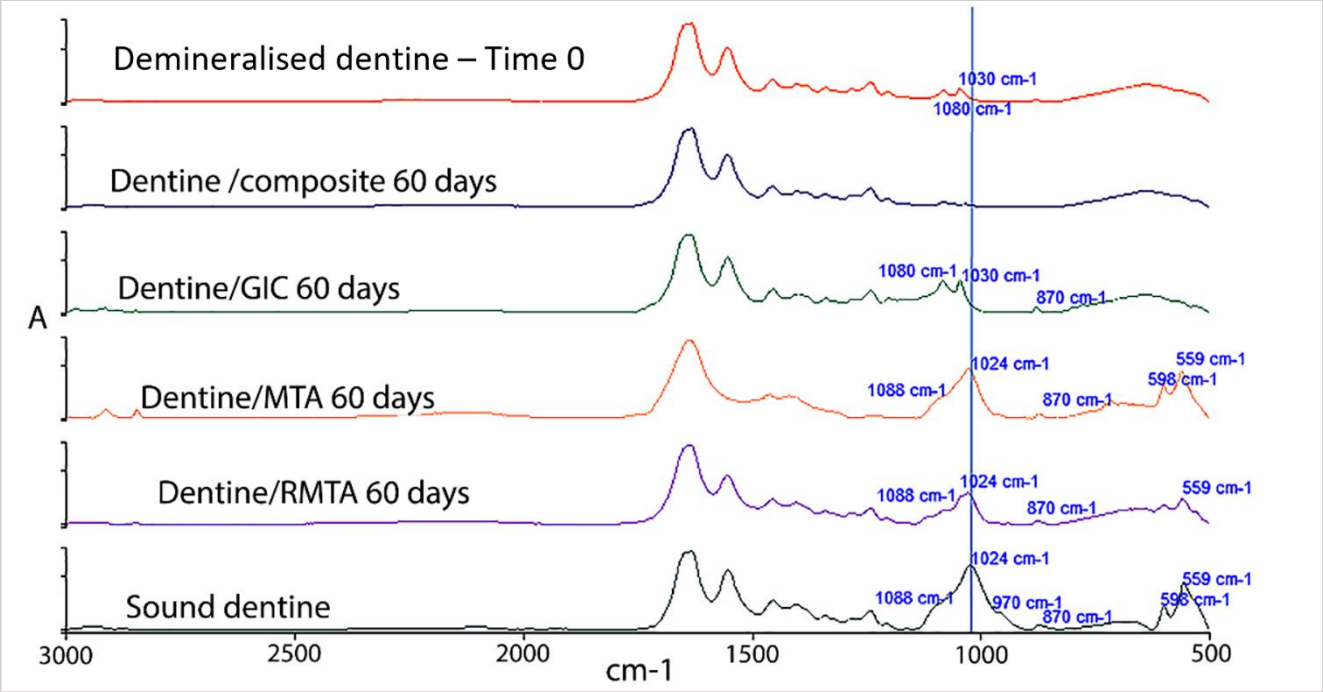

Figure 6: FTIR-ATR characterisation of demineralised dentine treated with the different tested materials and after 6 months storage in AS. The PO stretching of hydroxyapatite presents peaks at 559 cm^{-1}, 598 cm^{-1}, 970 cm^{-1}, 1024 cm^{-1} 1088 cm^{-1} and P–O(H) stretching at 870 cm^{-1}. The dentine collagen is visible at 1650 cm^{-1} (C O: amide I) and 1540 cm^{-1} (CNH: amide II) in MTA and RMTA groups.

Tables

Table 1: Description of materials used in the present study.

Group	Material	Commercial name	Composition	Manufacturer
control	Universal adhesive and flowable composite	ZipBond Aura	Adhesive: monomers including MDP, ethanol, water, fluoride Composite: Acrylic monomers as (6-46%), Diurethane dimethacrylate (6-46%), Triethylene glycol dimethacrylate (6-46%), 2,2-bis[4-(2-methacryloxy)ethoxy)phenyl] propane (6-46%)	SDI, Australia
GIC	Glass ionomer cement	Fuji IX	Powder: 95%w alumino-fluoro-silicate glass, 5% polyacrylic acid powder. Liquid: 50% distilled water, 40% polyacrylic acid, 10% polybasic carboxylic acid. Powder/liquid ratio: 3.6/1.0	GC, Japan
MTA	Trioxide mineral agreggate	Endo-Pass	Tricalciumsilicate, dicalciumsilicate, tricalciumaluminate, phyllosilicates (smectite and hydrotalcite), zirconium dioxide and barium sulfate	DEI Italia, Italy
RMTA	Resin-based trioxide mineral agreggate	TheraCal LC	Portland cement type III <60%, HEMA, polyethleneglycol dimethacrylate<50%, barium zirconate<10%	Bisco Inc, USA

4.5 Artigo 5: Effects of ions-releasing restorative materials on the dentine bonding longevity of modern universal adhesives after load-cycle and prolonged artificial saliva aging.

materials

MDPI

Article

Effects of Ions-Releasing Restorative Materials on the Dentine Bonding Longevity of Modern Universal Adhesives after Load-Cycle and Prolonged Artificial Saliva Aging

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Abstract: This study aimed at evaluating the microtensile bond strength (MTBS) and fractographic features of dentine-bonded specimens created using universal adhesives applied in etch-and-rinse (ER) or self-etching (SE) mode in combination with modern ion-releasing resin-modified glass-ionomer cement (RMGIC)-based materials after load cycling and artificial saliva aging. Two universal adhesives (FTB: Futurabond M+, VOCO, Germany; SCU: Scotchbond Universal, 3M Oral Care, USA) were used. Composite build-ups were made with conventional nano-filled composite (AURA, SDI, Australia), conventional resin-modified glass ionomer cement (Ionolux VOCO, Germany), or a (RMGIC)-based composite (ACTIVA, Pulpdent, USA). The specimens were divided in three groups and immersed in deionized water for 24 h, load-cycled (350,000 cycles; 3 Hz; 70 N), or load-cycled and cut into matchsticks and finally immersed for 8 months in artificial saliva (AS). The specimens were cut into matchsticks and tested for microtensile bond strength. The results were analyzed statistically using three-way ANOVA and Fisher's LSD post hoc test ($p < 0.05$). Fractographic analysis was performed through stereomicroscope and FE-SEM. FTB showed no significant drop in bond strength after aging. Unlike the conventional composite, the two RMGIC-based materials caused no bond strength reduction in SCU after load-cycle aging and after prolonged aging (8 months). The SEM fractographic analysis showed severe degradation, especially with composite applied on dentine bonded with SCU in ER mode; such degradation was less evident with the two GIC-based materials. The dentine-bond longevity may be influenced by the composition rather than the mode of application (ER vs. SE) of the universal adhesives. Moreover, the choice of the restorative material may play an important role on the longevity of the finalrestoration. Indeed, bioactive GIC-based materials may contribute to maintain the bonding performance of simplified universal adhesives over time, especially when these bonding systems are applied in ER mode.

Keywords: adhesion; cycling mechanical stress; dentine; longevity; glass-ionomer cements; universal adhesives

1. Introduction

Direct restorations in modern operative dentistry are frequently accomplished using conventional resin composites due to their excellent mechanical and aesthetic properties [1,2]. Nevertheless, such restorative materials are still characterized by important downsides associated to polymerization shrinkage; a phenomenon that may induce stress at resin–dentine interfaces during the light-curing procedures and jeopardize their longevity [3–5]. Indeed, it has been widely demonstrated that the volumetric contraction of conventional resin composites can transfer polymerization stress directly to the adhesive-bonded interface, causing its innermost deformation due to a lack of proper bonding performance of some adhesive systems [3,6,7]. Consequently, the sealing between composite and dental hard tissues (i.e., dentine and enamel) can be seriously compromised. This will result in gaps and marginal leakage formation, which are pathways for microleakage of oral fluids, bacteria, and enzymes penetration [3,8–10]. Such issues may translate into important clinical problems such as post-operative sensitivity, marginal discoloration, recurrent caries, and advanced pulp pathology in all those cases that are seriously compromised by the caries process [11,12].

The recently introduced universal adhesive systems are currently very popular in general dental practices, as well as in dental hospitals, due to the fact that they can be applied both in self-etching (SE) and etch-and-rinse (ER) modes. Considering their compositions, universal adhesives can be also classified as simplified systems because all ingredients, including acidic functional monomers and solvents, are incorporated into one bottle. They are similar to one-step self-etching systems, so that they might still present issues related to bonding performance, degradation, and longevity [9,13]. However, application in self-etching mode minimizes recontamination of the dentine by blood and saliva during etch washing and drying. This makes SE a less technique-sensitive procedure compared to ER application mode. Moreover, SE systems present further benefits such as less post-operative sensitivity due to residual smear plugs, which are usually only partially removed from inside the dentinal tubules because of the mild acidic nature of SE systems. Indeed, the tubules remain occluded and the dentinal fluid movement is less evident compared to that usually experienced with ER systems [9,11].

On the other hand, great attention has been given to improve the effectiveness and longevity of resin–dentine bonds through several clinical strategies that may abate stress concentration at the resin–dentine interface during polymerization [14]. For instance, the use of flowable composites or resin-modified glass-ionomer cements (RMGIC) as liners or as dentine substitute materials may represent a suitable method to provide a sort of "stress-absorption" effect at the bonding interface [15,16]. This has been advocated to prevent stress development at the dentine-bonded interface and reduce gap formation, microleakage, and degradation over time [14,17,18]. Although RMGIC are self-adhesive materials, they are also often applied in dentine after etching and adhesive application, especially in those situations where the structure of the dental crown is highly compromised and a lack of mechanical retention is encountered [19–21].

It is also important to consider that occlusal stress during mastication, swallowing, as well as in cases of parafunctional habits, can affect the integrity of the bonding interface, making such a structure more susceptible to "quicker" degradation in the oral environment [22]. This seems to be of particular interest in modern, minimally invasive therapeutic restorative dentistry since it has been demonstrated that cyclic mechanical stress can promote gap formation at the margins along the composite restorations; bacteria penetration into narrow marginal gaps might ultimately promote secondary caries formation [23]. Recently, it has been advocated that ion-releasing resin-based

restorative materials can reduce such biofilm penetration into marginal gaps of simulated tooth restorations; the risk for development and propagation of secondary caries is also reduced [24].

It is widely accepted that glass-ionomer cement (GIC)-based materials have a bioactive ability to release therapeutic ions such as fluoride. The presence of such ions has been associated with long-term caries inhibition when GIC-based materials are applied as a dentine substitute [25–27]. Moreover, GIC-based materials are an ideal dentine substitute as their physical properties, such as the coefficient of thermal expansion, dimensional stability, optical properties (i.e., opacity), and microhardness, are very close to that of dentine [28]. ACTIVA BioActive Restorative is a new type of restorative, bioactive, flowable, resin-based composite comparable to RMGICs. It contains fluoro-aluminum silicate particles and polyacid components of glass ionomer that undergo the acid-base setting reaction. Moreover, a bioactive ionic resin matrix is also contained in ACTIVA, which confers both light and chemical polymerization. According to the manufacturer, ACTIVA release calcium, phosphate, and fluoride when in contact with saliva. It has been advocated that restorative materials able to release specific "therapeutic" ions (e.g., calcium, phosphates, fluoride, strontium, and other minerals) into the dental hard tissues may buffer the constant assault of day-to-day ingestion of acidic food and beverages and encourage remineralization along the margins of the restoration with the tooth [29]. However, it is of great relevance that the use of ion-releasing materials in restorative dentistry may contribute to the reduced activity of proteases such as metalloproteinases (MMPs) and cathepsins involved in collagen degradation. Such enzymes are considered one of the main causes for reduction of bonding longevity when simplified bonding systems are applied in dentine with self-etching or etch-and-rinse protocols [30,31]. Moreover, there is a lack of knowledge about the effects of modern ion-releasing materials based on glass ionomer cements on resin–dentine interfaces created using current universal adhesives after mechanical load cycling and prolonged storage in artificial saliva.

Thus, the aim of this study was to evaluate, after short-term load-cycle aging or after load-cycle stress followed by prolonged aging (8 months) in artificial saliva (AS), the microtensile bond strength (MTBS) of resin–dentine bonded specimens created using universal adhesives applied in an etch-and-rinse or self-etching mode in combination with modern ion-releasing RMGIC-based materials. Fractographic analysis was also performed using field-emission scanning electron microscopy (FE-SEM).

The hypothesis tested was that compared to conventional resin composite, the use of modern ion-releasing materials would preserve the bonding performance of modern universal adhesives, applied in etch-and-rinse or self-etching, after mechanical load cycling and/or prolonged storage in artificial saliva (8 months).

2. Materials and Methods

2.1. Preparation of Dentine Specimens and Experimental Design

Sound human molars were extracted for periodontal or orthodontic reasons (ethical approval number: LEC Nº 11.18, 05/12/2018) and stored in distilled water at 5 °C for no longer than 3 months. The roots were removed 1 mm beneath the cemento–enamel junction using a diamond blade (XL 12205; Benetec, London, UK) mounted on a low-speed microtome (Remet evolution, REMET, Bologna, Italy). A second parallel cut was made to remove the occlusal enamel and expose mid-coronal dentine.

Three main groups (n = 72 specimens/group) were created based on the restorative materials used in this study: (i) RC: Resin composite (Aura SDI, Bayswater Victoria, Australia), applied in 2 mm increment layers up to 6 mm, and light-cured as per manufacturer's instructions; (ii) RMGIC: Resin-modified glass-ionomer cement (Ionolux; VOCO GmbH, Cuxhaven, Germany) mixed for 10 s in a trituration unit and applied in bulk. Two capsules of RMGIC were used and each one was light-cured as per manufacturer's instructions to obtain 6 mm build-ups; (iii) ACTIVA (ACTIVA BioActive Restorative, PULPDENT, Watertown, MA, USA) applied in 2 mm increment layers up to 6 mm and light-cured as per manufacturer's instructions. Light-curing was performed using an

light-emitting diode (LED) light source (>1000 mW/cm^2) (Radii plus, SDI Ltd., Bayswater Victoria, Australia). The experimental design of this study required that the specimens in each main group were subsequently subdivided into four sub-groups (n = 18 specimens/group) based on the protocol employed for bonding procedures. Two modern universal adhesives were employed in this study: SCU (Scotchbond Universal, 3M Oral Care, St. Paul, MN, USA); FTB: (Futurabond M+, VOCO, Cuxhaven, Germany). These adhesives were applied as per manufacturer's instructions in self-etching (SE) or in etch-and-rinse (ER) mode (Table 1). In groups SCU–ER and FTB–ER, dentine was etched with 37% orthophosphoric acid for 15 s and subsequently rinsed with distilled water (15 s) and blotted, leaving the substrate moist. Adhesives were light-cured for 10 s. In groups SCU–SE and FTB–SE, the adhesives were applied with a microbrush for 20 s and air dried for 5 s to evaporate the solvent. These were finally light-cured for 10 s using am LED light source (>1000 mW/cm^2) (Radii plus, SID Ltd., Bayswater VIC, Australia). The specimens were finally restored with the selected restorative materials as aforementioned in the main groups. At this point, the specimens in each sub-group were furtherly divided into three groups (n = 6 specimens/group) based on the aging protocol: CTR: no aging (control, 24 h in deionized water); LC: Load cycling (350,000 cycles in artificial saliva); LC–AS: Load cycling (350,000 cycles in artificial saliva), followed by prolonged water storage (8 months in artificial saliva). A detailed description of the test groups can be found in Table 2 (Experimental design). The composition of the artificial saliva was AS: 0.103 g L^{-1} of $CaCl_2$, 0.019 g L^{-1} of $MgCl_2 \cdot 6H_2O$, 0.544 g L^{-1} of KH_2PO_4, 30 g L^{-1} of KCl, and 4.77 g L^{-1} HEPES (acid) buffer, pH 7.4] [32]. The specimens in the subgroup LC and LC–AS were mounted in plastic rings with acrylic resin for load cycle testing. A compressive load was applied to the flat surface (3 Hz; 70 N) using a 5 mm diameter spherical stainless-steel plunger attached to a cyclic-load machine (model S-MMT-250NB; Shimadzu, Tokyo, Japan) while immersed in AS [18,33].

Table 1. Adhesive system, composition, and application procedures.

Name	Composition	Application
Scotchbond Universal, 3M Oral Care, USA (lot: 627524)	10-MDP, HEMA, silane, dimethacrylate resins, Vitrebond™ copolymer, filler, ethanol, water, initiators, and catalysts (pH 2.7)	1. Apply the adhesive on the surface and rub it for 20 s. 2. Gently air-dry the adhesive for approximately 5 s for the solvent to evaporate. 3. Light cure for 10 s (>500 mW/cm^2).
FuturaBond M+, VOCO, Germany (lot: 1742551)	HEMA, BIS-GMA, ethanol, Acidic adhesive monomer (10-MDP), UDMA, catalyst ethanol, water, initiators, and catalysts (pH 2.8)	1. Apply the adhesive homogenously to the surface. 2. Rub for 20 s. 3. Dry off the adhesive layer with dry, oil-free air for at least 5 s. 4. Light cure for 10 s (>500 mW/cm^2).

Abbreviations: 10-MDP 10-methacryloxydecyl dihydrogen phosphate, Bis-GMA bisphenol A diglycidyl methacrylate, HEMA 2-hydroxyethyl methacrylate, UDMA urethane dimethacrylate.

2.2. *Micro-Tensile Bond Strength and Failure/Fractographic Analysis*

The specimens were cut after the aging period using a hard-tissue microtome (Remet evolution, REMET, Bologna, Italy) across the resin–dentine interface, obtaining approximately 15–18 matchstick-shaped specimens from each tooth (Ø 0.9 mm^2). These were submitted to microtensile bond strength tests using a device with a stroke length of 50 mm, peak force of 500 N, and a displacement resolution of 0.5 mm. Modes of failure were evaluated at 50× magnification using stereoscopic microscopy and conveyed in a percentage of adhesive (A), mixed (M), or cohesive (C) bonding fracture. Five representative fractured specimens from each sub-group were mounted on aluminum stubs with carbon glue after the critical-point drying process. The specimens were gold-sputter-coated and

analyzed using field-emission scanning electron microscopy (FE-SEM S-4100; Hitachi, Wokingham, UK) at 10 kV and a working distance of 15 mm.

Bond strength values in MPa were initially assessed for normality distribution and variances homogeneity using Kolmogorov–Smirnov and Levene's tests, respectively. Data were then analyzed using a three-way Analysis of Variance (ANOVA Factors: restorative material, adhesive, and aging protocol) and Newman–Keuls multiple-comparison test ($\alpha = 0.05$). SPSS V16 for Windows (SPSS Inc., Chicago, IL, USA) was used.

Table 2. Experimental design. Distribution of specimens in groups and sub-groups for evaluation via microtensile bond strength (MTBS), interface confocal microscopy, and SEM fractographic analysis. CTR = control, no aging; LC = load-cycling; AS = artificial saliva.

Total Number of Specimens in Main Groups	**RESIN COMPOSITE (72 Specimens)**			**RMGIC (72 Specimens)**			**ACTIVA (72 Specimens)**		
Number of specimens in sub-groups (18/group)	Number of specimens in aging sub-groups (6/ group)								
SCU–ER: Scotchbond Etch and rinse	CTR 6 spec	LC 6 spec	LC+AS 6 spec	CTR 6 spec	LC 6 spec	LC+AS 6 spec	CTR 6 spec	LC 6 spec	LC+AS 6 spec
FTB–ER Futurabond M+ Etch and rinse	CTR 6 spec	LC 6 spec	LC+AS 6 spec	CTR 6 spec	LC 6 spec	LC+AS 6 spec	CTR 6 spec	LC 6 spec	LC+AS 6 spec
SCU–SE: Scotchbond Self-etch	CTR 6 spec	LC 6 spec	LC+AS 6 spec	CTR 6 spec	LC 6 spec	LC+AS 6 spec	CTR 6 spec	LC 6 spec	LC+AS 6 spec
FTB–SE: Futurabond M+ Self-etch	CTR 6 spec	LC 6 spec	LC+AS 6 spec	CTR 6 spec	LC 6 spec	LC+AS 6 spec	CTR 6 spec	LC 6 spec	LC+AS 6 spec

3. Results

Micro-Tensile Bond Strength (MTBS) and Failure Mode Analysis

There were no pre-test failures before the microtensile bond strength assessment. Three-way ANOVA revealed a significant effect of adhesive ($F = 28.75$, $p < 0.001$) and restorative material ($F = 6.68$, $p < 0.001$) on the bond strength, whereas the aging protocol was not statistically significant ($F = 8.17$; $p = 0.125$). The interactions between the three variables were significant ($p < 0.001$).

The results of the microtensile bond strength test (mean and ± SD) are depicted in Table 3. It was observed that there was no significant difference ($p > 0.05$) at 24 h testing between the two adhesives when applied in etch-and-rinse (ER) or self-etching (SE) mode and then restored using the conventional RC or the two RMGIC-based materials (IONOLUX and ACTIVA). Conversely, the specimens created with the conventional RMGIC presented no significant differences ($p > 0.05$) when bonded using the two adhesives applied in ER or SE mode. However, all the specimens created with the conventional RMGIC showed a significant lower bond strength compared to those created with RC or ACTIVA. The failure mode showed that all the specimens restored with the RMGIC failed mainly in the cohesive mode, leaving a clear presence of the material still bonded to the dentine. The specimens created with RC or ACTIVA failed mainly in the cohesive in composite and mixed mode, leaving part of the dentine still covered by the restorative material and the other part exposed.

The fractographic analysis showed that the restorative materials employed in this study had no influence on the outcomes in the control storage period (24 h), but all those specimens created with SCU in ER mode presented less resin infiltration within exposed acid-etched dentine collagen fibrils (Figure 1A,B), while the specimens bonded using the FTB applied in ER mode presented fractures mainly underneath the hybrid layer (Figure 1C). Moreover, in this latter case, there was mineralized peri-tubular dentine around the lumen of the dentine tubules and no demineralized and exposed collagen fibrils (Figure 1D). Conversely, all the specimens bonded with the two adhesives applied in SE or ER mode and then restored with RMGIC showed a surface still covered by the restorative material

(cohesive mode within RMGIC) with no exposure of the dentine (Figure 1E,F) after microtensile bond strength testing. Furthermore, the fractographic analysis showed that the specimens created both with SCU (Figure 1F) and FTB (Figure 1G) applied in SE, and that failed in mixed or adhesive mode, presented a dentine surface still covered by a smear layer with no presence of collagen fibrils and/or exposed dentinal tubules (Figure 1I).

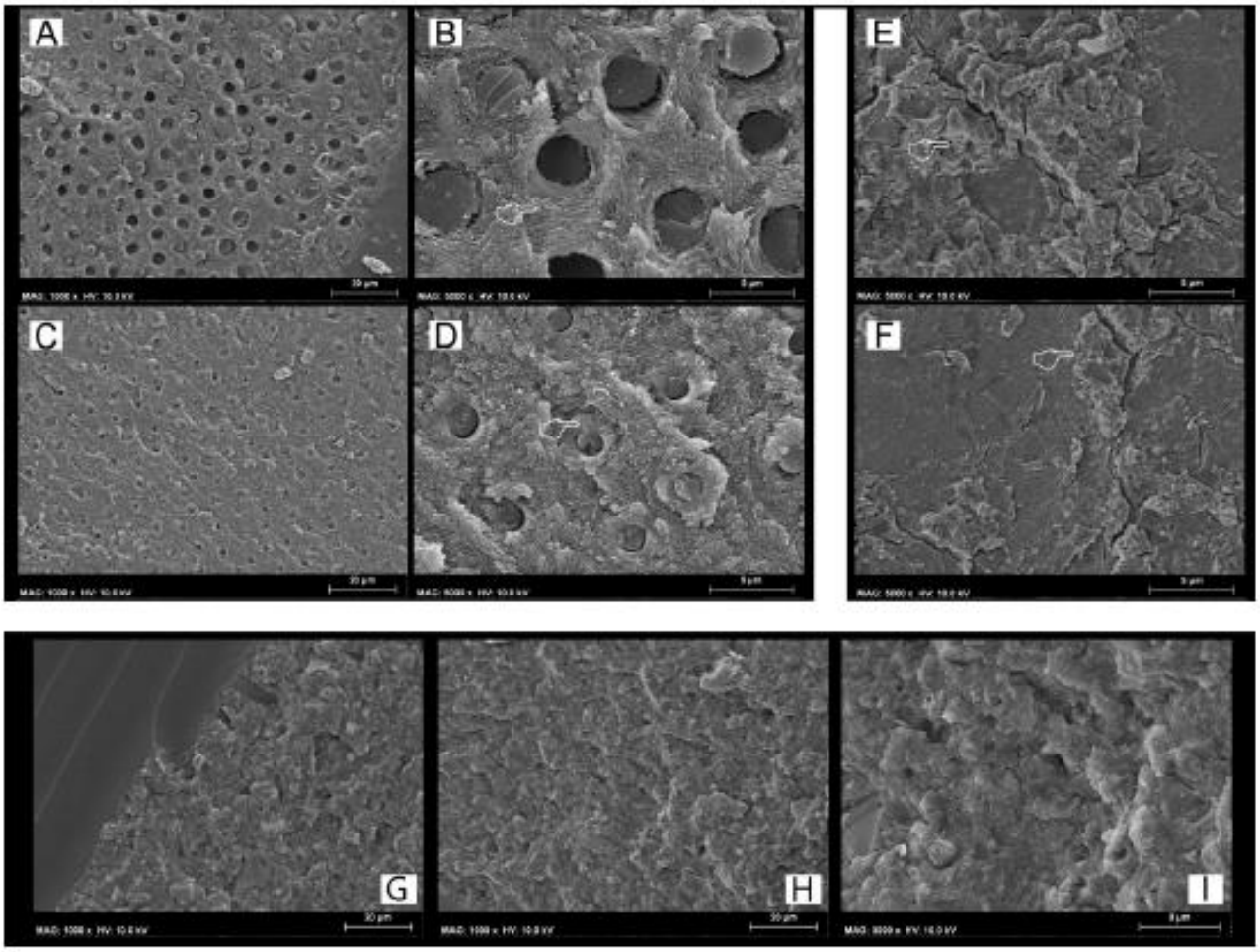

Figure 1. SEM fractographic analysis of the control specimens. (**A**) SEM fractography of a specimen created with SCU applied in ER mode and restored with resin composite (RC) showing the presence of exposed dentine and several resin tags still in the dentinal tubules. (**B**) At higher magnification, it is possible to note the presence of resin tags inside demineralized dentine tubules and collagen fibrils not well infiltrated by the SCU adhesive (pointer). This latter morphological characteristic may indicate that such resin–dentine interface would be affected by degradation over time and would drop in bond strength. (**C**) SEM fractography of a specimen created with FTB applied in ER mode and restored with ACTIVA showing the presence of exposed dentine and several resin tags still inside the small lumen of the dentinal tubules. (**D**) At higher magnification it is possible to observe a typical failure occurred at the bottom of the hybrid layer (HL) characterized by the presence of mineralized peritubular dentine (pointer), with tubules totally obliterated by resin tags and with no presence of demineralized exposed collagen fibrils. Conversely, the dentine specimens bonded with SCU (**E**) and FTB (**F**) applied in ER mode and restored with the RMGIC show the presence of the remaining RMGIC that totally covered the dentine surface. (**G**) SEM fractography of a specimen created with SCU applied in SE mode and restored with ACTIVA and (**H**) FTB applied in SE mode and restored with RC showing a characteristic failure in mixed mode. Note the presence of the remaining resin (**G**) and smear layer on the dentine surface; the latter was even more evident at higher magnification (**I**).

Table 3. The results show the mean (± SD) of the MTBS (MPa) to dentine and the percentage (%) of the failure mode analysis.

	RESIN COMPOSITE			RMGIC			ACTIVA		
	CTR	LC	LC+AS	CTR	LC	LC+AS	CTR	LC	LC+AS
SCU–ER:	48.9 (7.6)	33.5 (5.6)	28.1 (5.7)	35.1 (7.1)	33.4 (7.8)	31.1 (8.8)	55.3 (6.1)	53.1 (7.1)	50.1 (6.8)
Scotchbond	A1	B2	B2	B1	B1	B1	A1	A1	A1
Etch and rinse	50/45/5	15/55/30	10/50/40	80/20/0	75/20/5	50/35/15	45/55/0	55/40/5	30/50/20
FTB–ER	51.2 (5.9)	58.1 (7.3)	55.3 (6.5)	31.3 (6.7)	32.1 (6.6)	32.1 (7.1)	54.2 (5.7)	52.7 (6.2)	52.1 (5.6)
Futurabond M+	A1	A1	A1	B1	B1	B1	A1	A1	A1
Etch and rinse	55/40/5	45/50/5	20/65/15	70/30/0	65/35/0	60/30/5	45/55/0	55/40/5	30/50/20
SCU–SE:	45.1 (5.2)	44.4 (6.2)	34.1 (5.9)	32.3 (7.4)	34.4 (7.2)	29.6 (7.9)	46.1 (6.2)	49.8 (7.4)	49.5 (6.9)
Scotchbond	A1	A1	B1	B1	B1	B1	A1	A1	A1
Self-etch	45/50/5	40/50/10	10/55/35	70/30/0	65/30/5	50/45/5	40/55/5	30/65/5	45/50/5
FTB–SE:	49.2 (4.9)	48.3 (9.3)	45.6 (7.5)	34.1 (6.2)	31.5 (7.7)	30.5 (7.5)	48.1 (6.2)	51.1 (7.4)	50.5 (7.4)
Futurabond M+	A1	A1	A1	B1	B1	B1	A1	A1	A1
Self-etch	40/50/10	45/50/5	25/60/15	75/25/0	70/30/50	60/35/5	40/55/5	45/50/5	45/50/5

Failure mode [Cohesive/Mixed/Adhesive]. The same number indicates no significance in column, while the same letter indicates no significance in row ($p > 0.05$).

After submitting the specimens to load-cycle aging, the only group that showed a significant bond strength drop ($p < 0.05$) was that created with the SCU applied in ER mode and restored using the conventional RC. In this group, an important change in the failure mode was also observed; only 15% of the specimens failed in cohesive mode, while failure in mixed and adhesive modes were 55% and 30%, respectively (Table 3). This situation was not evident in the specimens bonded with the same adhesive but restored using IONOLUX (RMGIC) or ACTIVA; no significant bond strength drop ($p > 0.05$) and no radical change in failure mode was observed. The SEM fractography showed no important ultra-morphological changes in most of the fractured resin–dentine interfaces of these groups compared to the control group. Conversely, the specimens created with the SCU applied in ER mode (Figure 2A) and restored with the conventional RC, which failed prevalently in mixed and adhesive mode, showed that the fracture occurred underneath the hybrid layer with no sign of demineralized and/or poorly infiltrated collagen fibrils (Figure 2B,C).

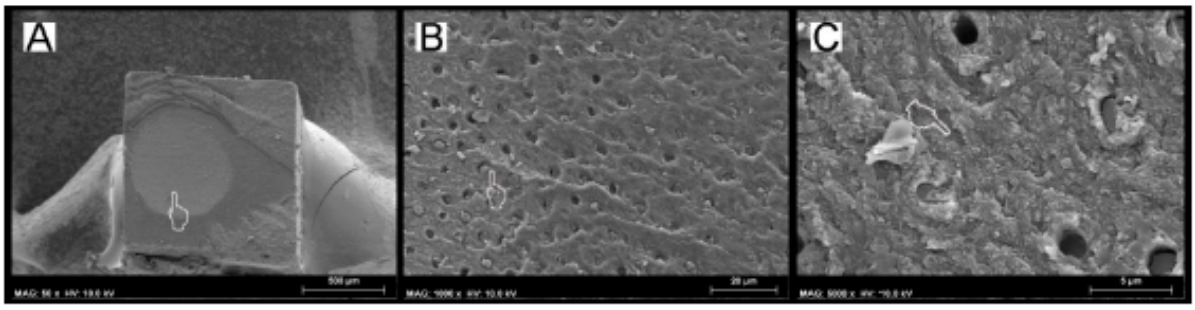

Figure 2. SEM Fractographic analysis after load-cycle aging. (**A**) SEM fractography of a specimen created with SCU applied in ER mode and restored with RC showing a characteristic failure in mixed mode. The finger pointer indicates a brighter area of greater and more evident aging (pointer), which was probably induced by the cycling load. However, when we observed that specific area at higher magnifications (**B**), it was possible to observe that the fracture occurred underneath the hybrid layer (pointer), which is characterized by the presence of mineralized dentine (pointer), with tubules totally obliterated by resin tags and with no presence of demineralized exposed collagen fibrils (**C**).

The prolonged aging in artificial saliva performed subsequent load-cycling stress induced important changes on microtensile bond strength as well as on the ultramorphology of the fracture of some specific groups. In particular, the specimens bonded with SCU applied both in ER and SE mode and then restored with the conventional composite had a significant drop in bond strength compared to the specimens in the groups CTR and LC ($p < 0.05$). Moreover, the number of failures in mixed and adhesive modes increased in the aforementioned groups compared to the control

(CTR) group. The SEM fractography showed evident signs of dentine degradation in the group of specimens created with SCU applied in ER mode and then restored with the conventional composite (Figure 3(A1,A2)). The SEM fractography showed that specimens created with SCU applied in SE mode and then restored with the RC presented degradation both of the adhesive (Figure 3B) and dentine hybrid layers (Figure 3C). Conversely, the same specimens restored with the RMGIC or ACTIVA presented a stable bond strength with no significant drop ($p < 0.05$), and the type of failure remained quite similar to the control group. The SEM fractography showed no drastic changes in all those groups for the ultramorphology of fractured resin–dentine interfaces compared to the control group (Figure 3D,E). In particular, the SEM fractography of a specimen created with SCU applied in ER mode and restored with ACTIVA and RMGIC showed the presence of dentine that was well mineralized with no sign of demineralized collagen fibrils, but with the presence of mineral debrides as a possible result of the bioactivity of such GIC-based materials (Figure 3D).

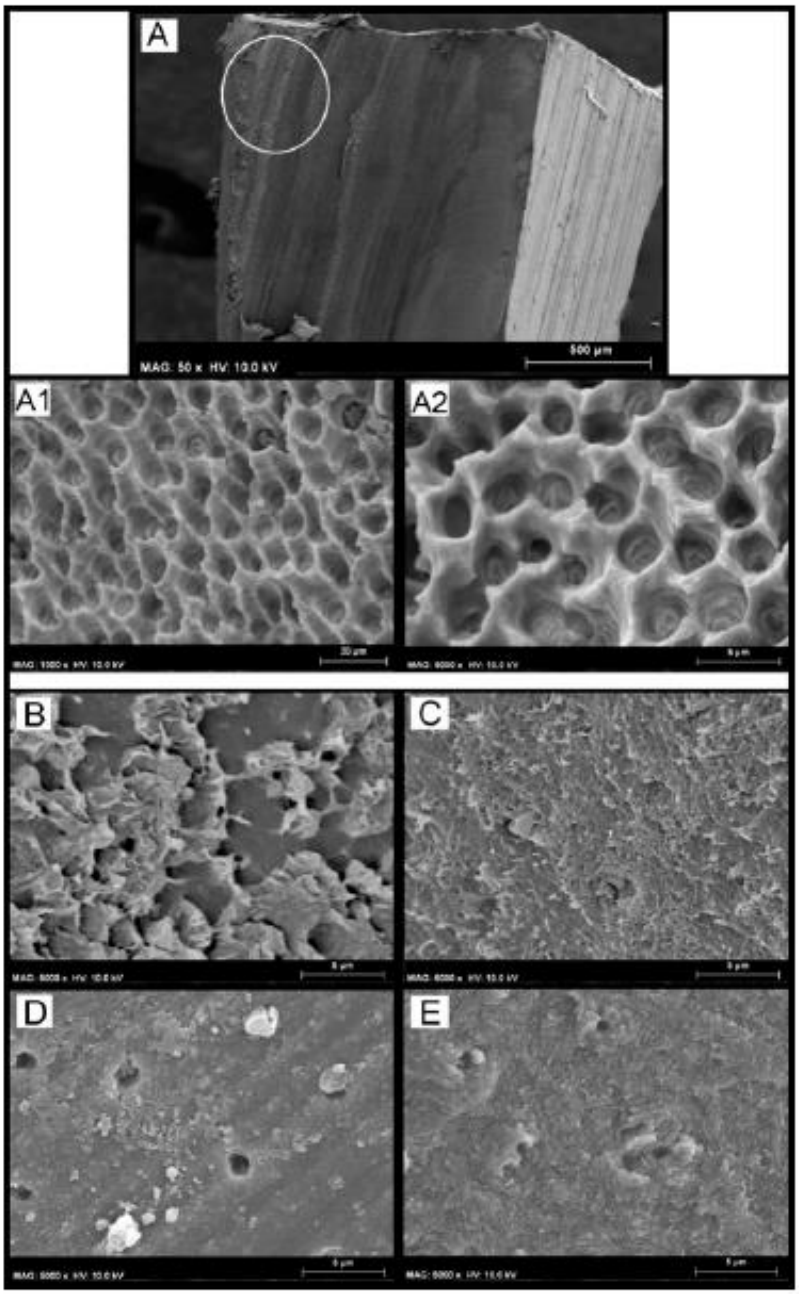

Figure 3. SEM Fractographic analysis after load cycling and aging in artificial saliva. (**A**) SEM fractography of a specimen created with SCU applied in ER mode and restored with RC showing

a characteristic failure in adhesive mode. Note that the white circle indicates no physical difference in the material; it was added to show the reader that images (**A1**,**A2**) were obtained by higher magnification in that zone. Indeed, in (**A1**,**A2**) it is possible to see severe collagen degradation without the presence of any resin residual. (**B**) SEM fractography of a specimen created with SCU applied in SE mode where it is possible to see a failure between composite and adhesive, probably due to degradation induced by excessive water sorption upon mechanical stress and prolonged AS storage. However, it was also possible to see, in those specimens that failed in mixed mode, signs of degradation of the collagen fibrils underneath the hybrid/interdiffusion layer (**C**). (**D**) SEM fractography of a specimen created with SCU applied in ER mode and restored with ACTIVA showing that the failure occurred underneath the hybrid layer, but the exposed dentine is well mineralized with no sign of exposed demineralized collagen fibrils. Note also the presence of mineral debrides that are a possible result of the bioactivity of ACTIVA, which released ions and diffused through the resin-bonded dentine. (**E**) SEM fractography of a specimen created with FTB applied in SE mode and restored with RMGIC. The specimens of this group failed mainly in cohesive and mixed mode; this latter zone is characterized by a fracture occurring underneath the hybrid layer, leaving behind a dentine surface completely mineralized with no sign of exposed, denatured, or demineralized collagen fibrils. Please note the presence of a well mineralized intratubular dentine inside the lumen of the dentine tubules.

4. Discussion

This study showed that the use of modern ion-releasing materials such as conventional RMGIC or RMGIC-based composite (ACTIVA) preserved the bonding performance of only one (SCU) of the two modern universal adhesives bonded to dentine in etch-and-rinse or self-etching mode, after the two aging protocols employed in the experimental design. Conversely, the dentine-bonded specimens created with the FTB universal bonding system applied in etch-and-rinse or self-etching showed no significant drop in bonding performance after aging, regardless the restorative material employed or the aging protocol. Hence, the hypothesis tested in this study needs to be partially accepted as the use of a specific new generation universal bonding systems may confer a stable dentine-bonded interface over time. Nevertheless, the use of modern ion-releasing restorative materials such as RMGIC or ACTIVA may preserve the bonding performance of those universal adhesives that are more prone to degradation after aging.

The effects of the load-cycle aging protocol on the bonding performance of the SCU system applied in ER mode and restored with the conventional RC were relevant; the bond strength of this group of specimens dropped significantly ($p < 0.05$). Moreover, only the specimens bonded using SCU applied both in ER and SE mode and then restored with the conventional composite showed a significant drop in bond strength compared to the specimens in the control (CTR, 24 h) group after prolonged aging in artificial saliva. The ultramorphology analysis performed in the specimens of the control group (24 h), created using the SCU system applied in ER mode and restored with RC showed the presence of demineralized-acid-etched dentine collagen that was not well resin-infiltrated (Figure 1A,B). While the same specimens submitted to LC aging showed no exposed collagen, but mineralized dentine with resin tags that obliterated the dentinal tubules (Figure 2). This was an interesting result, so we hypothesize that a possible explanation to the difference in bonding performance observed between these two latter situations (LC-only aging vs. CTR) may be attributed to the fact that the hybrid layer created using simplified adhesives applied in etch-and-rinse mode can represent the critical part of the resin–dentine interface, as it probably remains only partially polymerized [33–35]. Indeed, it has been advocated that during cycling loading such un-polymerized monomers within the hybrid layer, created with simplified, highly hydrophilic etch-and-rinse adhesives, may be mechanically "intruded" into the demineralized dentine causing a more compact and performant hybrid layer. However, such a morphological change within the resin–dentine interface may favor higher stress concentrations during the cycling load at the bottom of the hybrid layer, causing an accelerated mechanically-induced degradation phenomenon in this specific zone that often remains partially

demineralized and poorly infiltrated by adhesive monomers [34,35]. Indeed, the absence of a proper, partially demineralized bottom of the hybrid layer may explain why the dentine bonded with SCU or FTB in SE mode showed no bond-strength drop after load-cycle aging, regardless of the restorative material and the protocol employed for aging [33,34]. Our results seem to be in accordance with those of Dorfer et al. [36] who demonstrated water diffusion within the resin–dentine interface and hybrid layer during flexure; this promoted chemical/mechanical degradation and washout of "poorly" polymerized water-soluble monomers.

Apparently, such type of degradation mentioned above was improbable in dentine etched with phosphoric acid and bonded using the same simplified adhesive (SCU), but restored with RMGIC or ACTIVA. Indeed, such restorative materials may have absorbed some of the stress generated by the load-cycle aging due to their lower modulus of elasticity, thereby reducing the risk for degradation at the bonding interface [14,15]. The fact that the two GIC-based materials with lower moduli of elasticity may have distributed stresses within their bulk structure lowering the tension concentration at the interface created with the SCU adhesive, applied both in ER and SE mode and subsequently submitted to a cycling load followed by prolonged storage in AS. This observation was supported by the absence of reduction in bonding performance compared to those specimens restored with the conventional composite; this latter group presented a significant bond strength drop ($p < 0.05$) after such a prolonged aging protocol. In addition to the significant bond strength reduction (Table 3), the results of this current study also showed the presence of funneled dentinal tubules, with no presence of collagen fibrils and no residual of restorative material on the dentine surface (Figure 1), which are all typical morphological signs that indicate collagen hydrolysis and proteolytic denaturation caused by the activity of proteases such as MMPs and cathepsins [34,35,37]. Conversely, the SCU adhesive applied in ER mode and restored with ACTIVA failed mainly in mixed mode or in cohesive/mixed mode when restored with the RMGIC. The SEM fractographic analysis highlighted in those specimens the presence of exposed dentine due to a fracture that occurred underneath the hybrid layer, which left behind a well mineralized dentine with no sign of collagen degradation. Indeed, in this latter case, mineralized peri-tubular dentine around the lumen of the dentine tubules and with no demineralized and exposed collagen fibrils was often observed; this is a typical ultramorphological aspect of failure occurring away from the hybrid layer in resin–dentine interface characterized by high bonding stability [37].

Furthermore, mineral debris were detected as a possible result of the bioactivity of ACTIVA and RMGIC (Figure 3D). Indeed, glass-ionomer materials are considered the main bioactive ion-releasing restorative materials currently available in clinics, since they may be able to induce mineral growth within the bonded-dentine interface [18]. We speculate that the results of this study may be somehow correlated to the those hypothesized by Toledano et al. [22,33], who showed that when bioactive materials are submitted to mechanical cycling load, they may promote diffusion of ions through the adhesive-bonded dentine due to the permeable nature of simplified all-in-one bonding systems [37], increasing the mineral–matrix ratio, and reduce nanoleakage and permeability at the resin–dentine interface. Moreover, it has been demonstrated that fluoride ions may inhibit both pro- and active metalloproteinases (MMP-2 and MMP-9) [38], thus reducing the enzymatic degradation at the bonding interface. It may be also possible that in the case of diffusion of calcium and phosphate ions through permeable hybrid layers, these may precipitate and crystallize in complex calcium-phosphates and inhibit MMPs through the formation of a Ca-PO/MMP complex [39].

On the other hand, a possible explanation for the differences in bonding performance attained in this study with the two simplified universal adhesives when restored with a conventional RC may be related to their different chemical compositions. Unlike FTB, the SCU system, which was the only adhesive that both when applied in ER and SE mode in combination with RC presented a significant bond strength drop after prolonged aging protocol, contains a polyalkenoic acid copolymer (PAC). It has been shown that PAC contained in adhesives tends to accumulate primarily on the outer surface of the hybrid layer and creates "isles" between dentine and the adhesive layer [39]. It is also well known that PAC has multiple pendent carboxylic acids along a linear backbone that bind water, which

causes important water sorption and solubility. Moreover, the high molecular weight of PAC [40] precludes its penetration into interfibrillar spaces within the acid-etched dentine.

Several reports indicated that simplified adhesives containing relatively high amount of bisphenol A diglycidyl methacrylate (Bis-GMA) in combination with PAC and 2-hydroxyethyl methacrylate (HEMA) do not infiltrate well into acid-etched dentine, so creating HEMA-rich/Bis-GMA-poor hybrid layers. It is also believed that HEMA, mixing with water within the hybrid layer, may produce hydrogels able to absorb water, which in turn enable hydrolytic and enzymatic degradation processes that jeopardize the longevity of resin–dentine interfaces [41–43]. Furthermore, it is generally well known that water-containing and acidic, single-bottle, pre-hydrolyzed silane coupling agents have a relatively short shelf life because both water and lower pH media can cause silane to degrade over time [44]. A modern, universal adhesive such as SCU contains both free silane and silaned nanofillers. Thus, we believe that water sorption at the adhesive layer may have accelerated polymer hydrolysis and filler debonding, reducing the durability of its bonding performance [44,45].

The information obtained in this study, along with all the observations discussed above, may also be relevant to the contemporary philosophy in atraumatic restorative dentistry. This is based on the preparation of minimally invasive cavities in order to preserve as much sound dental tissue as possible. However, such an ultraconservative intervention should always be followed by restorative treatments performed using therapeutic restorative approaches that protect the resin–dentine interface from degradation processes and prevent the reoccurrence of secondary carious lesions [46,47]. It is well known that the bonding performance of adhesive systems applied to caries-affected dentine (CAD) is not as strong as that attained when such materials are used in sound dentine; the bonding performance seems correlated to the low biomechanical properties of CAD (e.g., modulus of elasticity) [47]. Therefore, such a situation leads to failure of the restoration over time, so that improvements and suitable alternative restorative procedures are necessary in order to improve the durability of the bonding between adhesives and CAD. Wang et al [48] demonstrated distinct differences in the depth of dentine demineralization and degree of adhesive infiltration in non-carious and CAD. Because of the structural alteration and porosities in CAD, deeper, demineralized layers occurred. The deeper the demineralized collagen, the poorer the resin infiltration into the deepest part of the CAD. This resulted in phase separation of resin adhesives and "weak" bond strength. However, Tekçe et al. [49] showed that in such circumstances, the use of flowable resin-based composites, RMGICs, and compomers may provide stronger dentine-bond strength and better margin sealing than conventional glass-ionomer cement and resin composites due to the ability of such materials to dissipate the occlusal stress and the therapeutic effect of ions released over time.

In conclusion, within the limitations of this study, it is possible to affirm that the choice of appropriate materials from a chemical and mechanical point of view can make a difference on the bonding performance/durability of dentine-bonded interfaces. Indeed, the application of well-formulated modern adhesive systems in combination with ion-releasing dentine-replacement materials might offer to clinicians the possibility to perform more long-lasting adhesive restorations. However, these concepts must be corroborated by future in vivo and/or clinical trial studies in order to evaluate their true suitability in a clinical scenario.

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4.6 Artigo 6: Contemporary restorative ion-releasing materials: current status, interfacial properties and operative approaches.

CLINICAL

BDJ Minimum Intervention Dentistry Themed Issue

VERIFIABLE CPD PAPER

Contemporary restorative ion-releasing materials: current status, interfacial properties and operative approaches

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Key points

Explores the mechanisms involved in the process that allows mineral deposition at the interface between such materials and dentine, and describes how conventional 'bioactive' restorative materials currently available on the market may be beneficial for treatments in minimally invasive (MI) dentistry.

Different carious tissue removal methods are currently available. However, chemo-mechanical methods reach a compromise between MI tissue removal to protect the pulp and an 'adhesion-friendly' substrate to enable successful restoration placement and interfacial longevity.

Contemporary 'therapeutic' bio-interactive materials should now be used for tissue replacement, as they may be able to reduce the susceptibility of tooth mineral to dissolution and/or to recover its mechanical properties via remineralisation.

Abstract

Minimally invasive (MI) concepts in restorative dentistry in the year 2020 request from the practitioner not only a scientifically supported rationale for carious tissue removal/excavation and defect-oriented, biological cavity preparation, but also a deep understanding of how to ensure a biomechanically stable and durable restoration in different clinical situations by applying different restorative options. Bio-interactive materials play an increasingly relevant role, as they not only replace diseased or lost tissue, but also optimise tissue mineral recovery (among other properties) when used in restorative and preventive dentistry. Indeed, this is of certain interest in MI restorative dentistry, especially in those cases where gap formation jeopardises the integrity of the margins along resin composite restorations, causing penetration of bacteria and eventually promoting the formation of secondary caries. Recently, the interest in whether ion-releasing materials may reduce such biofilm penetration into margin gaps and reduce such a risk for development and propagation of secondary caries is growing significantly among clinicians and scientists. The aim of this article was to explore mechanisms involved in the process that allow mineral deposition at the interface between such materials and dentine, and to describe how conventional 'bioactive' restorative materials currently available on the market may benefit treatments in MI dentistry.

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Introduction

Teeth are formed through a highly organised mineralisation process resulting in hierarchically arranged tissues, each one with specific properties.[1,2] They are composed of a combination of tissues with different embryologic origin and precise genetic regulation to result in a unique composition, size, shape and spatial distribution of minerals and organic components, comprising enamel, dentine, cementum and pulp.[3]

During a lifetime, teeth are exposed continuously to changing oral micro-environments with harsh conditions characterised by the presence of extrinsic and bacterial metabolic acids, while being required to perform optimally under variable and high masticatory loads. In combination, these are the fatigue-exposure factors that lead eventually to enamel and dentine breakdown.[4] In enamel, demineralisation induced by low pH can be counterbalanced when the biofilm fluid/acquired pellicle/saliva is super-saturated with calcium and phosphate ions.[5] In dentine, however, the higher organic content of this tissue and its complexity, including the collagen network, makes the process of mineral repair more complicated.[6]

Minimally invasive (MI) operative intervention approaches are focused on the sole removal of the diseased tissues and replacement by a biocompatible material. Contemporary interventions, driven by the advent of 'therapeutic' bio-interactive materials should now be used to broaden the application of this concept, resulting in tissue replacement which is able to reduce the susceptibility of tooth mineral to dissolution and/or able to recover its mechanical properties via remineralisation.

Loss and gain of mineral in enamel and dentine

The dental caries process is initiated by a drop in pH within the biofilm induced by specific metabolic activities of the organised bacteria.[7] Tooth remineralisation may be expected to occur to a certain extent in the presence of calcium-saturated saliva, and fluoride upregulates this process. Although a complete full-lesion remineralisation is unlikely, it is often also not required for arresting the caries process.[8] Hence, most advances in bio-interactive dental restorative material technology have focused on dentine remineralisation or dentine protection/replacement, also because the overall longevity of restorations is still of some concern in cavity margin sites located on dentine.

As previously stated, dentine is a complex tissue composed of mineral and organic phases. Dentine remineralisation is an intricate and dynamic process that entails highly orchestrated interactions of several cellular and matrix components.[9] Essentially, it involves the renovation of an organic phase (type I collagen) and inorganic apatite, leading to intrafibrillar mineralisation of collagen.[10] However, both stages must be in synergistic connection in order to allow precise mineral precipitation, both within the collagen intrafibrillar and interfibrillar spaces, and a recovery of the mechanical properties of the dentine tissue.[11]

For many years, the role of collagen in dentine was underestimated; it had been first considered only a passive organic scaffold.[12,13] However, recent evidence suggests that both the structure and assembly of type I collagen are essential in order to act as an active template for mineralisation, guiding the crystal deposition in parallel arrays, with preferential growth in the axial direction in the spaces between fibrils.[14] MI dentistry aims to minimise the amount of tissue removal and the maximal preservation of non-denatured collagen, which can still be protected by a hydroxyapatite coating.[15]

Background on the current guidelines for carious tissue removal

An updated approach to carious tissue removal has been recently reviewed and discussed.[16] Clinicians are still prompted to excavate lesions when a mechanically resistant tooth-restoration complex is needed to restore the patient's function and/or aesthetics. However, the traditional management for many years has been the complete or near-complete removal of the entire carious tissue biomass, in the belief that this would stop the caries process (non-selective removal). More recently, an improved understanding of the pathophysiology of the caries process and clinical trial evidence on carious tissue removal methods have supported the contemporary alternatives of 'prevention of extension' as opposed to 'extension for prevention'.[17] In selective carious tissue removal, for instance, carious issue is only completely removed in the periphery of a cavity, ensuring the stability and longevity of the restoration, while close to the pulp, affected and, in some cases, infected carious tissue may well be retained and sealed under the restoration if this prevents pulp exposure.

As a result, the sealed dentine beneath restorations placed following such carious tissue removal will be a combination of sound/translucent dentine at the cavity periphery and affected/demineralised dentine at the base, adjacent to the pulp.[18] Although conventional dental adhesives may achieve statistically lower bond strengths when applied to such affected dentine as compared to a sound dentine substrate,[19] the real values are still within the clinically safe standards for dental adhesion.[20] Importantly, the surface area of the cavity affected in that way is usually small compared with the overall surface of the whole cavity.

Moreover, different carious tissue removal methods result in different histological dentine substrates and morphology of the residual dentine.[21,22] In general, chemo-mechanical methods reach a compromise between MI tissue removal to protect the pulp[23,24] and an 'adhesion-friendly' substrate to enable successful restoration placement and interfacial longevity.[25] Figure 1 shows the morphology of residual dentine surfaces after different carious tissue removal techniques.

The use of conventional ion-releasing dental materials such as glass-ionomer cements (GICs) seems to provide a net mineral gain in carious dentine.[26] Using experimental biomimetic remineralising adhesive materials, it is possible to induce intrafibrillar mineralisation of collagen (Fig. 2). Indeed, it has been demonstrated that such a biomimetic

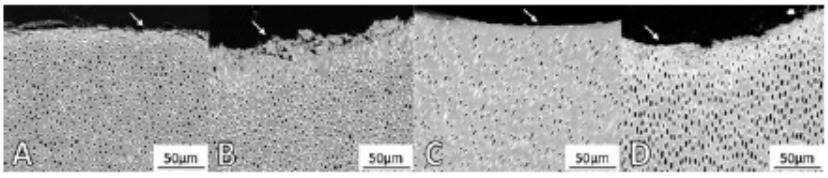

Fig. 1 Scanning electron microscopy (SEM) images of carious dentine cavities after carious dentine tissue removal with: a) Conventional tungsten carbide burs; b) Spoon hand excavator; c) Chemo-mechanical Papacárie (Fórmula & Ação, São Paulo, Brazil) (papain-based); d) Chemo-mechanical Brix 3000 (Brix Medical Sciences, Carcaraña, Argentina) (papain-based). Arrows point to the outermost excavated surface. Chemo-mechanical methods resulted in a relatively smooth dentine surface, with little dentine debris

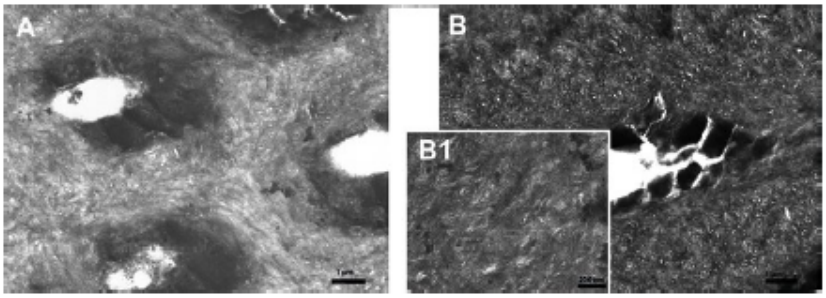

Fig. 2 a) Transmission electron microscopy (TEM) assessment of demineralised dentine created with an in vitro protocol to simulate caries-affected dentine. This image shows dentine collagen fibrils totally and partially demineralised. b) Images B and B1 (higher magnification of B) show the results obtained when such a substrate was treated using biomimetic analogues in combination with an experimental resin-based cement doped with fluoride-containing bioactive glass. In this case, it is possible to see clearly a dentine with a total dark appearance resembling that of sound dentine, and indicating both intrafibrillar and extrafibrillar remineralisation[26]

strategy for remineralisation may reinstate the mechanical properties of the demineralised dentine, as specific Ca/P compounds, such as amorphous calcium phosphate (ACP), can fill the nanometre-sized spaces within the collagen fibrils.[26,27] This mineral exchange results in remineralisation, protecting the collagen against further proteolytic degradation. At the clinical level, it results in increased restoration longevity and less incidence of secondary caries. Furthermore, they are biocompatible, acid-resistant and have thermal expansion coefficients similar to that of dentine.

The role of new materials in engineering demineralised dentine

Polymer, ceramics or resin composite biomaterials can be used to repair or replace damaged organs or tissues in the human body. Research currently has focused on developing nanoscale materials with biomimetic properties. In dentistry, the effect of these dentine-replacement materials is clinically relevant and represented by ion leaching/releasing from the bulk material and interaction with the underlying tissue. Furthermore, the application of such materials may provide feasible means to extend the longevity of material-dentine interface. For example, experimental adhesives containing calcium silicate-based, bio-interactive micro-fillers have been found promising to preserve the bond strength against ageing.[28]

Ultraconservative interventions aim to preserve the sound structure of the tooth as much as possible. However, the preparation of MI cavities must also be supported by therapeutic restorative techniques that induce protection of the material-dentine interface against hydrolytic or enzymatic degradation processes, avoiding deterioration of the bond and failure of the restoration over time.[29] This concept of removal is accomplished by leaving partial or demineralised caries-affected dentine as a residual substrate. As mentioned before, it is known that adhesion to this type of substrate is compromised compared to sound dentine. This is probably due to a combination of the reduced biomechanical properties of caries-affected tissue (for example, its modulus of elasticity), and the fact that the chemistry and structure of caries-affected dentine can affect the depth of dentine demineralisation and degree of adhesive infiltration.[30] Furthermore, irregular distribution and shallow penetration of adhesive monomers into demineralised collagen result in poor infiltration and adhesive phase separation. This hinders hybrid layer formation and results in a reduced bond strength.[31] In order to improve the durability of the bond between adhesives and caries-affected dentine, alternative restorative procedures are necessary, most of them involving biomimetic (phosphoprotein) analogues.[32]

The strategy of these materials to improve the durability of restorations relies on its ability to inhibit the activity of the collagenolytic enzymes present in dentine and cause mineral deposition.[33] The dentine collagenolytic enzymes, including matrix metalloproteinases (MMPs) and cysteine cathepsins, are responsible for the degradation of collagen fibrils exposed at the bonded interface. They are produced by odontoblasts and remain as inactive zymogens until a change in chemical morphology, resulting from application of adhesive systems or the biological caries process, results in its activation.[34]

Since restorations are (most of the time) placed over/on carious tissue, it is possible that they are present in the residual dentine in the activated form under the restoration. Their activity is downregulated by the presence of calcium and zinc, chelating or replacing the active site or by 'coating' the substrate.[35] Ionic dissolution products released from these materials have also been shown to reduce the degradation of exposed collagen and enhance the deposition of minerals.[36,37] It still remains unclear how this healing process occurs.[38] However, it seems that the Si concentration and the Ca/P ratio are critical. ACP is a precursor of hydroxyapatite and it has been reported that they can inactivate some MMPs.[39] The mechanisms involved in inhibition of proteolytic degradation of dentine-material interfaces are a key issue, which may be accomplished by ion-releasing materials to improve the dentine bonding durability and the therapeutic applications of these materials in caries prevention.

With improved understanding of the interaction between dentine and such bio-interactive 'smart' materials, it may be possible to develop routes for the synthesis of new functional materials with structural precision at different dimensional levels. The ultimate goal is to produce materials to replace or protect the exposed collagen, mimicking as best as possible the original sound tissue. Moreover, it would be interesting to investigate the possibility of extending their bio-interactivity by combining calcium phosphate (CaP) phases with different solubilities and/or developing controlled release approaches to expand their use in caries prevention.

Conventional 'bio-interactive' ion-releasing materials

MI operative dentistry concepts require, as part of restorative therapy, materials which are able to: 1) deliver mineral ions; 2) bind to collagen (acting as template of calcium and phosphorus and stimulating nucleation of apatite crystallisation); 3) protect collagen from degradation; 4) provide an adequate pH to favour new mineral formation; and 5) repel or constrain bacteria.[40] Ionic dissolution from ion-releasing materials may be the key factor in understanding their remineralisation potential. Calcium and phosphorous are the main components of the biological apatite. Other inorganic ions, such as fluoride, zinc, magnesium and silanol groups, may also act as substitutes in apatite crystal formation.

One description of 'bioactive/bio-interactive' materials postulates that these materials should be able to elicit a specific biological response at the interface, resulting in the formation of a bond between the tissue and the material.[41] Part of the interaction mechanism is due to ion release and, in this regard, some attention will be given to its laboratory and clinical properties applied to MI operative dentistry.

There are several materials already present on the market, which are able to release specific ions at the interface (Table 1). However, new 'smart' materials are being developed to facilitate dentine remineralisation, incorporating inorganic fillers (bioglass, CaP, hydroxyapatite/calcium silicate particles and silicon nanoparticles) in order to promote remineralisation at the bonded interface.[19,40] Although few of them cause full remineralisation, they play an important therapeutic role at the interface. Table 1 offers an overview of current commercially available bio-interactive restorative materials.

Zinc polycarboxylate cements

Zinc polycarboxylate cements (ZPCs) were the first dental cements showing some chemical adhesion to tooth structure. They were often used for luting restorations, intra-canal posts or orthodontic bands. It soon became clear that the use of zinc polycarboxylate resulted in more retention and less demineralisation in enamel under the bands compared to zinc phosphate cements.[42] The powder contains oxides of zinc, magnesium, tin, bismuth and/or alumina. Zinc and magnesium may act as a direct activator of the enzyme alkaline phosphatase and has been shown to inhibit osteoclast activity,

thus inducing the precipitation of poorly crystallised apatite.[43,44] Zinc is also known to induce collagen cross-link formation and may help to prevent enzymatic degradation.[45] The liquid is an aqueous solution of polyacrylic acid (PAA), a known non-collagenous protein surrogate for biomimetic intrafibrillar mineralisation of collagen fibrils[32] able to regulate the growth of the mineral crystallites during remineralisation processes. Indeed, this polymer has acidic characteristics and a predisposition to bind cations and stabilised ACP nanoprecursors.[46]

A recent study has shown the potential of these 'traditional' cements in increasing the mineral density in artificially induced carious dentine produced by a microbial protocol up to similar values achieved by GICs and calcium silicate cements.[47] ZPCs, in fact, have indeed slightly outperformed GICs in this regard (Fig. 3). Figure 4 illustrates the high contrast outer layer formed over the ZPC restored samples after 45 days of intra-pulpal pressure with simulated body fluid (SBF). In modern dentistry, such materials would be useful as pulp protection materials and/or as dentine replacement materials after deep selective carious tissue removal.

GICs

ZPCs were clinically replaced by GICs, which also contain PAA but, in addition, also exhibit fluoride release. They are water-based restorative materials composed of fluoro-alumino silicate powder which, by acid attack, forms polyalkenoate salts that interact with the subjacent dentine, forming an ion-interchange layer or diffusion zone. The formation of calcium polycarboxylate not only facilitates tissue remineralisation but also allows chemical bonding[48] at the interface. At the clinical level, this is a significant factor in the long-term adhesion and mineralisation ability, upgrading GICs as one of the most used restorative materials in paediatric dentistry, for example.

Modified forms of GICs, such as Glass Carbomers, have appeared on the market, with a similar composition and setting reaction to conventional GICs.[49] They are claimed to contain nanocrystals of calcium fluorapatite (FAp) and hydroxyapatite, which can act as nuclei for the remineralisation process and initiate the formation of FAp.[50] While they show reduced clinical success as a restorative material,[45] its use as a sealant or as pulp protection could be promising.

Table 1 Commercially available 'bio-interactive' ion-releasing materials (cont. on page 454)

Type of material	Brand	Manufacturer
Conventional GICs	IonoStar Plus	VOCO, Germany
	IonoFil Plus	
	Aqua Ionofil Plus	
	Ketac Universal	3M ESPE, St Paul, MN, USA
	Ketac Fil Plus	
	Riva Self Cure	SDI, Australia
	GC Fuji II	GC, Tokyo, Japan
High-viscosity GICs	Fuji IX Fast	GC, Tokyo, Japan
	Fuji IX GP	
	Fuji IX Extra	
	Chemfil Rock	Dentsply, Germany
	IonoStar Molar	VOCO, Germany
	Ionofil Molar	
	Ionofil Molar AC Quick	
	Ketac Molar	3M ESPE, St Paul, MN, USA
	Ketac Molar Quick	
Glass hybrid cements	Equia Forte Fil	GC, Tokyo, Japan
RMGICs	Activa	Pulpdent, USA
	Ionolux	VOCO, Germany
	Photac Fil Quick Aplicap	3M ESPE, St Paul, MN, USA
	Ketac Nano	
	Vitremer	
	Riva Light Cure UV	SDI, Australia
	Fuji II LC	GC, Tokyo, Japan
Metal-reinforced GICs	Ketac Silver	3M ESPE, St Paul, MN, USA
	Riva Silver	SDI, Australia
Glass carbomers	Glass Fill	GCP Dental, Netherlands
Giomers	Beautifil II	Shofu Dental Corporation, Japan
	Beautifil II Gingiva Shades	
	BeautiSealant	
Composites	Activa Presto	Pulpdent, USA
	Re-Gen Flowable Composite	Apex, USA
	Re-Gen Bulk Fill Composite	
Adhesives	Re-Gen Bioactive Adhesives	Apex, USA
Resin-modified glass-ionomer adhesives	Riva Bond	SDI, Australia
	Fuji Bond LC	GC, Tokyo, Japan

Table 1 Commercially available 'bio-interactive' ion-releasing materials (cont. from page 453)

Type of material	Brand	Manufacturer
Calcium silicate-based cements	Endo-PASS	DEI Italia, Italy
	Biodentine	Septodont, France
	ProRoot MTA	Dentsply, USA
	Angelus MTA	Angelus, Brazil
	MTA Bio	
	BioAggregate*	Innovative BioCeramix
	RetroMTA	BioMTA, Republic of Korea
	MTA Plus	Avalon Biomed Inc., USA
	Neo MTA	
	Endosequence BC sealer	Brasseler, USA
	Generex A	Dentsply, USA
Resin-modified MTA	TheraCal LC	Bisco, USA
ZPCs	Poly Zinc +	Prevest Denpro, India
	G.C.R.	Acrostone, Egypt
	Durelon	3M ESPE, St Paul, MN, USA
	HY-Bond	SHOFU INC, Japan
	SQ-ZPC	Aescu Pharma Co., Hong Kong
SDF	Riva Star	SDI, Australia
	Cariestop	Biodinâmica, Brazil
	FAgamin	Tedequim, Argentina
	Advantage Arrest	Elevate Oral Care, USA
	e-SDF	Kids-E-Dental, India
	Saforide	Toyo Seiyaku Kasei Co. Ltd, Japan

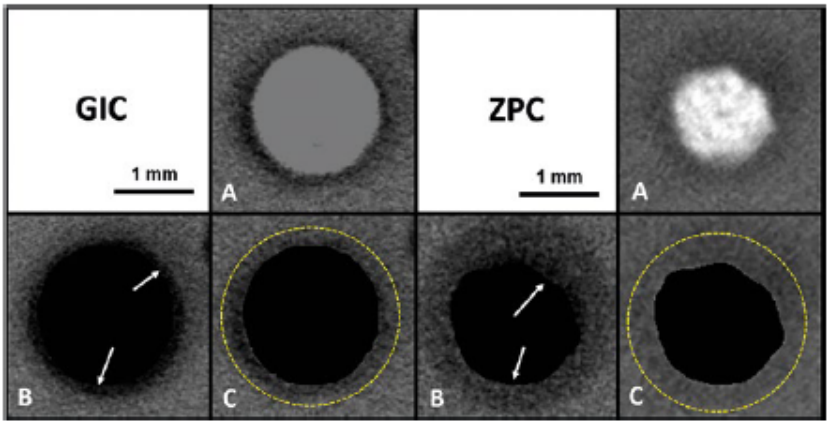

Fig. 3 a) Micro-CT slices of glass-ionomer cement (GIC) or zinc polycarboxylate (ZPC)-treated carious dentine cavities. b) Initial dentine caries cavity. Arrows point to carious dentine. c) Same region of the carious cavity after 45 days' restoration with the experimental cement under simulated pulp pressure conditions (restorative cement is removed manually so as not to interfere with density evaluation). Although both materials were able to increase the carious dentine density (yellow dashed circle) after the period, ZPC showed a 19% higher increase in density values compared to the GIC sample (unpublished data)

Resin-modified glass-ionomer cements (RMGICs) possess improved clinical properties. They are also considered self-adhesive materials and contain methacrylate-based monomers (HEMA, TEGDMA, UDMA), vinyl-modified polyalkenoic acid (VPA), photo-activators (such as camphorquinone) and tertiary amines (co-initiators), in order to allow photopolymerisation.[51,52] RMGICs can bond micromechanically to dentine due to the resin infiltration of exposed collagen after PAA conditioning. They are also able to bond chemically to dentine by ionic interaction of carboxyl groups from the acid with calcium ions of the remaining hydroxyapatite crystals in the tooth substrate.[53]

The longevity of resin-dentine bonds may also be improved by using materials or clinical measures that may reduce the stress concentration at the interface between resin and dentine during light-curing procedures.[54] RMGICs can be used in deeper cavities in order to provide a 'stress absorption' layer that will absorb part of the shrinkage of the resin composite used for the restoration.[55,56] This has been advocated to prevent stress development at the dentine-bonded interface,[57,58] thus decreasing the risk for gap formation and microleakage.

One more factor to consider as a source of degradation is the occlusal stress during mastication and in cases of parafunctional habits; all these factors can affect the integrity of the bond interface.[59] It has been shown that the use of RMGICs can provide a more stable bond to dentine, as well as provide a longer-lasting marginal sealing compared to resin composites. This seems to be correlated to the ability of such materials to dissipate the occlusal stress and to the beneficial result of the ions released over time. Indeed, it is of particular interest in modern MI therapeutic restorative dentistry, since it has been demonstrated that cyclic mechanical stress can promote gap formation at the margins of resin composite restorations. Bacterial penetration into narrow margin gaps might ultimately promote secondary caries formation.[60]

From RMGICs, other material classes have been developed. Giomers, for example, are resin composite materials where a pre-reacted glass-ionomer (PRG) filler technology has been incorporated.[61] The main advantage of this material would be its improved fluoride release, but otherwise their clinical performance can be compared to conventional resin composites.[62] More recently, a new type of bioactive flowable resin-based restorative GIC, containing fluoro-alumino silicate particles and polyacid

components along with a bioactive ionic resin matrix, has been developed (Activa, Pulpdent, USA). One study[63] has demonstrated that the use of a conventional RMGIC or the Activa restorative GIC/resin-based material can reduce the degradation during load cycling and/or prolonged storage in artificial saliva of the hybrid layer created with modern universal adhesive applied in etch and rinse mode (Fig. 5).

Bioactive glasses

Among other known bioactive and ion-releasing materials are bioactive glasses (also known as bioglass, often shortened to BAG).[64] The basic components of bioactive glasses are calcium oxide, sodium, phosphorous and silica.[65] The surface reaction is a complex, multistage process derived from their reactions with tissue fluids, which results in the formation of a biologically active hydroxy-carbonate apatite layer.[66] It has been found that this reaction releases critical concentrations of soluble Si, Ca, P and N ions, which induce intracellular and extracellular responses.[67] Although most information about this material has been acquired through bone research, this material has been used in dentistry especially for dentine mineralisation[68] and for the treatment of dentine hypersensitivity.[69] However, the most common way to use bioactive glasses in dentistry is via air-abrasion/polishing procedures. Indeed, the pre-treatment of dental substrates using Bioglass 45S5 (Sylc, Velopex, London UK) in air-abrasion devices is currently used in restorative dentistry to create a 'bioactive smear layer' within the interface, which can be incorporated into the hybrid layer during application of RMGICs and self-etch adhesives. This bioactive smear layer remains available at the bonding interface, and induces remineralisation and protection of the dentine-bonded interface.[19]

Moreover, it has been demonstrated that CaP has the ability to mediate MMP-2 and MMP-9 by forming a high-molecular-weight aggregate, CaP-MMP, which immobilises MMPs by binding to fibrin.[70] The binding capacity can also be influenced by the alkaline pH generated by the bioactive glasses during water immersion. A reduction in this activity is expected at around pH 10, since the ideal activity of MMP occurs at neutral pH. In addition, the surface created by Bioglass 45S5 is a SiO_2-rich gel layer.[43,71] The sequestration of calcium and phosphate ions from the glass and their diffusion through the SiO_2-rich layer can induce their transformation into ACPs. After that, hydroxyapatite can be formed and it is well known that they may inhibit MMP activity.[72] The interactions between CaP complexes and amino acids indicates an involvement in bone mineralisation regulation. Although few studies focus on amino acids bound to surfaces, this appears to be due to an affinity of exposed collagen for the glass surface and chemical interaction between the dentine and glass, likewise in bone regeneration, leading to apatite formation at the interface.[73]

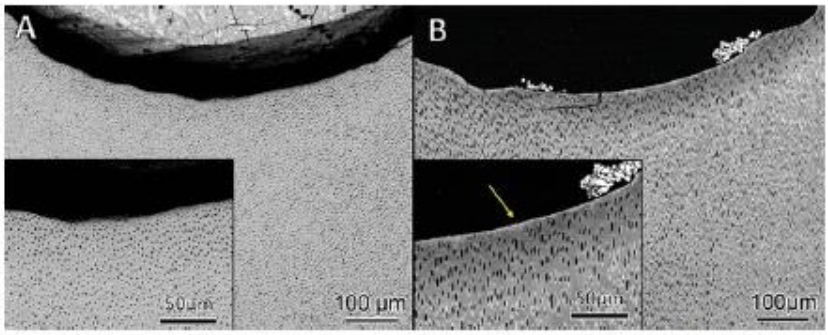

Fig. 4 SEM images of dentine carious cavities restored with either GIC or ZPC after 45 days of intra-pulpal pressure with simulated body fluid (SBF). a) GIC-restored carious interface. b) ZPC-restored interface. For ZPC-restored cavities, a high contrast outermost surface layer is detected (yellow arrow), indicating an increase of mineral density probably due to the interaction of dentine and the minerals released by ZPC

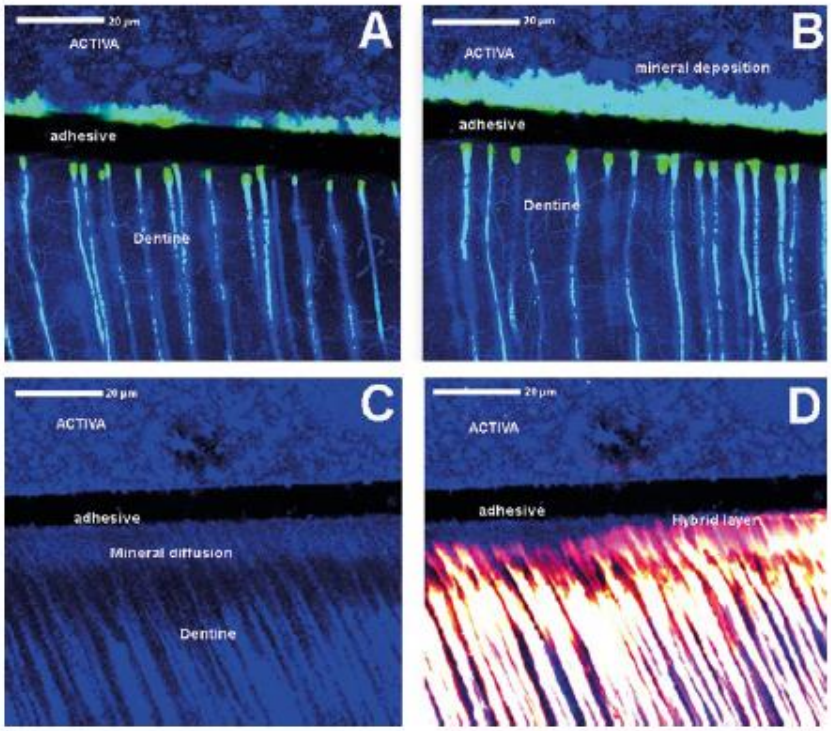

Fig. 5 Confocal reflection/fluorescence single projection images of bonding interfaces created using universal bonding systems in combination with Activa Restorative. a, b) It is possible to see the interface created with the universal adhesive (Scotchbond 3M Oral Care, MN, USA) applied in self-etching mode before and after load cycling and storage in artificial saliva, respectively. It is possible to recognise the increased presence of a porous reactive zone characterised by mineral deposition between the interface with Activa. c, d) An interface created with the universal adhesive (Scotchbond) applied in etch and rinse mode before and after load cycling and storage in artificial saliva, respectively. In this case, it is possible to note that the porosities within the hybrid layer are clearly reduced (nanoleakage in image D) by mineral precipitation (reflective signal in image C)

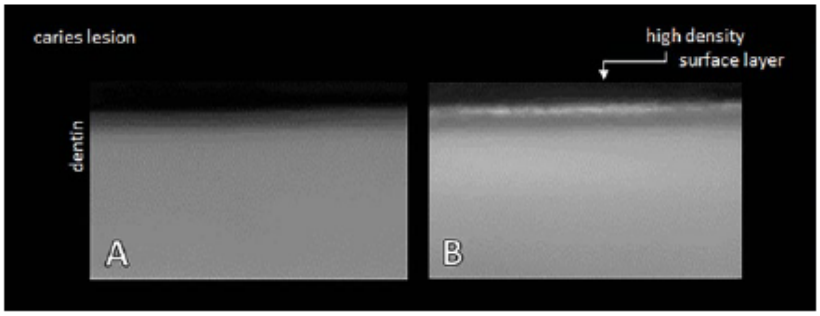

Fig. 6 Bovine tooth specimens after a microbial cariogenic challenge at the dentine. a) Baseline tooth specimen with carious dentine surface (test surface). b) Same tooth specimen immediately after SDF application showing a high-density layer and a subjacent relatively dense area below the lesion (images courtesy of Dr Andréa Fonseca-Gonçalves and Gabriella Fernandes Rodrigues)

Calcium silicate cements

The first calcium silicate dental cement, mineral trioxide aggregate (MTA), was developed in the 1990s as a repair material for endodontic perforations and root-end fillings due its biocompatibility and ability to induce mineralised tissue formation.[74] This cement is primarily composed of di- and tri-calcium silicate, tri-calcium aluminate, tetra-calcium aluminoferrite and bismuth oxide. Calcium silicate cements are hydrophilic materials that can tolerate humidity and release calcium and hydroxyl ions into surrounding fluids (saliva, blood, dentinal fluid). These materials set by a hydration and precipitation mechanism, the remineralisation mechanism differing due to the alkaline nature of these materials. Degradation of collagen fibrils occurs and leads to the formation of a porous structure, which facilitates the penetration of high concentrations of calcium and carbonate ions, leading to increased mineralisation in this zone.[75] It is important to note that they cannot induce biomimetic remineralisation by re-establishing functional properties. Their ability is to induce mineral precipitation and induce formation of a reparative/osteo-dentine.

Its clinical indications have been expanded to include pulp-capping procedures, pulpotomies or root apical barrier formation.[76] Due to its biocompatibility and sealing ability, they have become an important material in supporting the concept of MI dentistry. As mentioned before, the alkaline setting reaction of these cements can reduce MMP activity and also has beneficial antibacterial effects on caries-affected (and infected) dentine.[77,78] Studies also demonstrated optimal healing responses, with dentine bridge formation in the pulp space[79,80,81] confirming the biocompatibility of calcium silicate cements. They also exhibit expansion and contraction properties similar to dentine, which results in higher resistance to margin leakage and subsequent bacterial migration.[78] Despite some of these materials potentially being affected by colour change or staining, all these properties together facilitate its successful clinical use.

Silver diamine fluoride

Silver diamine fluoride (SDF) was first approved for dentistry use in Japan in the 1960s[82] and, since then, has been used in China, Brazil, Argentina and Australia. In 2014, USA licensed an SDF product for therapeutic use in tooth sensitivity.[83] Its main application is, however, directly related to MI dentistry concepts to arrest carious lesion progression or prevent lesion establishment.[84] Nevertheless, SDF has a major adverse effect regarding the black stained appearance left after its use, raising aesthetic concerns from patients and/or parents.

SDF has a very alkaline pH (around 10) and the solution contains diamine-silver and fluoride ions. The interaction of silver and fluoride has a synergistic effect on the tooth structure, favouring the synthesis of fluorohydroxyapatite,[85] which is chemically more stable than hydroxyapatite in acid environments, with important implications in remineralisation/re-hardening of the carious lesion.[86] Another *in vitro* study found that SDF increases the mineral density of artificial carious lesions;[87] however, the mechanism behind it is still not completely clear. It has been proposed that it would rather occur due to a reaction between silver and dentine minerals, rather than the classic fluoride-mediated remineralisation,[88] and some recent micro-CT investigations (unpublished data) may support this assumption, since a high-density superficial layer has been found in carious dentine after SDF application, which may even be extended into deeper dentine parts (Fig. 6).

Furthermore, fluoride and silver also inhibit MMP and cathepsin activity and, therefore, may also inhibit dentine/collagen degradation.[88] Finally, SDF also has a well-known antibacterial effect, with inhibition of cariogenic biofilm formation.[89]

Limited data is still available regarding advanced characterisation of SDF-tooth interactions. At present, more attention is being given to trying to mitigate the staining problem.[90] A saturated potassium iodide solution has been used to minimise this side effect and/or restoration with GICs over SDF-stained dentine to mask the stained tissue. Some SDF solutions are already commercially available in combination with self-cured GICs.

Future prospects – dentine interface biomineralisation

Two different models of *in vitro* remineralisation can be found in the literature, classified as the top-down/classical and bottom-up/non-classical approaches. A major criticism in the classical approach is that it results in extrafibrillar remineralisation without remineralisation of the intrafibrillar components.[32] Therefore, in this approach, conventional remineralisation does not occur by spontaneous nucleation of mineral matrix, but rather by the growth of residual apatite crystals in demineralised dentine. If there are only a few residual crystals, there is no remineralisation.[10] On the other hand, the bottom-up approach was suggested as an alternative and is independent from apatite crystallites that may have remained. This biomimetic remineralisation is driven by analogues, leading to hierarchical remineralisation of dentine,[32,91] resulting in a highly ordered intrafibrillar nanoapatite assembly.

Dentine biomineralisation occurring within the restorative interface could be accomplished following the bottom-up strategy, where the crystals and structures formed can incorporate organic macromolecules.[6,14] It is known that, in demineralised dentine, the collagen intrafibrillar gap regions are spaces which hydroxyapatite mineral precursors occupy; eventually nucleate and hydroxyapatite crystal plates grow.[32] It is therefore important to have mineral re-incorporation when the dentine is exposed to demineralisation (from erosion, caries or restorative procedures).

A mineral crystal is formed through a nucleation event in which a cation and anion pair bond and create nuclei for crystal growth. Many biominerals are formed by an amorphous precursor pathway mediated by a non-collagenous protein. Several inorganic materials have been shown to be bio-interactive and able to deliver remineralising ions. Once such biomineralisation processes are better understood and their place in the MI operative approach is recognised, the interaction between materials and tooth surfaces, namely 'bio-interactivity', should also be considered in the longevity of the tooth-restoration complex.[6,92] Development of biomaterials able to catalyse remineralisation of incompletely resin-infiltrated collagen matrices created by resin adhesives will represent a great advance in dental care.

Conclusions

There are different methods available to perform carious tissue removal. The first important concept to consider is the type of substrate that these methods leave to be further treated. Thereby, a good diagnosis and the planned treatment could act together with the 'smart' materials to heal the tissue left behind. Hence, in MI dentistry, the 'bio-interactivity' is important to create a therapeutic surface for adhesive procedures.

As current commercially aesthetic resin composite materials have no ability to remineralise the collagen network after acid demineralisation, ion-releasing materials need to be used in association. Unfortunately, they are also not able to immediately remineralise the remaining caries-affected dentine. However, they have specific therapeutic benefits that could improve the protection of collagen fibrils until the remineralisation process occurs.

Zinc polycarboxylate was the first cement to show chemical ability to bond chemically to dental hard tissues. They are nowadays used quite rarely, as GICs and RMGICs have a wider range of applications along with an ability to release fluoride in the micro-environment. However, both have the ability to induce mineral precipitation at the interface in specific ways.

Quick-setting calcium silicate-based cements may be indicated for deeper cavities due to their ability to stimulate the pulp cells to produce a reparative dentine bridge and create calcium carbonate and/or apatite-like crystallisation layers along the interface. Moreover, they also possess antibacterial properties against eventual remaining microorganisms left after selective carious tissue removal, reducing the risk for secondary caries and improving the longevity of restorations.

As opposed to dentine-replacement materials, SDF can be applied without caries removal as it is able to simultaneously prevent and arrest lesion progression by a synergistic interaction between released ions and tooth tissue. Using this association of different materials to restore the cavity, it is possible to reduce the stain effect and keep the therapeutic benefit of the hardened carious lesion.

Application of modern adhesive systems in combination with ion-releasing dentine-replacement materials may offer to practitioners the possibility to perform adhesive restorations with long-lasting performance. Furthermore, understanding the ion-releasing process of materials may be the key factor for the development of a therapeutic bonding system, being a promising alternative way to reduce the degradation of the resin-dentine interface.

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5. CONSIDERAÇÕES FINAIS

A pesquisa, dentro de todas as áreas, tem como finalidade encontrar lacunas de conhecimento e supri-las com embasamento científico. Porém, isso demanda tempo e requer esforços de múltiplos profissionais que, juntos, vão associando ideias e concretizando novas descobertas. Na área odontológica, o mesmo princípio dinâmico acontece, entretanto, existe uma grande diferença, principalmente no quesito de linguagem entre os pesquisadores, empresas e profissionais clínicos. Esse estudo foi conduzido no intuito de aproximar essas áreas, realizando um levantamento bibliométrico dos materiais bioativos mais estudados, experimentos laboratoriais que compararam diferentes materiais em diversas simulações de situações clínicas e uma narrativa sobre os materiais disponíveis comercialmente, suas interações com os tecidos biológicos e suas indicações clínicas.

No primeiro estudo, verificou-se que três tipos de materiais são os mais estudados quanto às modificações bioativas de suas composições, sendo eles os adesivos, os compósitos e os cimentos. A maioria apresentou bioatividade com relação às análises laboratoriais testadas, porém, eram em sua grande maioria estudos *in vitro*, indicando que estudos clínicos ainda precisam ser realizados para investigar a aplicabilidade clínica desses novos materiais. Além disso, o tipo de substrato mais testado foi a dentina hígida, o que não condiz com os princípios clínicos de remoção minimamente invasiva (BANERJEE et al., 2017). É possível observar, um aumento na produção de artigos científicos nessa área após o ano 2000, e isso deve-se principalmente aos avanças da nanotecnologia (YAZDANIAN et al., 2021), mas também pode estar associada à busca por materiais bioativos capazes de promover resposta biológica que evitem ao máximo o desgaste das estruturas dentárias (SCHWENDICKE et al., 2016; TORRES et al., 2021).

Visando comparar as técnicas de remoção que auxiliam na seletividade da remoção do tecido cariado e a interação de cimentos considerados bioativos com este tecido, conduziu-se o segundo estudo, testando o método rotatório convencional com o uso de curetas e agentes químico-mecânicos à base de papaína. O tamanho das cavidades pode ter sido um fator limitante, bem como a experiência do operador, porém esta não resultou em diferenças ao comparar

os métodos de remoção. Estudos prévios mencionam que um operador bem preparado é capaz de realizar com acuidade a remoção seletiva da cárie, desde que conheça os princípios biológicos do substrato dentinário e suas características clínicas (SCHWENDICKE et al. 2018). A microscopia eletrônica de varredura após a remoção, revelou diferenças na morfologia da dentina remanescente, porém, isso não interferiu na recuperação da densidade mineral pelos cimentos de polialquenoato. Entretanto, quando comparados entre si, o cimento de policarboxilato de zinco apresentou um desempenho melhor que o ionômero de vidro, reforçando os resultados já encontrados anteriormente (PIRES et al., 2018).

Com o objetivo de verificar a seletividade de remoção apenas do colágeno degradado, não passível de remineralização, conduziu-se o terceiro estudo, testando dois géis a base de papaína em dentina hígida: Papacárie Duo Gel® and Brix3000®. Esses géis foram comparados com ácidos pré-condicionantes da dentina (ácido fosfórico 37% e ácido poliacrílico 11,5%), responsáveis por expor as fibras de colágeno e garantir a adesividade dos compósitos restauradores à dentina (WANG et al., 2004). Comparando a morfologia e a rugosidade superficial dos materiais testados, comprovou-se que os agentes químico-mecânicos à base de papaína não interferem nas características da dentina hígida, diferentemente dos ácidos. Contudo, nesse estudo, não é possível extrapolar clinicamente qual interação esse substrato deixado pelo Papacárie Duo Gel® ou Brix3000® terá com os materiais restauradores.

Já o quarto estudo, investigou a interação *in vitro* de materiais liberadores de íons com a dentina artificialmente cariada. Comparou-se um compósito resinoso inerte (Aura®) com um cimento de ionômero de vidro (Fuji IX®), cimento de silicato de cálcio (Endo-pass®) e cimento de silicato de cálcio modificado por resina (Theracal LC®). Nesse estudo, foi possível observar a gradação de interação dos materiais de acordo com suas composições, e como isso pode influenciar na interação com a dentina, promovendo diferentes respostas na interface. Este estudo mostrou que os compósitos não apresentaram nenhuma interação, exceto pela formação da camada híbrida e a presença de colágeno exposto, tonando a restauração por esses materiais susceptíveis à degradação enzimática e hidrolítica (NAKABAYASHI et al., 1982; WANG et al., 2004). Já o

CIV apresentou a preservação do colágeno e deposição mineral na interface, bem como o Theracal LC®. Estes poderiam, portando, serem considerados materiais intermediários quando comparados com a ação cáustica provocada pela alta alcalinidade do cimento de silicato de cálcio puro (ATMEH et al., 2012, 2015). Isso, possivelmente, é devido à alteração na composição do Theracal LC®, que apresenta uma matriz hidrofóbica à base de resina (MERAJI et al., 2018), tornando-o um material com adesão à estrutura dentinária capaz de liberar íons que promovem a precipitação mineral, bem como um material polimerizável, o que melhora sua aplicabilidade clínica quando comparado ao demorado tempo de presa dos cimentos a base de silicato de cálcio puros (TORABINEJAD et al., 2010).

Seguindo essa linha, o quinto estudo comparou cimentos de ionômero de vidro modificados por resina (Ionolux® e ACTIVA®), aderidos à dentina através de sistemas adesivos universais simplificados (Scotchbond® e Futurabond®), aplicados com ou sem condicionamento ácido prévio. Foi possível observar que a composição dos sistemas adesivos pode influenciar mais na longevidade da adesão do que seu modo de aplicação, possivelmente porque o Scotchbond® apresenta em sua composição partículas de silano, que tem sua ação diminuída quando em contato com umidade, presente tanto na composição quanto nos fluidos dentinários (YOSHIHARA et al., 2016).

Mesmo com essas intercorrências, a seleção de um bom material restaurador pode suprimir essas desvantagens, como foi o caso dos ionômeros de vidro modificados por resina testados nesse estudo. Eles aumentaram a longevidade das restaurações unidas pelo Scotchbond®, especialmente quando aplicados com condicionamento ácido prévio. Isso pode ser explicado pela liberação de íons por esses materiais, capazes de provocar deposição mineral e preencher as camadas de colágeno expostos (SAMPAIO et al., 2011; SAURO et al., 2018).

Além das novas composições de materiais restauradores que vêm sendo testadas, já existem no mercado materiais intitulados bioativos ou liberadores de íons. Porém, a indicação clínica de cada material ainda é uma lacuna a ser transpassada. O sexto estudo foi conduzido através de uma revisão voltada para praticantes da odontologia com o intuito de resumir como esses materiais

interagem com o dente e, com isso, aumentar o conhecimento científico nessa área, estimulando um questionamento crítico para a utilização desses materiais em cada situação clínica.

Pode-se dizer que a odontologia minimamente invasiva e o desenvolvimento de novos materiais, são uma via de mão dupla. Visto que, a expansão da mínima intervenção só foi possível com o advento dos materiais restauradoress com propriedades adesivas. Em contrapartida, a instauração desse novo conceito, proporciona e estimula a pesquisa de novos materiais com propriedades cada vez mais preventivas e biologicamente mais ativos. Portanto, é um processo dinâmico, em que várias áreas trabalham em conjunto, fornecendo informações específicas guiando a pesquisa em prol desse melhoramento.

6. CONCLUSÕES

Mediante aos estudos conduzidos nesse trabalho sobre a remoção, recuperação e restauração da dentina cariada, podemos concluir que:

- Os biomateriais modificados mais testados são os adesivos, os compósitos resinosos e os cimentos. Sendo que, a maioria é realizada em estudos *in vitro*, testados em dentina hígida e apresentando potencial bioativo como principal ferramenta de análise;
- Quando bem empregados, os conceitos de remoção seletiva de cárie, podem ser alcançados por qualquer técnica aplicada. O substrato remanescente após a remoção não influencia o potencial de recuperação mineral dos cimentos de polialquenoato. Entretanto, o cimento de policarboxilato de zinco apresenta um potencial de recuperação da densidade mineral mais elevado quando comparado ao cimento de ionômero de vidro;
- Os agentes químico-mecânicos à base de papaína não alteram a morfologia ou a rugosidade superficial da dentina hígida;
- As interações na interface material/dentina são diferentes para cada tipo de material e variam de acordo com suas composições. Os compósitos resinosos são materiais inertes e que conjuntamente com o sistema adesivo promovem uma zona de colágeno exposto na interface. Já o CIV e Theracal LC®, são capazes de preservar o colágeno e promovem deposição mineral. O oposto ocorre com os cimentos a base de silicato de cálcio, que provocam uma degradação cáustica na interface;
- As formulações dos sistemas adesivos universais influenciam mais na longevidade das restaurações do que o seu protocolo de aplicação. Os CIVs modificados por resina, são capazes de melhorar essa longevidade, especialmente quando os sistemas adesivos são aplicados com condicionamento ácido prévio;
- Muitos materiais liberadores de íons já se encontram disponíveis no mercado, entretanto, é importante a elucidação de seus mecanismos de ação e aplicabilidades clínicas.

7. RECOMENDAÇÕES E PERPECTIVAS FUTURAS

Os conceitos minimamente invasivos na odontologia restauradora requerem um maior conhecimento científico pelos professionais clínicos, principalmente no que diz respeito a remoção do tecido cariado e princípios biológicos do substrato dentinário remanescente, mas também uma profunda compreensão de como garantir e estabelecer uma restauração biomecanicamente estável e duradoura em diferentes situações clínicas.

A comercialização dos sistemas adesivos biomodificados ainda é limitada, mesmo sendo os materiais mais estudados quanto à bioatividade. A busca pelo desenvolvimento e aprimoramento desse tipo de material, possivelmente, é por estarem em íntimo contato com a superfície dentinária. Além disso, também se deve ao fato de serem, juntamente com os compósitos resinosos, a opção restauradora de maior escolha pelos profissionais clínicos. Entretanto, ultrapassar a barreira dos avanços laboratoriais, interesses mercadológicos e utilização clínica é um desafio para essa classe de materiais que dependem de uma boa resistência de união, estabilidade da composição química e das propriedades mecânicas, bem como aperfeiçoamento estético.

Já para a classe de materiais que apresentam adesão química ao dente, as biomodificações se tornam mais possíveis. Como é o caso dos CIVs, sendo largamente utilizado com diferentes intuitos, desde forradores cavitários a materiais restauradores definitivos; promovendo reparação tecidual por meio de liberação de íons flúor, ação antimicrobiana, barreira térmica, adesão à estrutura dentária, dentre outros. As variações na composição, visam melhorar algumas propriedades específicas, como é o caso dos ionômeros modificados por resina, que apresentam uma maior resistência mecânica e melhor tempo clínico de trabalho devido à fotoativação, mas que por outro lado, há uma diminuição na liberação de íons flúor e são mais sensíveis quanto à umidade, que pode~~m~~ comprometer sua presa.

Para os materiais à base de silicato de cálcio, a incrementação de uma matriz inorgânica, é justamente com o intuito de promover adesão ao dente, já que esses materiais puros são porosos e sem adesividade. Como a princípio esses materiais foram desenvolvidos para tratamentos endodônticos, a propriedade adesiva ou de resistência mecânica não tinha tanta importância.

Porém, percebeu-se que esses materiais são capazes de liberar íons cálcio e com isso promover deposição mineral na interface, sendo uma característica importante na recuperação e proteção do tecido dentinário.

Podemos dizer que os materiais hidráulicos são os mais indicados para entrarem em contato diretamente com a dentina, seja ela afetada ou infectada, pois devido à sua característica alcalina, o colágeno degradado é rapidamente substituído pela deposição de íons cálcio. Desta maneira, são ótimos materiais para promoverem a proteção do complexo dentinho-pulpar. Os cimentos vítreos, possuem como característica, a preservação do colágeno que pode ser remineralizado pela deposição de minerais, devido a isso, sua melhor indicação seria na presença de dentina afetada, sendo que sua adesão a esse tipo de substrato também é alcançada. O mesmo já não ocorre com os sistemas adesivos e os compósitos resinosos. Mesmo para os sistemas autocondicionantes, o substrato ideal para uma perfeita adesão e longevidade da restauração ainda é o tecido hígido. Conhecendo esses mecanismos básicos de ação por esses principais compostos, fica mais fácil entender a indicação e aplicabilidade clínica de cada material, mesmo quando há alteração em sua composição.

Com o desenvolvimento de novos materiais uma otimização da recuperação mineral tende a ser alcançada, indo ao encontro dos conceitos de mínima intervenção e possibilitando o surgimento de um material que contemple os aspectos físicos, químicos, biológicos, mecânicos e estéticos da restauração.

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ANEXOS

Anexo 1

HOSPITAL UNIVERSITÁRIO
CLEMENTINO FRAGA FILHO
/HUCFF/ UFRJ

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Potencial bioativo de cimentos à base de silicato de cálcio e policarboxilato de zinco em lesões de cárie artificiais em dentina

Pesquisador: Paula Maciel Pires

Área Temática:

Versão: 2

CAAE: 54941416.9.0000.5257

Instituição Proponente: UNIVERSIDADE FEDERAL DO RIO DE JANEIRO

Patrocinador Principal: UNIVERSIDADE FEDERAL DO RIO DE JANEIRO

DADOS DO PARECER

Número do Parecer: 1.588.462

Apresentação do Projeto:

Protocolo 078-16. Respostas recebidas em 2.6.2016.

INTRODUÇAO:

O dente é o órgão mais mineralizado do corpo humano, composto de aproximadamente 88-95% de hidroxiapatita, que é essencialmente um sal de fosfato de cálcio (Sousa et al., 2009). Sendo assim, apesar de possuir alta resistência mecânica, atribuída ao conjunto da microestrutura e ao gradiente de propriedades mecânicas, que se inicia na superfície do esmalte até a dentina subjacente (An et al., 2012), os dentes são extremamente

vulneráveis aos processos de desmineralização que podem ocorrer durante toda a vida útil do mesmo. Lesões de cárie dentária são caracterizadas pela perda mineral, no qual a ação de ácidos, principalmente o ácido lático, produzido pela fermentação bacteriana leva à desmineralização do esmalte e da dentina(Kidd & Fejerskov, 2004). Os conceitos mais atuais em odontologia preventiva prezam por tratamentos minimamente invasivos e conservadores dos dentes afetados por lesões de cárie no sentido de preservar o potencial para remineralização do tecido desmineralizado, mas não completamente destruído pelos processos bacterianos(Dai et al., 2011). Nesse contexto, sabe-se que a diminuição do conteúdo mineral leva à redução das propriedades mecânicas do tecido

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Continuação do Parecer: 1.588.462

Pesquisadores	apresenta_da_equipe0001.pdf	03:37:02	Paula Maciel Pires	Aceito
Folha de Rosto	folhaderosto.pdf	03/04/2016 03:34:33	Paula Maciel Pires	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

RIO DE JANEIRO, 14 de Junho de 2016

Assinado por: Carlos Alberto Guimarães (Coordenador)

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Anexo 2

TERMO DE CESSÃO DE DENTES HUMANOS

Eu, _____, portador do RG: _____, nascido em ___/___/_____, sexo (M) (F), residente à rua: _____ nº_____, bairro:_____ na cidade de _____, Estado _____, CEP: _____, telefone: _____/_____, estou consciente de que de que o(s) dente(s) abaixo descrito(s) foi (foram) extraído(s) por indicação terapêutica para a melhoria da minha saúde , como documentado em meu prontuário.

Após ter sido informado e ter minhas dúvidas suficientemente esclarecidas, concordo em doar de forma voluntária o(s) dente(s) humano(s) _____ (identificação do(s) dente(s)) para o Biorrepositório criado exclusivamente para a pesquisa de estudo da aluna de pós- graduação da Universidade Federal do Rio de Janeiro (UFRJ).

Rio de Janeiro, _____ de _____ de _____.

_____

Assinatura do doador ou seu representante legal