UNIVERSIDADE FEDERAL DE PELOTAS
Faculdade de Odontologia
Programa de Pós-Graduação em Odontologia

Dissertação de Mestrado

Caracterização físico-mecânica e atividade antibacteriana de resinas adesivas contendo grupamentos metálicos

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Pelotas, 2015

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Dissertação apresentada ao Programa de Pós-Graduação em Odontologia da Faculdade de Odontologia da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Mestre em Odontologia, área de concentração em Dentística.

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Pelotas, 2015

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Dissertação aprovada como requisito parcial, para obtenção do grau de Mestre em Odontologia (área de concentração: Dentística), Programa de Pós-Graduação em Odontologia, Faculdade de Odontologia, Universidade Federal de Pelotas.

Data da Defesa: 20 de fevereiro de 2015

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Universidade Federal de Pelotas / Sistema de Bibliotecas

Catalogação na Publicação

C659c Cocco, Alexandra Rubin

Caracterização fisico-mecânica e atividade antibacteriana de resinas adesivas contendo grupamento metálico / Alexandra Rubin Cocco ; Rafael Guerra Lund, orientador ; Evandro Piva, coorientador. — Pelotas, 2015.

124 f . : il.

Dissertação (Mestrado) — Programa de Pós-Graduação em Dentística, Faculdade de Odontologia, Universidade Federal de Pelotas, 2015

1. Sistemas adesivos. 2. Atividade antibacteriana. 3. Propriedades mecânicas. 4. Citotoxicidade. I. Lund, Rafael Guerra, orient. II. Piva, Evandro, coorient. III. Título.

Black : D236

Elaborada por Claudia Denise Dias Zibetti CRB: 10/932

Dedicatória

Dedico este trabalho à minha família,
à Deus
e à todas as pessoas que me ajudaram a
construí-lo.

Agradecimentos

Não fizemos nada nesta vida sozinhos. E este trabalho não foi diferente. Por isso, agradeço todos os professores, alunos, funcionários da Faculdade de Odontologia. Cada um de uma forma ou outra me ajudou a construir essa dissertação. Alguns me ajudaram na parte teórica, outros me ajudaram na parte prática. Teve aqueles que com um simples bom dia, um simples sorriso e um abraço me proporcionaram dias melhores, trabalhos mais tranquilos.

Quero agradecer em especial:

Meus pais que me proporcionaram a melhor escola da vida: educação moral, espiritual e lições de vida. Obrigada pelo apoio e ajuda para eu seguir estudando. Não tenho palavras para dizer o quanto me orgulho de vocês. Por tudo o que vocês fizeram e fazem por mim, muito obrigada.

Meu irmão **Gabriel** pela preocupação que tem comigo e com meu bem estar. Também ao meu irmão caçula, **Rodrigo** por compreender minha falta física em muitos jogos de futebol. Porém sempre estive com pensamentos positivos e torcendo pela tua vitória.

Como dizia Vinicius de Moraes "A gente não faz amigos, reconhece-os". A amizade é fruto de escolhas e agradeço aos amigos **Cácia e Wellington** por vocês terem me escolhido e me reconhecido como amiga. Agradeço a Cácia pela amizade, pelos conselhos e por ter me dado à oportunidade de dividir muitos momentos de alegria. Agradeço Wellington (Tom) pelas horas de trabalho juntos, pela dedicação e pela companhia. Obrigada pela amizade, pelos ensinamentos.

Agradeço a PPGO pela oportunidade de realizar o mestrado em uma ótima instituição com conceito 6. Agradeço a CAPES pela bolsa de estudo. Agradeço também a todos os professores, colegas e demais colaboradores do PPGO pela convivência e aprendizado e, principalmente as amigas que conheci e convivi nesses 2 anos: Tamires, Carine, Querén, Karine, Andréia, Camila e demais.

Agradeço demais amigos: Fernanda Amado, Liana, Luana, Thiana, Melina, Dionéia, Carine Pires, Andressa Spohr, Andressa Gastman, Tamara, Aline Simões, Camila Amaral, Maura, Alícia Soares, Taiane, Ana Paula,

Fernanda Grill, Janine, Marina Bernd, Bruna Victória, Carol Silva, José Porto, Rafael Onofre, Lucas Brondani, Tómas Recuero pela paciência nos momentos difíceis e por aqueles de felicidade que compartilhamos. Agradeço as minhas amigas de Cruz Alta que mesmo de longe sempre estavam torcendo por mim. Agradeço por entenderem minha falta em muitos momentos.

Agradeço a **Sônia, Tamires** e todas aquelas pessoas que me ajudaram nos experimentos de microbiologia. Agradeço aos funcionários **Tatiana, Carmem, Lizangela, Junior e Leandro** por tudo que fizeram por mim, pela paciência, pela preocupação e pelas palavras carinhosas que me motivavam em muitos dias. A equipe CDC-Bio, Laboratório de Microbiologia e Laboratório de Cultivo Celular e de Biologia Molecular (NCT-BIO).

Agradeço meus primos, em especial **Isadora,** minha madrinha **Naíze**, minha cunhada **Josi** e toda minha família. Sei que todos estavam torcendo por mim.

Agradeço a meus orientadores **Rafael Guerra Lund e Evandro Piva** por me fazerem me sentir uma pessoa de valor, pela confiança e pelas oportunidades. Agradeço pelas inúmeras conversas e conselhos. Agradeço pela paciência, pelos ensinamentos. Vocês me ensinaram muito mais do que teorias. Vocês são exemplos para mim de caráter, honestidade, profissionalismo e de ser humano. Agradeço aos professores Rafael Moraes, Josué Martos, Adriana Silva, Giana Lima, Rudimar, Luiz Fernando, Neno, doutora Eliana Torres e doutoranda Fernanda Leal pela ajuda em trabalhos paralelos. Vocês também são exemplos de educadores e pesquisadores, e serão sempre minha referência.

Agradeço o **Goiabinha** pela companhia e pelos inúmeros momentos de alegria.

“Em tuas mãos coloquei minhas preocupações, cuidados e problemas. Em tua sabedoria coloquei meu caminho, minhas direções e meus objetivos. Em teu amor, coloquei minha vida.”

Por fim, agradeço **a Deus** pela vida, pela sabedoria e por todas vezes que pedi a Ele tranquilidade e força para seguir em frente.

“Por vezes sentimos que aquilo que fazemos não é senão uma gota de água no mar. Mas o mar seria menor se lhe faltasse uma gota.”

Madre Teresa de Calcutá.

Resumo

COCCO, Alexandra Rubin. **Caracterização físico-mecânica e atividade antibacteriana de resinas adesivas contendo grupamentos metálicos**. 2015. 123f. Dissertação (Mestrado em Dentística) - Programa de Pós-Graduação em Odontologia, Faculdade de Odontologia, Universidade Federal de Pelotas, Pelotas, 2015.

A presença de microrganismos na cavidade dental após a remoção do tecido cariado pode levar ao fracasso do tratamento restaurador. Com o objetivo de eliminar microorganismos da cavidade dental, sistemas adesivos com propriedades antibacterianas têm sido desenvolvidos. Para abordar este tema, foi realizada uma revisão sistemática dos monômeros antibacterianos incorporados em sistemas adesivos e um estudo laboratorial a fim de caracterizar físico-mecanicamente resinas adesivas experimentais com potencial antibacteriano. Inicialmente, a revisão sistemática e o monitoramento tecnológico foram realizados para avaliar a efetividade de monômeros antibacterianos incorporados em sistemas adesivos, bem como analisar perspectivas futuras para o desenvolvimento tecnológico do setor. Uma busca em sete bases de dados foi realizada: *MedLine (PubMed), Lilacs, Ibecs, Web of Science, Scopus, Scielo e The Cochrane Library.* Adicionalmente, a informação tecnológica foi resgatada por meio do sistema online Questel Orbit (Paris, France), que permite a busca e análise de patentes em mais de 90 autoridades. Enquanto isso, um estudo laboratorial foi conduzido com o objetivo de desenvolver e determinar a atividade antimicrobiana, bem como caracterizar físico-mecanicamente resinas adesivas de sistemas adesivos autocondicionantes de 2 passos experimentais, contendo um de dois metacrilatos metálicos (prata ou estanho), em concentrações mol % de 0,5, 1 ou 2. Um sistema adesivo experimental, sem adição de metacrilatos metálicos na resina adesiva, serviu como controle negativo. Testes físico-mecânicos (grau de conversão e resistência de união à dentina) e testes biológicos (modelo de biofilme e citotoxicidade) foram realizados. Na revisão sistemática, um total de 1341 artigos e 240 patentes foram resgatados inicialmente. Desses documentos, 33 artigos e 9 patentes estavam relacionados a monômeros com atividade antibacteriana e foram incluídos no estudo. O monômero antibacteriano mais relatado na literatura foi o MDPB, encontrado no sistema adesivo comercial Clearfil Protect Bond® (Kuraray Co. Ltda., Japão). Já no estudo laboratorial, as resinas adesivas com os metacrilatos de prata e de estanho, nas concentrações de 1% e 2%, apresentaram ação antibacteriana. Resinas adesivas contendo 2% dos dois metacrilatos testados apresentaram menor viabilidade celular. A incorporação de 1% de metacrilatos metálicos de prata ou estanho nas resinas adesivas experimentais proporcionaram ação antibacteriana, sem comprometer as propriedades físicas e biológicas das resinas adesivas desenvolvidas. Apesar de serem necessários estudos clínicos para confirmar a efetividade desses materiais na prevenção e controle de patologias dentais, como a cárie, a revisão sistemática e o estudo laboratorial demonstraram que há evidência de atividade antibacteriana desses adesivos *in vitro*.

Palavras-Chave: efeitos antibacterianos; sistemas adesivos; bactérias orais; propriedades mecânicas; toxidade; monômeros antibacterianos; revisão sistemática

Abstract

COCCO, Alexandra Rubin. **Physical mechanical characterization and antibacterial activity of adhesive resins containing metal groups**. 2015. 123f. Dissertation (Master Degree em Dentística) - Programa de Pós-Graduação em Odontologia, Faculdade de Odontologia, Universidade Federal de Pelotas, Pelotas, 2015.

The presence of microorganisms in the dental cavity after caries removal can lead to failure of the restorative treatment. Aiming to eliminate microorganisms of dental cavity, adhesive systems with antibacterial properties have been developed. To approach this issue, was performed a systematic review of antibacterial monomer incorporated into adhesive systems and a laboratory study to physical-mechanically characterize experimental adhesive resins with antibacterial potential. Initially, the systematic review and technological monitoring were conducted to evaluate the effectiveness of antibacterial monomer incorporated in adhesive systems, and analyze future prospects for the technological development of the sector. A search in seven databases was performed*: MedLine (PubMed), Lilacs, IBECS, Web of Science, Scopus, SciELO and The Cochrane.* In addition, the technology information is rescued by of the online system Questel Orbit (Paris, France), which allows the patent search and analysis in 90 authorities. Meanwhile, a laboratory study was conducted in order to develop and determine the antimicrobial activity as well as mechanically characterize physical-adhesive resin adhesive systems of two experimental steps, containing two metal methacrylates (silver or tin). An experimental adhesive system without addition of metal methacrylates in adhesive resin, served as a negative control. Physical and mechanical tests (degree of conversion and bond strength to dentin) and biological tests (biofilm model and cytotoxicity) were performed in these new adhesive systems. In the systematic review, a total of 1341 articles and 240 patents were rescued first. Of these documents, 33 articles and 9 patents were related to monomers with antibacterial activity and were included in the study. The monomer more reported in the literature was MDPB, antibacterial monomer incorporation commercial adhesive system Clearfil Protect Bond^{TM} (Kuraray Co. Ltd., Japan). Resin adhesive with methacrylates of silver and tin at concentrations of 1% and 2% showed antibacterial action. The concentration of 2% of two methacrylates showed less cell viability. The incorporation of 1% silver or tin methacrylate in the experimental adhesive resins provided antibacterial action without compromising the physical and biological properties. Although being necessary clinical studies to confirm the effectiveness of these materials in the prevention and control of dental diseases such as caries, the systematic review and the laboratory study showed that there is evidence of antibacterial activity in vitro of these adhesives.

Keywords: antibacterial effects; adhesive systems; oral bacteria; mechanical properties; toxicity; antibacterial monomers; systematic review

Sumário

1. Introdução geral

A realização da técnica minimamente invasiva é atualmente preconizada, pois além de preservar a estrutura dentária (CHENG et al., 2013), pode evitar danos pulpares, principalmente em cavidades muito profundas em que pode haver risco de exposição pulpar ou a pré-existência de uma pulpite reversível (MALTZ; ALVES, 2013). Esta técnica visa apenas à remoção de uma camada da lesão de cárie, deixando outras camadas na cavidade dentária (MALTZ; ALVES, 2013).

A lesão de cárie em dentina é composta por três camadas distintas:

1. Infectada: camada mais superficial; clinicamente aparenta tecido amolecido de coloração amarela ou marrom claro. Possui grande quantidade de bactérias (MASSARA; ALVES; BRANDAO, 2002).
2. Camada contaminada: dentina um pouco mais rígida do que a anterior, podendo ser removida por lascas ou escamas. Existe menor quantidade de bactérias (MASSARA; ALVES; BRANDAO, 2002).
3. “Dentina normal”: apresenta-se com consistência dura e coloração castanho escura, apresentando uma grande redução de bactérias (MASSARA; ALVES; BRANDAO, 2002).

A remoção parcial de tecido cariado tem como objetivo interromper a progressão de cárie, através da remoção da dentina infectada. Porém, após a remoção e selamento da cavidade, bactérias permanecem na cavidade podendo levar ao fracasso do tratamento restaurador, não permitindo a recuperação pulpar e proporcionando uma possível ocorrência de cárie secundária e descoloração marginal (MALTZ, 2011).

Para minimizar estes problemas, partículas e/ou monômeros com ação antibacteriana têm sido incorporados aos sistemas adesivos dentinários (CHENG et al., 2012). Partículas como prata (CHENG et al., 2012), zinco (HENN et al., 2012; HENN et al., 2011) ou até mesmo óleos essenciais de origem vegetal (PERALTA et al., 2013) têm sido utilizados, porém são lixiviados da matriz polimérica e sua durabilidade é questionável. Além disso, podem afetar as propriedades mecânicas do material e serem tóxicos aos tecidos pulpares (IMAZATO; RUSSELL; MCCABE, 1995; NAMBA et al., 2009).

Os monômeros, diferentemente das partículas, são imobilizados na matriz polimérica, reticulam-se no polímero através da polimerização,

dificultando a lixiviação (IMAZATO; MCCABE, 1994). Dessa forma, os monômeros antibacterianos atuam sobre as bactérias quando em contato com a superfície (partículas atuam a longa distância). O mecanismo de ação antibacteriano de monômeros ainda é especulado, sendo que pode ser a ruptura da membrana da parede celular bacteriana que leva à morte celular desses microrganismos (IMAZATO et al., 1998; KENAWY EL; WORLEY; BROUGHTON, 2007). O primeiro monômero antibacteriano relatado foi o MDPB (12-*methacryloyloxydodecylpyridinium bromide*), o qual é um dos componentes do sistema adesivo comercial Clearfil Protect Bond® (Kuraray Co. Ltd., Japan). Posteriormente, outros monômeros foram sintetizados com a mesma finalidade antimicrobiana, como o DMAE-CB (*methacryloxylethyl cetyl dimethyl ammonium chloride*) (XIAO et al., 2009).

Muitos autores, para complementar a ação antibacteriana dos monômeros, têm incorporado em sistemas adesivos pequenas porcentagens de partículas antibacterianas, ocorrendo assim ação por contato e à longa distância (CHENG et al., 2013; ZHANG et al., 2013; ZHANG et al., 2013). O monômero DMADDM (*dimethylaminododecyl methacrylate*) quando combinado com partículas de prata, teve resultados satisfatórios com relação ao seu efeito antibacteriano. Além de ter aumentado a ação antimicrobiana, este efeito se manteve após seis meses (ZHANG et al., 2013).

Partículas de metais nobres têm chamado à atenção devido às suas propriedades físico-químicas, óticas e antibacterianas. Por causa disso, têm sido estudados metacrilatos metálicos como prata, zinco e estanho. A prata é bem conhecida pela sua ação antibacteriana e tem sido empregada em materiais dentários como o amálgama (ALLAKER, 2010; MONTEIRO et al., 2009). Seu mecanismo de ação é desconhecido, mas tem sido relatado que as partículas de prata poderiam desnaturar o DNA e o RNA das bactérias, inibindo a replicação bacteriana (CASTELLANO et al., 2007; LIAO et al., 2010). Já o zinco inibe o metabolismo do açúcar, podendo diminuir a produção de ácidos por *Streptococcus mutans* (HE; PEARCE; SISSONS, 2002; WANG; SHEN; HAAPASALO, 2014). Além disso, estudos recentes demonstraram que o metacrilato de zinco foi ainda capaz de inibir metaloproteinase da matriz (HENN et al., 2012), um componente da dentina que degrada o colágeno, causando hidrólise dos sistemas adesivos ao longo do tempo. Já o estanho tem sido usado juntamente com fluoretos em materiais odontológicos, mas o

seu mecanismo de ação antimicrobiano ainda é desconhecido. Estudos *in vivo (ATTRAMADAL; SVATUN, 1984) e in vitro* (SVATUN, 1978; SVATUN; ATTRAMADAL, 1978) mostraram que soluções e dentifrícios contendo estanho foram capazes de inibir a formação da placa dental e reduzir a produção de ácidos, provavelmente produzidos por *Streptococcus mutans.* Além disso, o estanho demonstrou *in vivo* um profundo e duradouro efeito inibitório na microbiota oral (ATTRAMADAL; SVATUN, 1984). Os íons metálicos, em geral, têm como mecanismo de ação perturbar o sistema respiratório e de transporte de elétrons em células bacterianas. Além disso, são considerados seguros por não serem absorvidos pelo corpo (BARRY; TROGOLO; PASTECKL, 2001).

Partindo deste princípio, o objetivo deste trabalho foi: (1) revisar sistematicamente a existência e a eficácia de monômeros antibacterianos incorporados em sistemas adesivos, bem como os avanços e prospectivas futuras no desenvolvimento de materiais adesivos antibacterianos; (2) desenvolver sistemas adesivos contendo metacrilato metálico (prata e estanho) e avalia-los quanto à ação antibacteriana, citotoxicidade e propriedades mecânicas. A hipótese é que tanto os monômeros antibacterianos quanto os metacrilatos metálicos apresentam ação antibacteriana e a incorporação deles não afetam as propriedades mecânicas.

2. Projeto de Pesquisa

1 Introdução /Justificativa

As principais falhas de restaurações de resina composta são causadas por fratura e cárie secundária (DEMARCO et al., 2012; KOPPERUD et al., 2012; MJOR, 2005; ZHANG et al., 2013). Os principais fatores que podem estar associados com o desenvolvimento de cárie secundária são: a presença de grandes *gaps*, entre restauração e dente, e o biofilme acumulado, ocorrendo a desmineralização ao longo da parede da cavidade do dente restaurado (CENCI et al., 2009; HE et al., 2011; KOPPERUD et al., 2012; KUPER et al., 2013; ZHANG et al., 2013). Consequentemente surge a necessidade de substituir restaurações ou repará-las, o que representa elevados custos econômicos anuais e perda de tempo, sendo classificado como o tratamento mais comum em prática odontológica geral (DEMARCO et al., 2012; PALLESEN et al., 2013; ZHANG et al., 2013).

Além disso, para evitar gastos de tempo em consultório, muitos profissionais preferem materiais simplificados, como por exemplo, os sistemas adesivos autocondicionantes. Os sistemas adesivos são compostos por monômeros e os mais utilizados são os metacrilatos como bisfenol glicidil metacrilato (BisGMA) e o trietilenoglicoldimetacrilato (TEGDMA) (HE et al., 2012; Jingwei He 2012). Sabe-se que esses monômeros utilizados em sistemas adesivos podem não ser compatíveis com os tecidos pulpares. Dentre eles, o HEMA é um monômero hidrofílico que possui a facilidade de difundir através dos túbulos dentinários e atingir a polpa, uma vez que possui um coeficiente de difusão intratubular maior do que outros monômeros, devido ao seu baixo peso molecular, tendo como consequência a hipersensibilidade (130 g/mol) (ZANCHI et al., 2010). Além disso, em cavidade profunda, a dentina é mais úmida, e o HEMA apresenta propriedade hidrolítica, atraindo água para a interface adesiva, podendo causar degradação da mesma e diminuição das propriedades mecânicas (ZANCHI et al., 2011). Uma alternativa para esses problemas relatados tem sido o uso de surfactantes, como o bisfenol A glicil di-metacrilato etoxilado (Bis-EMA). O Bis-EMA é um dimetacrilato que resulta em um polímero com menor suscetibilidade de hidrólise em meio aquoso. A vantagem do Bis-EMA é que ele possui um comportamento bipolar o que pode

levar ao aumento da solubilidade dos monômeros hidrófobos dos sistemas adesivo, facilitando a entrada dos demais e reduzindo a fase de separação (ZANCHI et al., 2011; ZANCHI et al., 2013).

Ademais, os sistemas adesivos atuais não possuem monômeros que apresentam componentes antibacterianos. (MCCABE, 1994; XIAO et al., 2008; ZHANG et al., 2013). O primeiro desenvolvido foi o MDPB (brometo de metacriloiloxidodecilpiridínio) o qual possui como agente antibacteriano o sal de quartenário de amônio (QA). A ação antibacteriana do QA ainda não esta bem relatada. Apresentam-se três explicações para os mecanismos de ação desses compostos: 1. Contato da carga negativa da bactéria com a carga positiva do sal quartenário de amônio, resultando em pressão osmótica; 2. Difusão do QA através da parede celular da bactéria e ligação à membrana citoplasmática e 3. Disrupção da membrana citoplasmática, com a liberação dos componentes citoplasmáticos e morte celular (ANTONUCCI et al., 2012; HE et al., 2011).

O MDPB encontra-se disponível comercialmente em resinas compostas e sistemas adesivos (IMAZATO et al., 2006). O Clearfil Protect Bond® (Kuraray Co. Ltd., Japan), primer que possui 5% de MDPB em sua composição tem demonstrado maior atividade antibacteriana quando comparado com outros primers (IMAZATO et al., 2006; FEUERSTEIN et al., 2007). Compósitos contendo MDPB também têm mostrado inibir o avanço de cárie secundária (MCCABE, 1994; IMAZATO et al., 1994) e sua ação antibacteriana pode durar até 6 meses (IMAZATO et al., 1998; IMAZATO et al., 2001). No entanto, a sua durabilidade continua incerta. Algumas evidências demonstram que o sistema adesivo Clearfil Protect Bond® (Kuraray Co. Ltd., Japan), após foto-ativação, reduz significativamente a ação antibacteriana (FEUERSTEIN et al., 2007; GONDIM et al., 2008; DA SILVA et al., 2010). Isso indica que o componente antibacteriano incluído no MDPB não é lixiviado (TURKUN et al., 2006).

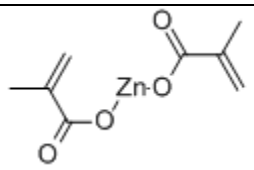
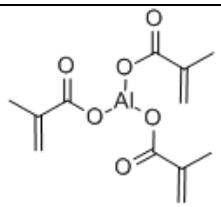
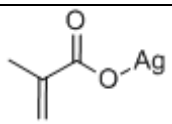
Por isso, novos agentes antibacterianos têm sido investigados visando prolongar o tempo de atividade antimicrobiana dos materiais odontológicos sem afetar suas propriedades físico-mecânicas e uma alternativa para isso está na investigação de monômeros com grupamentos metálicos potencialmente antibacterianos. Através da síntese destes monômeros e mistura em resinas monoméricas odontológicas proporciona-se a inclusão de monômeros em moléculas funcionalizadas que irão reticular-se no polímero através da fotoativação. Outra vantagem é a relativa baixa solubilidade ou

lixiviação/erosão que essas moléculas estão sujeitas por estarem integradas ao polímero se comparado aos demais sais com efeito antibacteriano utilizados em materiais odontológicos. A possibilidade de funcionalização e consequentemente integração através da fotopolimerização é um parâmetro particularmente importante para a manutenção de propriedades físico-mecânicas de sistemas adesivos dentinários, uma vez que a aplicação em finas camadas deve resistir à tensão de contração inicial de compósitos e incidência das pressões da oclusão sobre a interface adesiva.

Monômeros com grupamentos metálicos como o metacrilato de prata e zinco (Tab. 1) podem exibir propriedade antibacteriana. A prata é bem conhecido por ter atividade antibacteriana, antifúngica e antiviral e é efetiva contra uma gama de bactérias (MORONES et al., 2005; MONTEIRO et al., 2009), além de apresentar baixa toxidade e boa biocompatibilidade com células humanas (RO¨SCH, 2007). Porém pouco é utilizada como material dentário devido ao seu alto custo e sua cor desfavorável (YOSHIDA, 2009). Já o zinco, em recente estudo do grupo, demonstrou ter atividade antibacteriana em uma resina modelo, além de proporcionar resultados interessantes na área de Odontologia, como a inibição de metaloproteinase da matriz (HENN et al., 2011; HENN et al., 2012).

Por isso, o objetivo deste estudo será: (1) investigar sistematicamente a atividade antibacteriana de monômeros antibacterianos incorporados em sistemas adesivos; (2) bem como desenvolver sistemas adesivos contendo metacrilato metálico, investigando atividade antibacteriana, citotoxicidade e propriedades mecânicas.

Tabela 1 – Fórmula molecular e estrutural de diferentes metacrilatos com grupamento metálico

Monômero	Fórmula molecular	Fórmula estrutural
Metacrilato de zinco	$C_8H_{10}O_4Zn$	
Metacrilato de alumínio	$C_{12}H_{15}Al\ O_6$	
Metacrilato de prata	$C_4H_5AgO_2$	

*Dados forcenidos Aldrich Chemical Co. (Milwaukee, WI, USA).

2 Objetivos

2.1 Objetivos Gerais

O objetivo do presente estudo será formular e avaliar sistemas adesivos autocondicionantes experimentais, livres de HEMA, contendo metacrilatos com grupamento metálico potencialmente antimicrobianos.

2.2 Objetivos Específicos

1. Realizar uma revisão sistemática para verificar a existência e a eficácia de monômeros com ação antibacteriana incorporados em sistema de adesivos.

2. Avaliar o desempenho físico-mecânico do sistema adesivo experimental através de ensaios *in vitro;*

3. Determinar a atividade antimicrobiana e anti-biofilme destes sistemas adesivos experimentais;

4. Verificar a biocompatibilidade destes sistemas adesivos experimentais através de ensaio de citotoxicidade.

3 Hipótese

A hipótese testada será de que há uma ação antibacteriana dos monômeros incorporados aos sistemas adesivos relatados na revisão sistemática e nos sistemas adesivos experimentais formulados.

4 Materiais e métodos

4.1 Metodologia para a realização da Revisão sistemática

Uma revisão sistemática sobre monômeros antibacterianos incorporados em sistemas adesivos será realizada para verificar a existência e a efetividade antibacteriana dos mesmos.

4.1.1 Estratégia de busca e seleção dos artigos

A revisão sistemática será feita de acordo com as normas PRISMA (MOHER et al., 2009). A busca de artigos será feita por duas pessoas independentemente (ARC e WOR) em sete bases de dados: *MedLine (PubMed), Lilacs, Ibecs, Web of Science, Scopus, Scielo* e *The Cochrane Library.* Os descritos usados para realizar a estratégia de busca serão: monômeros antibacterinos, sistemas adesivos, agentes antibacterianos.

Depois do resultado de cada base de dado, os artigos serão transferidos para um programa EndnoteX5 (Thompson Reuters, Philadelphia, PA, USA), onde as referências duplicadas serão individualmente removidas. Os artigos serão selecionados pelo título e resumo pelas mesmas pessoas individualmente. Caso existir alguma diferença na seleção dos artigos, os autores entrarão em consenso. Aqueles que se enquadrarem aos critérios de seleção serão lidos por completo (Fig. 1). Haverá restrição de língua, porém não de data.

Alguns dados serão extraídos de cada artigo e tabulados no Microsoft Office Excel 2013 (Microsoft Corporation, Redmond, Washington, USA) pelos dois revisores: autor, ano, país, monômero antibacteriano, principais resultados. Caso algum artigo ou dado não for encontrado, os revisores entrarão em contato com os autores do artigo e caso não responderem em um mês, o artigo será excluído.

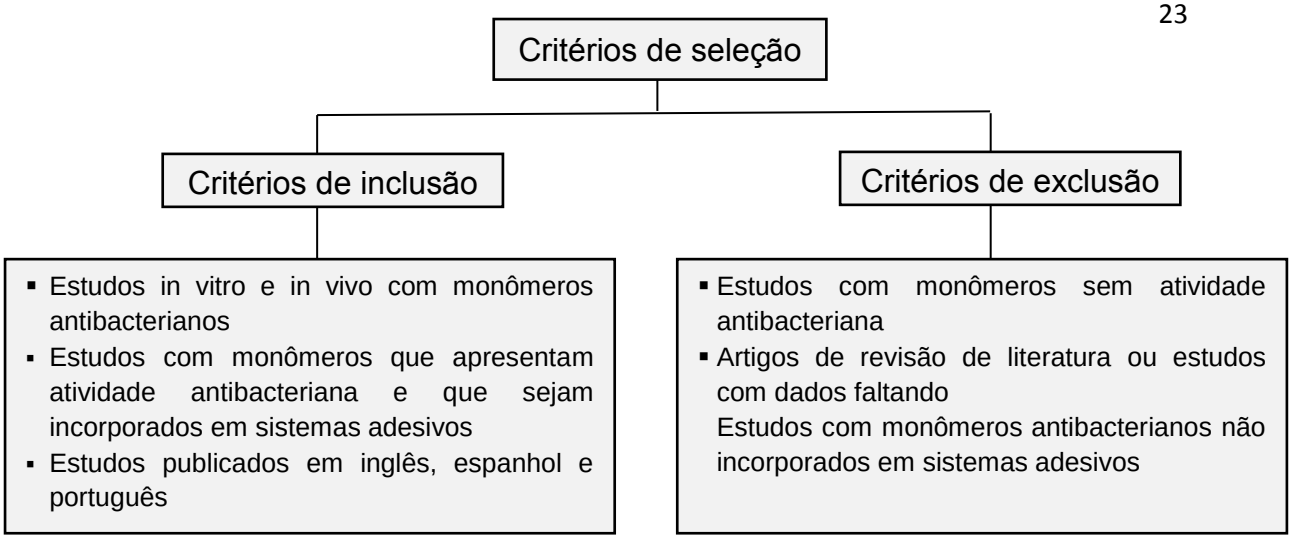


Figura 1 – Critérios de seleção

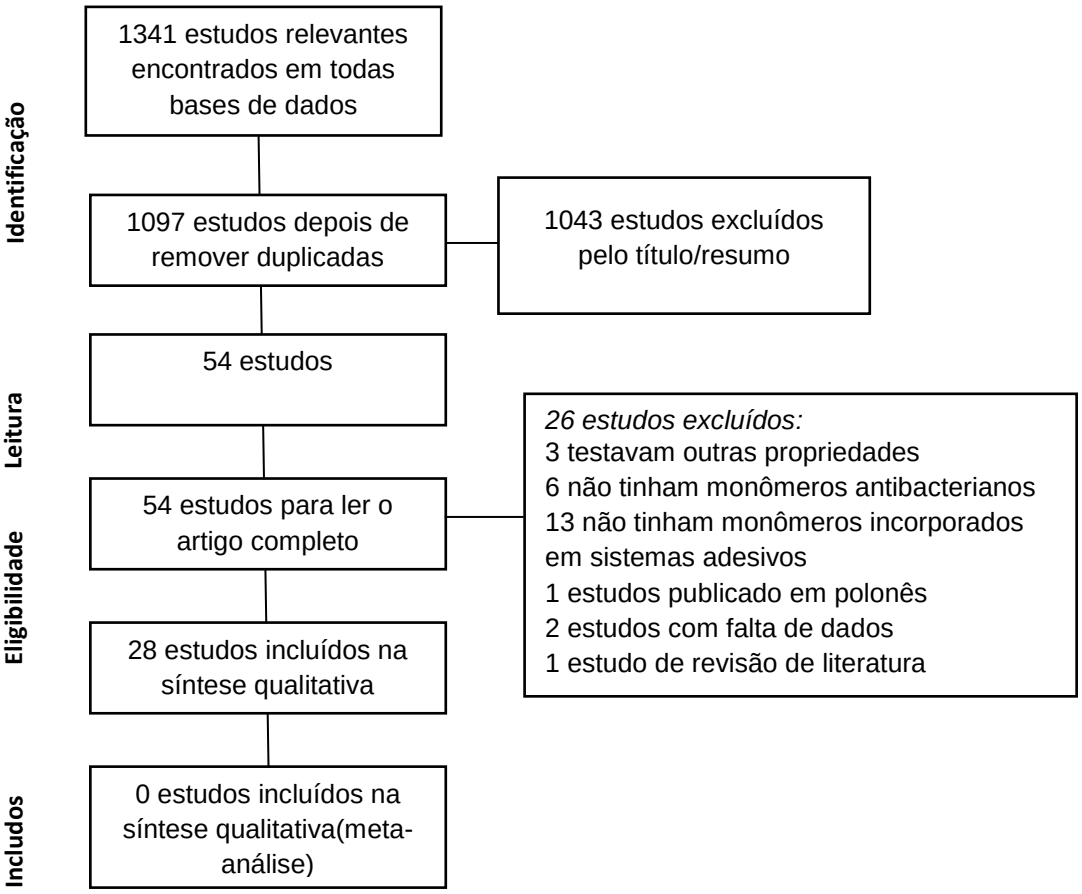


Figura 2 - Representação esquemática do delineamento do estudo (conforme descrito em PRISMA)

4.2 Formulação dos sistemas adesivos autocondicionantes experimentais

Todos os reagentes utilizados para formular o sistema adesivo experimental serão adquiridos junto à Esstech Inc. (Essignton, PA, USA), com exceção do álcool etílico absoluto (etanol) que será adquirido junto à Labsynth® Produtos para Laboratório Ltda. (Diadema, SP, Brasil). Os metacrilatos com grupamento metálicos serão adquiridos junto à Aldrich Chemical Co. (Milwaukee, WI, USA).

Um sistema adesivo experimental autocondicionante de 2 passos, livre do monômero HEMA (Tab. 2) será formulado. Serão incorporados em resinas adesivas experimentais monômeros com grupamentos metálicos na quantidade de 1% em massa (Tab. 3). Uma resina adesiva, AD-50, sem a incorporação dos metacrilatos será utilizado como controle (Tab. 4). E como referência comercial será utilizado o sistema adesivo Clearfil Protect Bond® (Kuraray Co. Ltd., Japan) (Tab. 5). Todos os sistemas adesivos passarão pelos seguintes testes (Fig. 3):

Tabela 2 – Componentes usados para sintetizar o sistema adesivo autocondicionante de 2 passos experimental: *primer e Bond*

Primer experimental	*Bond* experimental
30% GDMA 20% água 20% etanol 30% Bis-EMA 10	48% de Bis-GMA 25% TEGDMA 25% Bis-EMA 10 0,4 %CQ 0,8% EDAB

Tabela 3 - Formulação de resinas adesivas experimentais com adição de metacrilatos com grupamento metálicos

Metacrilato de zinco (mZn)		Metacrilato de prata (mAg)		Metacrilato de alumínio (mAl)	
Primer experimental	Adesivo + mZn	*Primer* experimental	Adesivo + mAg	*Primer* experimental	Adesivo +mAl

Tabela 4 – Componentes usados para sintetizar o sistema adesivo experimental controle, AD-50

Primer experimental	Bond experimental
30% GDMA 20% água 20% etanol 30% Bis-EMA 10	49% BISGMA 25% TEGDMA 25% HEMA 0,4 %CQ 0,8% EDAB

Tabela 5 – Descrição da referência comercial utilizada no estudo

Produto	Composição
Clearfil Protect Bond; Kuraray Medical, Tóquio, Japão	**PRIMER** (primer auto-condicionante) 6ml -MDP, MDPB, HEMA, dimetacrilato hidrofílico, foto-iniciador, água **BOND (** agente adesivo) 5ml -MDP, HEMA,Bis-GMA, dimetacrilato hidrofóbico, foto-iniciador, água, sílica coloidal, superfície tratada com NaF

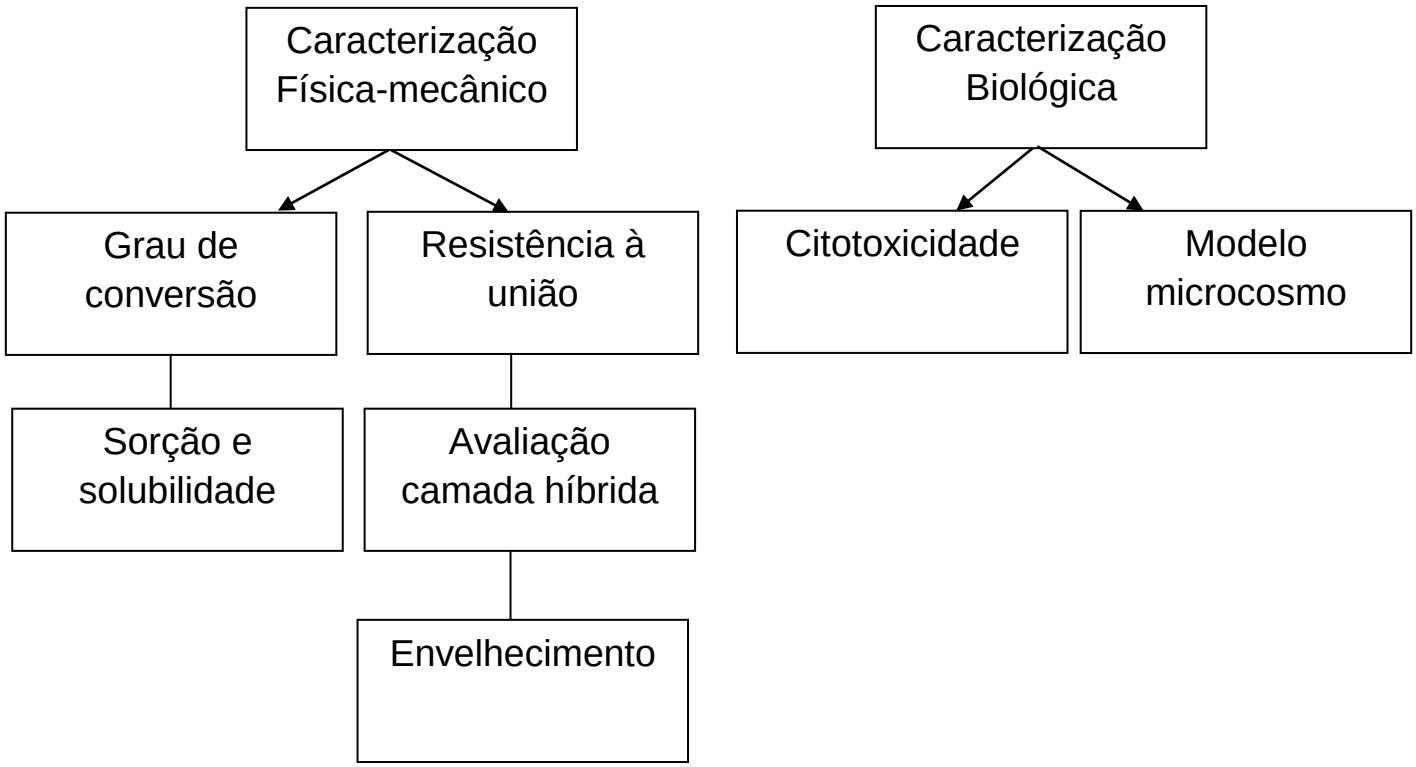


Figure 3 - Fluxograma dos testes a serem realizados

4.3 Ensaios físico-mecânicos

4.3.1 Grau de conversão

O grau de conversão das resinas adesivas será avaliado por meio de aparelho espectrofotômetro infravermelho por Transformada de Fourier (RT-FTIR Shimadzu Prestige21 Spectrometer, Shimadzu, Tóquio Japão) equipado com dispositivo de refletância total atenuada (ATR), composto por um cristal de diamante, com espelhos de angulação de 45º (PIKE Technologies, EUA). Um suporte será acoplado para a fixação da unidade foto-ativadora LED (Radii® Curing Light, SDI, Bayswater, Victória, Austrália) ao espectrofotômetro, permitindo uma distância uniforme de 5 mm entre a extremidade da ponteira de fibra ótica e a amostra. A irradiância será mensurada por intermédio de radiômetro portátil (model 100, Kerr, EUA).

Uma gota de cada resina adesiva será despejada diretamente no cristal de diamante e uma leitura será feita. Após, cada resina adesiva experimental

será fotoativado por 20 segundos (s) e analisada três vezes. O grau de conversão, por segundo, será calculado considerando a intensidade da vibração do tipo de estiramento da dupla ligação carbono-carbono (C=C) na freqüência de 1635cm^{-1}. Posteriormente, os dados obtidos serão plotados em uma curva ajustada pelo parâmetro regressivo não linear de Hill 3.

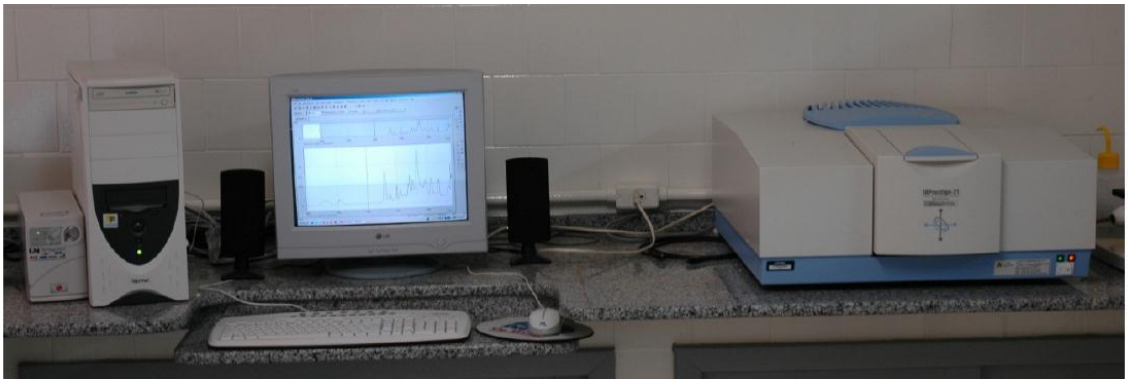


Figura 4 - Aparelho de Espectroscopia no Infravermelho por transformada de Fourier a ser utilizado no estudo

4.3.2 Ensaio de microtração (Resistência de união à dentina)

Cinqüenta incisivos bovinos serão obtidos em um frigorífico na cidade de Pelotas- RS (n=10). Estes serão limpos e armazenados em solução desinfetante de cloramina T a 0,5% por sete dias. Após este período de desinfecção, serão removidas raiz e polpa de cada dente e armazenados em água destilada, acondicionados em freezer, onde permanecerão congeladas até o momento da utilização. Os procedimentos aqui descritos serão baseados na normalização ISO TS 11405:2003 (ORGANIZATION, 2003). A porção correspondente ao esmalte será removida com o objetivo de expor a dentina superficial. A regularização do tecido e padronização da lama dentinária será realizada através de polimento com lixa de carbeto de silício (SiC) granulação 600 por 1 minuto (min) em politriz (Aropol-E, Arotec, Brasil), sob refrigeração com água.

Todos os procedimentos adesivos serão executados por um único operador previamente treinado e cegado. O sistema adesivo será aplicado em dentina bovina conforme a instrução de um sistema adesivo autocondicionate de 2 passos: aplicação do primer por 20s, o qual será aplicado com auxílio de um micropincel descartável. Em seguida, será realizada a evaporação do

solvente com jatos seco de ar por 10s, a uma distância de 10cm. Uma fina camada da resina adesiva será aplicada uniforme e regularmente sobre toda a superfície de dentina e fotoativada por 20s com aparelho LED (Radii, SDI, Austrália) com intensidade de 1400 mW/cm^2. Três camadas de no máximo 2mm serão realizadas com uma resina composta Z250, fotopolimerizando cada. Os dentes serão armazenados em água destilada em um refrigerador pelo período de 24horas (h).

Após esse período, usando uma cortadeira de precisão (Isomet 1000, Buehler, EUA), os dentes serão seccionados perpendicularmente a interface de união com disco diamantado sob refrigeração, com velocidade de corte de 400RPM, resultando em espécimes de aproximadamente 1mm^2 de área de secção transversal cujas dimensões serão aferidas com um paquímetro digital (Digimatic Caliper 500-144B, Mitutoyo Sul Americana, Suzano, SP, Brasil) com precisão de 0,01mm para cálculo da área de união. Serão obtidos em média 20 palitos que serão armazenados aleatoriamente em água destilada a 37°C em uma estufa por 24h, 6 meses e 12 meses. Após o tempo de armazenamento, os espécimes serão fixados em dispositivo para ensaio de microtração através do adesivo de cianocrilato (Loctite Super Bonder®, São Paulo, SP, Brasil), de modo que a interface resina/adesivo/dentina permaneça livre de qualquer contato. O ensaio será realizado em máquina de ensaios mecânicos (DL500; EMIC, São José dos Pinhais, PR, Brasil) com velocidade de 0,5mm/min até a fratura. Os resultados da resistência de união serão calculados em MPa de cada espécime e será oriundo da divisão da carga empregada no momento da fratura pela área de união (cm^2) de cada amostra, utilizando o programa Tesc (Versão Standard, EMIC® Equipamentos e Sistemas de Ensaio.

Figura 5- Máquina de ensaios mecânicos a ser utilizada no estudo, EMIC DL 500

4.3.3 Análise do modo de fratura

Após o teste de resistência de união à microtração, as amostras correspondentes à dentina serão cuidadosamente removidas do dispositivo para serem analisados em microscópio óptico disponível em microdurômetro digital (Microhardness Tester FM 700, Future-Tech Corp., Kawasaki, Tóquio, Japão), com aumento de 100 e 500x e, os modos de falha classificados em: coesivas, quando ocorrerem exclusivamente em dentina ou em compósito restaurador; adesivas, quando ocorrerem na região de interface dentina/ resina;

mistas, quando os dois tipos de fraturas ocorrerem simultaneamente, ou seja, as falhas acontecem na interface adesiva, estendendo-se para um dos substratos vizinho.

4.3.4 Sorção e solubilidade em água

Todas as etapas do desenvolvimento desse estudo seguem a especificação ISO 4049 (2000). Dez espécimes de cada resina adesiva serão confeccionados dispensando-se o material em um molde circular metálico com 1mm de espessura e 15mm de diâmetro. Sobre o material será colocada uma tira de poliéster, e em seguida fotopolimerizado durante 20s com um aparelho LED (Radii, SDI, Austrália) com intensidade de 1400mW/cm^2. Em seguida, todos os espécimes terão seus bordos polidos com lixas de granulação 600 e depois 1200.

Os espécimes serão colocados em um “dessecador” contendo sílica e armazenados em estufa a 37°C deixando-os 24h. Após, serão mensurados em uma balança com precisão de 0,1 mg. A mensuração será repetida até que uma massa constante, m1, for obtida, ou seja, até a massa seca (sem sofrer sorção do meio) do espécime não for superior a 0,1mg, em qualquer período de 24h.

Após a definição da m1, os espécimes serão colocados em água destilada (concentração de 10ml para cada corpo de prova), de tal forma que fiquem verticais e no mínimo 3mm de separação entre amostras. Os espécimes serão armazenados em estufa a 37°C, e após uma semana, serão mensurada novamente para obter uma segunda massa (m2), a qual representará a massa úmida do espécime após este ter sorvido água do meio em que foi armazenado.

Em seguida, os corpos de prova serão armazenados novamente no “dessecador” com sílica, dentro de uma estufa a 37°C, a fim de uma terceira massa (m3), a qual representa a massa final do espécime após o fenômeno de solubilidade ser adquirida. A sorção de água (WS) e solubilidade (SL) serão calculados através da seguinte forma:

$$WS = (m2 – m3) / V, SL = (m1 – m3) / V,$$

Figura 6 – Corpos de prova e dessecador empregados na metodologia de sorção e solubilidade

4.3.5 Avaliação da qualidade da camada híbrida

Serão utilizados 10 dentes bovinos para a realização do teste (n=2). A superfície vestibular será removida utilizando lixas grossas sob refrigeração à água para expor a dentina superficial. Após, uma padronização será realizada utilizando lixa de carbeto de silício de granulação 600.

Os sistemas adesivos experimentais serão aplicados conforme as técnicas descritas anteriormente e o mesmo compósito restaurador será usado para realizar uma restauração de 1mm. Após a realização da restauração, um disco de dentina será sobreposto ao outro, construindo um “sanduíche de resina”. Cada bloco será armazenado em água destilada por 24 horas a 37°C. Após esse período, os blocos serão cortados no sentido mésio-distal com disco de diamante sob refrigeração à água obtendo dessa maneira 4 interfaces por dente. Estas interfaces serão incluídas em resina epóxica e, após a sua polimerização, será realizado acabamento com lixa de carbeto de silício de granulação 600 para remover todas as irregularidades presentes. Para o polimento serão utilizadas lixas de carbeto de silício de granulação 1200, 1500, 2000 e 2500 com pastas suspendidas de diamante com granulação decrescente de 3,0; 0,25 e 0,10µm (Arotec Ind. e Co, Granja Viana, SP, Brasil). A cada etapa, os espécimes serão limpos em água deionizada sob ultrassom durante 10min.

Após as etapas de acabamento e polimento, as interfaces serão desmineralizadas com solução de ácido fosfórico a 50% por 5-6s e posteriormente, serão lavadas com água deionizada e desproteinizadas em

hipoclorito de sódio a 2,5% por 10min. As interfaces serão submetidas à limpeza em água sob ultrassom por 10min e, em seguida, elas serão imersas em 20 mL de álcool a 100% por 10min e desidratados em sílica gel por 2h.

Por fim, os espécimes serão montados sobre os *stubs* e metalizados com liga de ouro-paládio para observação em microscopia eletrônica de varredura (SHIMADZU SSX550, Scanning Electron Microscope, Tóquio, Japão) (Fig. 7), operando no modo alto vácuo e utilizando detector de elétrons secundários.

Figura 7 – Aparelho usado para a realização da caracterização da camada híbrida, microscópio eletrônico de varredura

4.3.6 Envelhecimento/ tempo de prateleira (shelf-life)

Os sistemas adesivos serão transferidos para frascos plásticos impermeáveis à luz e armazenados com o intuito de promover o envelhecimento acelerado. Serão armazenados em uma estufa com umidade de 50% e temperatura de 40ºC por até 12 semanas. Decorrido este prazo, será realizado o protocolo para preparo de amostras e ensaio de resistência de união conforme descrito anteriormente para o grupo testado após 24h.

4.4 Ensaios Biológicos

4.4.1 Ensaios de citotoxicidade

O ensaio de citotoxicidade será desenvolvido no Laboratório de Biologia Celular e Molecular da Faculdade de odontologia da UFPel.

As resinas adesivas experimentais, o AD-50 (controle negativo) e a referência comercial serão suspensos em meio de Eagle modificado por Dulbecco (DMEM), em concentrações de 0.001 a 10mM. As resinas adesivas serão testadas na forma polimerizada.

4.4.1.1 Cultivo celular

Uma linhagem celular de odontoblastos MDPC 23 será utilizada no estudo. O meio de cultura celular utilizado será DMEM suplementado com 10% de soro fetal bovino (SFB), 2% L-glutamina, penicilina (100U/ml) e estreptomicina (100mg/ml). Em cada poço teste de uma placa de 96 poços serão colocados $2x10^4$ células em 200µl de DMEM acrescido de 10% de SFB. A placa será incubada em uma estufa de CO_2 com controle de temperatura e pressão, em ambiente úmido a 37ºC, 95% de ar e 5% de CO_2 por 24h de forma a permitir a adesão das células no fundo da placa de cultivo.

4.4.1.2 Teste de Citotoxicidade (Ensaio MTT)

A viabilidade celular será avaliada pelo ensaio colorimétrico MTT, o qual se baseia na capacidade das células viáveis reduzirem metabolicamente o MTT (brometo de 3-(4,5-dimetiltiazol-2-ilo)-2,5-difeniltetrazólio) por meio da enzima mitocondrial desidrogenase succínica em um cristal de formazan de cor azul 29 púrpura que se acumula no citoplasma celular. Uma vez que os produtos estiverem nos poços testes, a placa será incubada (37ºC, 5% de CO_2) por um período de 24h permitindo que os produtos atuem na monocamada celular. Após esse período, o meio será sugado e será preparado 2ml de solução de MTT, os quais serão adicionados em cada poço teste (20µl por poço) e incubados novamente por 24h de forma a permitir o metabolismo do MTT. Passado o período, o meio será sugado e o formazan ressuspendido em 200µl de dimetil sulfóxido (DMSO).

Os resultados serão lidos em um espectrofotômetro com um comprimento de onda de 560nm, onde serão considerados os valores de absorbância como indicador da viabilidade celular (FERNANDEZ et al., 2010; KOULAOUZIDOU et al., 2009).

4.4.2 Efeito antibacteriano do adesivo em modelo de microcosmos

4.4.2.1 Coleta da saliva

A saliva coletada será de um voluntário adulto, saudável e que não esteve sob terapia antibiótica por 1 ano. O voluntário não sofrerá nenhum dano e após a coleta poderá realizar sua higiene oral, sendo orientado pelo pesquisador. O voluntário receberá um termo de consentimento livre e esclarecido (Apêndice 1), o qual explicará e esclarecerá o objetivo do trabalho.

Através de filme de parafina (Parafilm "M"®, American National CanTM, Chicago, IL, EUA) será realizada a estimulação da saliva. O doador suspenderá a higiene oral por 24h previamente às coletas, que serão realizados no período matutino (em jejum). A saliva será depositada em um coletor graduado estéril e transportada em ambiente refrigerado ao Laboratório de Microbiologia (FO-UFPel). Uma alíquota de saliva (0,5mL) será separada para quantificação microbiana (UFC/mL). O restante será homogeneizado em agitador de tubos tipo Vortex e imediatamente utilizado como inóculo.

4.4.2.2 Confecção de saliva artificial – DMM

A obtenção do meio DMM (meio definido enriquecido com mucina) será realizada conforme protocolo descrito por Wong & Sissons (2001), o qual contém mucina gástrica de suíno (2,5g/l), uréia (1.0mmol/l), sais (em mmol/l: de $CaCl_2$, 1,0; $MgCl_2$, 0,2; KH_2PO_4, 3,5; K_2HPO_4, 1,5; NaCl, 10,0; KCl, 15,0; NH_4Cl, 2,0), mistura de 21 aminoácidos livres, 17 vitaminas e fatores de crescimento. O meio contém aminoácidos para o equivalente em proteína/ peptídeo (em mmol/l) em concentrações baseadas nas da saliva humana: alanina (1,95), arginina (1,30), asparagina (1,73), ácido aspártico (1,52), cisteína (0,05), ácido glutâmico (5,41 mmol/l), glutamina (3,03), glicina (1,95), histidina (1,08), isoleucina (2,38), leucina (3,68), serina (3,46), treotonina (1,08), triptofano (0,43), tirosina (2,17), valina (2,38), e caseína (5,0g/l).

4.4.2.3 Protocolo de obtenção e crescimento dos biofilmes

Cinqüenta dentes bovinos (n=10) serão desgastados para obter um cilindro de esmalte e dentina através de uma furadeira industrial com broca de núcleo de diamante (tipo trefina) (Fig. 8). Após, a porção correspondente ao esmalte será desgastada até a dentina em uma politriz (Aropol-E, Arotec, Brasil) com lixa de 600. Os espécimes serão armazenados em água destilada em um recipiente para serem esterilizados em radiação gama 4080 em um período de aproximadamente 5min.

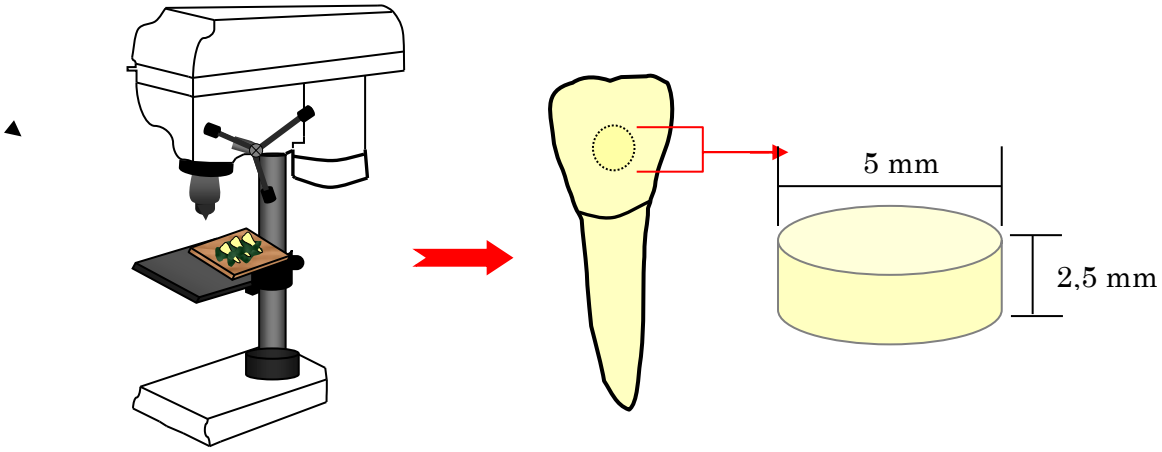


Figura 8 – Furadeira industrial com broca de núcleo de diamante utilizada para obter discos de esmalte/dentina.

Após esse período, sobre os discos de dentina será aplicado o sistema adesivo experimental e fotopolimerizado com um aparelho LED (Radii, SDI,Austrália) com intensidade de 1400mW/cm^2. Será inoculada a saliva em um volume de 400µL por micro-poço (24 poços). Após 1h em repouso a 37°C, será adicionado 1,8mL de meio DMM em cada micro-poço, com sacarose em concentração de 1%, permanecendo por um período de 6 horas. Após esse período, os espécimes serão transferidos para outros micro-poços com DMM sem sacarose, que permanecerão por 18 horas. O modelo usado será de regime semi-dinâmico. Os biofilmes serão formados independentemente sobre os discos de dentina suspensos através de dispositivos de fio ortodôntico, em cada micro-poço. As placas serão incubadas em condição atmosférica de anaerobiose (5-10% CO_2 e menos que 1% O2) em jarras (Probac do Brasil

produtos Bacteriológicos Ltda., Santa Cecília, SP) com geradores de anaerobiose (Anaerobac - Probac) sob temperatura controlada (37ºC), e mantida em repouso na incubadora. Os meios, DMM com e sem adição de sacarose, serão renovados diariamente durante 3 dias. Após o período de desafio cariogênico será realizado o peso seco. Será removido o biofilme dos discos de dentina e serão colocados em um *eppendorf*, o qual foi pesado anteriormente. O eppendorf com o biofilme será pesado e os valores serão transferidos para uma tabela no programa Excel, o qual irá calcular a quantidade de salina que será adicionada conforme a quantidade de biofilme, ou seja, 1mg de biofilme é igual a 1 ml de salina. Após os *eppendorf* serão sonificados (LI et al., 2009).

4.4.2.4 Viabilidade bacteriana

Será realizada diluição seriada das suspensões de biofilme para contagem de microrganismos totais, acidúricos, *Estreptococcus mutans* e lactobacilos, obtendo diluições de 10x (1), 100x (2), 1000x (3), 10000x (4), 100000x (5), 1000000x (6) e 10000000x (7) e imediatamente inoculadas em duplicata nos seguintes meios de cultura: Ágar sangue para microrganismos totais; Ágar mitis salivarius com 0,2 unidades de bacitracina/mL (MSB), para quantificação de *Estreptococcus mutans*; Ágar Rogosa SL para lactobacilos; e BHI com pH ajustado a 4,7 para quantificação de microorganismos totais acidúricos. As placas serão incubadas em condição de anaerobiose (80% N_2, 10% CO_2 e 10% H_2), a 37ºC por 96h. Após o período de incubação, será realizada a contagem das Unidades Formadoras de Colônias (UFC). Para estimar as UFC será aplicada a seguinte fórmula:

$$[UFC \times 1000/20] \times diluição$$

4.5 Análise estatística

Para a realização da análise estatística, o método escolhido será feito a partir da obtenção dos resultados.

O método estatístico mais apropriado será escolhido com base na aderência ao modelo de distribuição normal e igualdade de variâncias. Para todos os testes será considerado o valor $p<0,05$ como estatisticamente significante. Para a realização da análise estatística, será utilizado o programa estatístico SigmaStat 3.01.

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Orçamento

Os recursos para adquirir os materiais necessários descritos abaixo serão financiados pelo Edital 2013/01 PQG-FAPERGS (R$ 24.759,11) e pelo PROAP (R$ 1500,00).

Descrição	Custo unitário $RS	Custo total $RS
luvas para procedimento	18,00	36,00
Touca	8,1	8,1
cera pegajosa em bastão	25,00	125,00
compósito restaurador	89,00	890,00
microbrush	14,00	56,00
tiras de poliéster	4,8	4,8
cola cianocrilato	4,95	4,95
lixas para desgaste	1,00	100,00
Resina epóxica	100,00	100,00
meio ágar BHI	155.05	155.05
placas de cultura de 96 poços	4.5	45,00
100 dentes bovinos	30,00	30,00
placa p/ cult 24 poços	5,49	247,05
ponteira neutra 0,5 a 10ul c/1000 t-300 axygen	50,60	50,60
ponteira amarela 1-200ul c/1000 t-200y axygen	50,60	50,60
tubo eppendorf cap 1,5ml c/1000 unidades	29,90	29,90
tubo eppendorf cap.2,0ml c/1000 unidades	34,80	34,80
anaerobac c/10 – probac	101,40	405,60
placa petri desc est	2,98	104,30
agar sangue nr.2 c/500gr	24,35	121,75
agar mitis salivarius c/ 500g, acumedia	360,30	360,30
rogosa sl agar c/ 500g	1.253,00	1.253,00
reagentes para confecção de DMM	4610,00	4610,00
canforoquinona	410,00	410,00
EDAB	300,00	300,00
Etanol	10,00	10,00
Bis-GMA	350,00	350,00
Bis-EMA 10	350,00	350,00
TEGDMA	350,00	350,00
HEMA	350,00	350,00
GDMA	350,00	350,00
metacrilatos-exportação	280.00	1000
despesas com impressão		500,00
congressos		1000,00
total		13.792,80

Cronograma

Ano	Mês	Revisão de Literautura	Revisão sistemática	Qualificação	Espera dos materiais	Testes físico-mecânicos	Testes biológicos	Dissertação da tese	Defesa e submissão de artigos
2013	Março	X			x				
	Abril	X			x				
	Maio	X			x				
	Junho	X			x				
	Julho	X			x				
	Agosto	X	x	x	x				
	Setembro	X	x		x				
	Outubro	X				x			
	Novembro	X				x			
	Dezembro	X				x			
2014	Janeiro	X				x			
	Fevereiro	X							
	Março	X					x		
	Abril	X					x		
	Maio						x		

	Junho						**x**		
	Julho							**x**	
	Agosto							**x**	
	Setembro							**x**	
	Outubro								**x**
	Novembro								**x**
	Dezembro								

Apêndice

Apêndice 1.

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE)

Convidamos o (a) Sr (a) para participar da Pesquisa "**Caracterização físico-mecânica e atividade antibacteriana de resinas adesivas contendo grupamentos metálicos**", sob a responsabilidade da pesquisadora Alexandra Rubin Cocco, a qual pretende avaliar o efeito antibacteriano de sistemas adesivos experimentais. Sua participação é voluntária e se dará por meio de doação de saliva na Faculdade de Odontologia da UFPel. Os procedimentos realizados durante a pesquisa não oferecem riscos à sua saúde. Se depois de consentir em sua participação o (a) Sr (a) desistir de continuar participando, tem o direito e a liberdade de retirar seu consentimento em qualquer fase da pesquisa, seja antes ou depois da coleta dos dados, independente do motivo e sem nenhum prejuízo a sua pessoa. O (a) Sr (a) não terá nenhuma despesa e também não receberá nenhuma remuneração. Os resultados da pesquisa serão analisados e publicados, mas sua identidade não será divulgada, sendo guardada em sigilo.

Consentimento Pós–Informação

Eu,_____,
RG nº_____ fui informado sobre o que o pesquisador quer fazer e porque precisa da minha colaboração, e entendi a explicação. Por isso, eu concordo em participar do projeto, sabendo que não vou ganhar nada e que posso sair quando quiser.

Este documento será revisado para aprovação pelo Comitê de Ética em Pesquisa desta Instituição de atenção à saúde.

Data __/__/____.

_____

Nome e assinatura

3. Relatório do trabalho de campo

Neste capítulo estão relatadas as complementações e alterações baseadas no Projeto de Pesquisa aprovado no exame de qualificação.

O projeto inicialmente submetido à qualificação junto ao Programa de Pós-Graduação em Odontologia consistiu no trabalho intitulado: "Caracterização físico-mecânica de blendas monoméricas contendo metacrilatos com grupamentos metálicos e avaliação da atividade antibacteriana".

Foram aceitas as sugestões da banca de qualificação do projeto e as modificações foram realizadas. O título foi modificado para "Caracterização Físico-Mecânica e Atividade Antibacteriana De Resinas Adesivas Contendo Grupamentos Metálicos" e na introdução, alguns parágrafos foram alterados, pois estavam sem conexão. Além disso, na revisão sistemática, a qual inicialmente seria sobre metacrilatos metálicos incorporados em sistemas adesivos, foi sugerido pela banca ampliar o assunto e fazer sobre monômeros antibacterianos incorporados em sistemas adesivos. Ao redigir o artigo, algumas complementações foram necessárias para torná-lo mais interessante. Entre as alterações, o acréscimo de patentes sobre o assunto, as inovações do setor e prospectivas futuras foram adicionados.

O primeiro problema enfrentado durante a execução do projeto completo foi decorrente ao atraso do material (metacrilatos), os quais foram importados da Alemanha. Os materiais chegaram em maio de 2014: metacrilato de zinco, metacrilato de prata, metacrilato de estanho, metacrilato de cobre, metacrilato di-n-butildimetacrilato de estanho, metacrilato de ferro, dimetacrilato de zircônia, metacrilato de sal de cromo ácido, metacrilato de níquel e metacrilato de cálcio hidratado. O segundo problema enfrentando foi que o metacrilato de alumínio não foi encomendado.

O objetivo principal do trabalho é verificar a ação antibacteriana dos metacrilatos. Para isso, foi realizado um *screening* de todos os metacrilatos nas concentrações: 0,5%, 1% e 2%. Concentrações maiores como 5% ou 10% não foram realizadas, pois estudos prévios indicam que poderiam alterar as propriedades mecânicas (baseado no trabalho de mestrado da aluna Sandrina

Henn, Henn et al. 2011). O teste de halo de inibição mostrou que apenas o metacrilato de prata e o metacrilato de estanho, nessas concentrações, tiveram halo de inibição. Assim, o projeto foi alterado em relação aos materiais. Além disso, o sistema adesivo comercial Clearfil Protect Bond® seria usado como controle, porém sua compra foi dificultada devido a esse adesivo não ser comercializado no Brasil. A compra foi realizada através de terceiros, porém não tem previsão de chegada. Devido ao prazo, resolvemos não esperar o adesivo para começar as metodologias, ficando assim sem um referência comercial.

Outro objetivo do presente trabalho era usar um sistema adesivo livre do monômero HEMA. Este monômero seria substituído pelo Bis-HEMA 10. Ao realizar os testes físico-mecânicos, valores baixos de resistência de união à microtração foram encontrados. Várias tentativas foram feitas, adicionando e removendo monômeros. Desse modo, um sistema adesivo autocondicionante de 2 passos já estabelecido e utilizado no CDC-Bio foi empregado para realização deste trabalho.

Além disso, o teste de citotoxicidade foi realizado com WST-1 ao invés do MTT. Apesar de ser mais caro, ele apresenta vantagens em relação ao MTT por ser em solução e mais rápido de fazer a leitura das placas. Ademais, algumas metodologias não foram realizadas (sorção e solubilidade e envelhecimento) devido ao prazo de defesa. E o teste de avaliação da camada híbrida não pode ser realizado devido problemas no equipamento MEV local, o qual está estragado. Devido ao prazo, essas análises adicionais deverão ser posteriormente realizadas. Surgiu a oportunidade de realizar o teste na cidade de Rio Grande-RS, porém decidimos não fazer devido ao prazo e achamos que os testes realizados eram suficientes para obter um artigo de bom nível.

Artigo 1*

*Artigo formatado conforme as instruções para autores do periódico Biofouling
http://www.tandfonline.com/loi/gbif20#.VMvNHdLF91Y
acesso em 30/01/2015

A systematic review e rastreamento tecnologico about antibacterial monomers used in dental adhesive systems: current status and further prospects

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Abstract

This study systematically review the literature to assess the effectiveness of antibacterial monomers incorporated into dental adhesive systems; as well as the research advances and the future prospects of this technology. The following 7 databases were screened. The online system Questel Orbit (Paris, France) was accessed to obtain patent data. The inclusion criteria were articles and patents that investigated the antimicrobial activity of antibacterial monomers in adhesive systems; and only documents in the English, Spanish or Portuguese languages were sought. After screening, 33 studies and 9 patents were included. All studies reported the inclusion of antimicrobial monomers to be an effective dental treatment strategy. MDPB was the only antimicrobial monomer incorporated into a commercially available adhesive system, Clearfil Protect Bond™. Although there is evidence of antibacterial activity from *in vitro* studies, clinical studies must be conducted to confirm the effectiveness of these materials in the prevention of dental pathologies.

Keywords: antibacterial effects; antibacterial monomer; bacteria; dental adhesive

Introduction

Nowadays, there is a trend to use the minimally invasive dentistry to restaurant treatments approach to promote preservation of the tooth structure with conservative techniques in an effort to avoid damage to the dental pulp complex (Cheng et al. 2013). Incomplete removal of infected dentin is currently recommended, especially in clinical situations of deep carious lesions. However, the viable bacteria that may be left in the dentin after restorative treatment continues to be a clinical concern buscar referência atual

Restorations performed with composites accumulate more biofilm and are subject to faster degradation than those made with other materials (Zalkind et al. 1998, Beyth et al. 2007, Li, Weir, Chen, et al. 2014), such as ceramics and glass ionomer cements (Barbosa et al. 2012). This can lead to the formation of secondary caries, damage to the pulp and consequently lead to restoration failure (Mjor et al. 2000). Furthermore, polymerization shrinkage can result in the formation of gaps between the adhesive resin and the primed dentin, or between the adhesive resin and the hybrid layer (Turkun et al. 2006), which can lead to penetration of bacteria that cause secondary caries (Cenci et al. 2008).

Several studies (Feuerstein et al. 2007, Gondim et al. 2008, Esteves et al. 2010,) indicated that self-etching adhesive systems containing acidic monomers with lower pH in their formulation, may have antibacterial effect. However, this effect is limited to 24 or 48 hours (Feuerstein et al 2007). Therefore, materials with antibacterial effect lasting for a longer time were developed: the antibacterial-agent-releasing and non-antibacterial-agent-releasing materials (Leung et al. 2005). The incorporation of chlorhexidine, fluoride and silver particles are considered antibacterial-agent-releasing materials, with their effect attributed to the release of antibacterial products (Leung et al1980). Substances presented in this material are only simply dispersed in the matrix phase, and it is impossible to control the kinetics of release. Consequently, antibacterial activity decreased over the course of time. Moreover, release of the agent may adversely influence the physical properties and result in toxic effects (Ribeiro and Ericson 1991, Kurata et al. 2011,).

To overcome the disadvantages of these products, researchers have attempted to develop non-antibacterial-agent-releasing materials. The current

trend involves the development of monomers with quarternary ammonium salts (Li et al.2014), which present relatively low toxicity and a broad antimicrobial spectrum (Xiaoet al. 2008). It is reported that the quarternary ammonium (QA) is bactericidal due to three possible processes: 1.Contact of the negatively charged bacterial and positively charged QA, resulting in osmotic pressure; 2.Diffusion through the cell wall and binding to the cytoplasmic membrane; and 3.Disruption of the cytoplasmic membrane, release of cytoplasmic constituents and cell death (Figure 1.) (Guiqian Lu 2007, He et al. 2011, Xu et al. 2012).

Quarternary ammonium salts are able to copolymerize with other methacrylate monomers and could provide long-term antibacterial activity (Cheng, Weir, Zhang, Arola, Zhou and Xu). The following monomers with QA incorporated have been synthesized**:** 2-dimethyl-2-dodecyl-1-methacryloxyethyl ammonium iodine (DDMAI); 2-methacryloyloxyethyl dimethylammonium (IDMA1); 2,2-bis(methacryloxyloxyethyl dimethylammonium) (IDMA2); dimethyl amino dodecylmethacrylate (DMADDM)**;** dimethylamino hexylmethacrylate (DMAHM); methacryloxyl ethylcetyldimethylammonium chloride (DMAE-CB); and the compound 12-methacryloyloxy dodecypyridinium bromide (MDPB). MDPB was the first antibacterial monomer incorporated into a commercially available adhesive system Clearfil Protect Bond^{TM} (Kuraray Co. Ltd., Japan) (Imazato et al. 1995, Imazato et al. 1999).

There is a trend towards developing dental materials with antibacterial activity; however their effectiveness in the reduction of oral bacteria has not been completely elucidated. Therefore, the aim of this systematic review was to evaluate the antimicrobial activity of dental antibacterial adhesive systems; as well as the advances in research, and future prospects for the development of antimicrobial dental materials.

Materials and methods

Electronic searches

This systematic review is described according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al. 2009). The literature search was carried out by two independent reviewers (ARC and WLOR) until 25th September of 2014. The following seven databases were screened: *MedLine (PubMed), Lilacs, Ibecs, Web of Science, Scopus,*

Scielo, and *The Cochrane Library*. Moreover, the online system Questel Orbit (Paris, France) was accessed to recover patent documents related to antibacterial monomers in dental adhesive systems. This system allows the patent search and analyzes over 90 authorities. The search strategy is described in Table 1. The references cited in the articles included were also checked. After the identification of articles in all databases, they were imported to the software Endnote X7 (Thompson Reuters, Philadelphia, PA, USA) to remove duplicates.

Screening and study selection

All titles and abstracts of articles and patents initially found were analyzed and selected in accordance with the eligibility criteria (Figure 2.). There was a limit of language (English, Portuguese and Spanish), but no limit on year of publication was applied. Reference lists of studies included were hand searched for additional articles. Full copies of all potentially relevant studies were identified. Those that appeared to meet the inclusion criteria, or for which there were insufficient data in the title and abstract to make a clear decision, were selected for full analysis. The full text papers and patents were assessed independently and in duplicate by two reviewers (Figure 3). Any disagreement on the eligibility of studies included was resolved through discussion and consensus, and in case of disagreement, a third reviewer (RGL) decided whether or not the article should be included.

Data extraction

Items of scientific and technological information were tabulated and analyzed by the software program Microsoft Office Excel 2013 (Microsoft Corporation, Redmond, Washington, USA). In addition, the search for and analysis of patent applications was conducted by the online system Questel Orbit (Paris, France). Two reviewers (WLOR and ARC), who received training in the use of these software programs conducted the analyses independently. If there were some items of information missing, the authors of these papers were contacted via e-mail to retrieve any missing data. If no answer was received within 2 weeks after the first e-mail message was sent, then a second e-mail

was forwarded. If authors had given no answer one month after the first contact, the missing information was not included.

Reviewers tabulated data of interest from the documents included, containing: the demographic data (year, country), the applicant or author's names, the inventor's name, the types of dental adhesives used, priority countries and the type of study conducted. Furthermore, data referring to monomers and microbiological method used, main findings of studies included were also extracted (Table 2). Due to the high degree of heterogeneity in terms of different studies and methodologies, it was considered inappropriate to conduct a meta-analysis.

Assessment of Risk of Bias to articles

The methodological quality of each included study was independently assessed by the two reviewers adapted from other systematic review of in vitro studies (Sarkis-Onofre, 2014)

Risk of bias was assessed according to the description of the methodology of articles as regards the following items: blinding of the operator, sample size calculation, positive control, negative control, use of more than one microorganism, description of components of the experimental and commercial adhesive. If the writer reported these items, the article received “Y” (yes). If it was not possible to find the information, the article received “N” (not). Articles reporting one item were classified as having high risk of bias, two or three items as medium risk of bias, and four or five items as low risk of bias, as shown in Appendix 1.

Results

Study selection

The last electronic search was conducted on September 25th, 2014. Figure 3 is a flowchart that summarizes the article and patent selection process. Of the 1341 articles initially recovered from all databases, 1043 articles were excluded because they were not related to antibacterial monomers incorporated into adhesives. Twenty-six studies were excluded because they did not satisfy the selection criteria: 3 tested properties other than monomers (Chai et al. 2011, Imazato et al. 2008, Li et al. 2013), 6 had no antibacterial monomer (Al-

Musallam et al. 2006, Cehreli et al. 2003, Mehdawi et al. 2009, Ohmori et al. 1999, Paradella et al. 2009, Walter et al. 2007), 13 did not use monomers in adhesive system (Bakhshi et al. 2013, He, Soderling, Osterblad, Vallittu and Lassila 2011, Huang et al. 2011, Imazato, Ebi, Tarumi, Russell, Kaneko and Ebisu 1999, Imazato and McCabe 1994, Imazato, Russell and McCabe 1995, Imazato et al. 1994, Izutani et al. 2011, Izutani et al. 2010, Kurata, Hamada, Kanazawa and Endo 2011, Li et al. 2011, Xiao, Chen, Fang, Xing, Wang, Wang and Li 2008, Xu, Wang, Liao, Wen and Fan 2012), 1 studied was published in the Polish language (Ã‡ehreli et al. 2003), there were 2 studies with missing data (Li, Chen, et al. 2009, Xiao, Chen, Fang, Xing, Li, et al. 2008) and 1 review study (Imazato 2009).

In the patent database, the search strategy initially retrieved 240 patents, with 200 being excluded after reading the title and abstract (Figure 3) since they were not related to antibacterial monomers. Of the remaining 38 patents, 29 patents were excluded: 26 had no antibacterial monomer, 2 were written in Japanese and 1 document was related to a method for preparing antibacterial materials. A total of 9 patents were included in the analysis (Table 3).

Study characteristics

Table 2 shows the demographic data from articles considered in this study. All articles were published between 1997 and 2014. The majority of monomers had QA incorporated. Figure 4 shows the different types of monomers with their molecular formula. The efficacy of MDPB monomer was evaluated in 23 articles. Only 2 studies were *in vivo* and one *in situ*. All *in vitro* studies demonstrated reduction of bacterial activity with the incorporation of antibacterial monomers. Moreover, seven studies presented high risk of bias. Out of all the studies included, only one (Gondim, Duque, Hebling and Giro 2008) presented low risk of bias (Appendix 1).

With regard to adhesive systems, Clearfil Protect Bond™ was the only one that MDPB monomer incorporated into the commercial presentation. This material was tested in 23 articles (Table 2). Other studies synthesized monomers similar to MDPB, such as DMAE-CB, IDMA-1 and IDMA-2, DDMAI, DMADDM and DMAHM. These monomers were added in experimental (42% of studies) and commercial (58%) adhesives systems. As regards patent

documents, Table 3 shows data of 9 patents deposited from 1994 to 2012. Antibacterial agents such as QA, MDPB, DMAHM and DMADDM were found in these documents, which claimed their incorporation into adhesives systems, as well as into dental materials for prosthesis and operative dentistry (i.e. adhesive compositions, adhesive primers, dental cements, composite resins).

Discussion

Effectiveness of antibacterial monomers in dental adhesives system

Through this systematic review it was possible to demonstrate that incorporation of antibacterial monomers into adhesives system could be beneficial to reduce biofilm accumulation. It is hypothesized that dental adhesive systems with these substances could minimize or reduce the progression of dental caries. However, other factors must be taken into consideration to determine the clinical success of these adhesives, such as different oral hygiene habits of patients in order to control biofilms, presence of microgaps in the restoration and patient's caries risk (Demarco et al. 2012). Therefore, the clinical effectiveness of these materials needs to be further studied, since few clinical study were conducted to confirm the antibacterial effect of these substances.

Among the studies included in this review, two were conducted *in vivo* (Imazato et al. 2004, Uysal et al. 2011) and one *in situ* (da Silva et al. 2010). The monomer incorporated MDPB into the experimental primer, and exhibited antibacterial effect on infected cavities in dog's teeth, suggesting a possible clinical benefit (Imazato et al.2004). Moreover, the commercial adhesive Clearfil Protect Bond™ (Kuraray Co. Ltd., Japan), which contains MDPB, reduced enamel demineralization around orthodontic brackets, with a significant effect after 30 days (Uysal, Amasyali, Ozcan, Koyuturk and Sagdic 2011). However, the only *in situ* study with these commercial adhesive systems, demonstrated that none of the antibacterial materials tested reduced caries formation in dentin (da Silva, Franca, Florio and Basting 2010). All the other studies were *in vitro* and the majority of them used the agar diffusion method in their methodology (12 studies). However, the current trend is the use of the microcosm model, because it offers the advantage of coming closer to the physical-chemical,

microbiological and nutrient conditions, in addition to maintaining the complexity and heterogeneity of *in vivo* plaques (McBain 2009).

All *in vitro* studies showed a positive antibacterial effect of these monomers. However, the evidence of the results must be considered with caution because the majority of studies were classified as high or medium risk of bias. Many methodologies need to evaluate the antibacterial activity in longer periods, and in animal models to confirm the longevity of this effect. Furthermore, some studies only assessed the antibacterial activity against *Streptococcus mutans* (Imazato et al.1999, Kuramoto et al. 2005, Li, Chai, et al. 2009) or *Enterococcus faecalis* (Imazato et al. 2006). It is imperative to evaluate this effect with other microorganisms (i.e. *Lactobacillos sp.)* that are involved in the dental caries process.

The main antibacterial substance incorporated into the monomers of adhesive systems was QAS. MDPB was the most studied monomer (23 articles), and it is a compound of QA and a methacrylate group. In an unpolymerized state, this monomer acts only as a disinfectant. When the material is polymerized, the antibacterial agent is immobilized in the polymer matrix by the copolymerization of MDPB with other monomers, and inhibits the growth of the bacteria in contact with it (Izutani et al.2011). Another property of MDPB that was evaluated was cytotoxicity, which demonstrated that the unpolymerized monomer with 40 $\mu g ml^{-1}$ was cytotoxic to cell viability (Imazato et al.1999). Other agents also inhibited bacterial growth in a manner similar to that of MDPB, such as the monomer IDMA-1 incorporated into experimental adhesive (Antonucci et al. 2012). Moreover, among the studies included, MDPB and DDMAI were shown to have an inhibitory effect against *Streptococcus mutans,* a well-known microorganism involved in dental caries (He et al. 2012, He et al. 2013, Imazatoet al. 2012). MDPB also presented an antibacterial activity against many other oral microorganisms, such as the facultative anaerobic bacteria *Lactobacillus acidophilus* and *Lactobacillus casei* (Korkmaz et al. 2008), *Actinomyces sp.* and *Candida albicans* (Yoshikawa et al. 2007). Other studies showed that the Clearfil Protect Bond™ did not present an antibacterial effect after 14 days (Feuerstein et al. 2007, Polydorou et al. 2013). Among the other monomers, 3 % DMAE-CB was demonstrated to have antibacterial activity at least 6 months after the adhesive was polymerized *in*

vitro (Xiao et al. 2009). However, this monomer showed cytotoxicity with LC_{50} (lethal concentration, 50 %) between 2-5 µg $mL^{-1.}$ The eluents of adhesive with polymerized DMAE-CB-incorporated, did not exhibit stronger cytotoxicity than the eluent of its parent adhesive (Chai et al.2011). Furthermore, IDMA-1 also showed cytotoxicity above 20 % (Antonucci et al. 2011); and for DMADDM the median lethal concentration of LC_{50} was between 20-40 µg mL^{-1} (Li et al. 2013). In the development of dental materials, it is important to correlate the antibacterial potential of monomers with cytotoxicity and other physical and mechanical properties of the adhesive system (i.e. degree of conversion, bond strength).

Adhesive systems containing antibacterial agents have shown an important effect on the reduction of bacterial activity *in vitro* (Table 2). With data obtained *in vivo* it has been demonstrated that the presence of antibacterial agents could help to reduce demineralization around brackets or adjacent restorations (Uysal et al. 2010). These advances in research may be important to control demineralization, since fixed orthodontic appliances are sites that allow plaque accumulation, and favor colonization by cariogenic bacteria, such as *Streptococcus mutans* and *Lactobacillus sp.*, which can also cause periodontal complications (Imazato et al. 2007). These materials could have a similar effect to that of fluoride released from dentifrices or other materials, such as glass ionomer cements, capable of reducing demineralization (Cenci et al.2008).

The antibacterial monomers were incorporated into commercial (58 %) and experimental (42 %) adhesives that demonstrated compatibility with other monomers used. The majority of monomers tested did not affect the material properties (Imazato et al.2009, Zhanget al. 2013), however in some studies a reduction of mechanical properties was observed, due to the large amount of incorporated monomer (He et al. 2013). They were able to inactivate oral cavity bacteria *in vitro* (Esteveset al. 2005, da Silva et al.2010,), however there are no longitudinal clinical studies that confirm this antibacterial effect on the control of secondary caries. Probably these products would have no effects on controlling the progression of these lesions, since it has been demonstrated that secondary caries is related to poor oral hygiene rather than to microleakage (Cenci et

al.2008). Without adequate plaque control, these monomers would have little effect on decreasing caries progression.

Future prospects for antibacterial agents in dentistry

The release of antibacterial particles in system adhesives could be improved with delivery systems in the future, as exemplified in Figure 5 with nanofibers, nanospheres or nanotubes (Jie-Xin Wang 2006). The immobilization of this particles in porous hosts can delay the release time for longer periods (Toshikazu 1999). One study found that silver nanoparticles showed better dispersion and antibacterial ability when immobilized in hollow silica tubes in comparison with hollow silica spheres (Jie-Xin Wang 2006). Furthermore, the current trend involves the incorporation of particles (i.e. silver) and monomer antibacterial into dental adhesive systems to improve the antibacterial activity (Cheng, et al. 2013, Zhang, et al. 2014). Some recent studies have shown that the use of nano-silver together with an antibacterial monomer increased the antibacterial activity and did not interfere in the mechanical properties (Cheng, et al. 2013, Zhang, et al. 2013). Nano-silver complemented the effect of MDPB, since MDPB inhibits bacterial growth on contact, and nano-silver at long-distance (Zhang et al. 2013). Another study showed that when DMADDM was combined with silver particles, it also provided satisfactory results with respect to antibacterial effect (Cheng, et al. 2013).

Silver is well known for its low toxicity and good biocompatibility with human cells. It also has antibacterial, antifungal and antiviral activity (Monteiro et al. 2009, Allaker 2010,). Therefore, silver particles have been incorporated into dental materials to improve the antibacterial activity (Imazato, Ebi, et al. 2003, Li, Weir, Fouad, et al. 2014) or to potentiate this effect (Cheng et al.2013). The incorporation of silver into adhesive systems demonstrated low biofilm viability and metabolic activity, and not affect color and mechanic proprieties (Cheng et al. 2013). Silver can be incorporated as nanoparticles or microparticles, in a concentration of 0.1 % to 1 % into primers or bond (Melo et al. 2013, Zhang, Cheng, et al. 2013). It has been reported that the antibacterial mechanism of silver ions is the inactivation of vital bacterial enzymes, causing the loss of DNA ability to replicate and leading to cell death (Morones et al. 2005).

Other types of particles, such as calcium phosphate have been incorporated into adhesive system with the objective of preventing caries and achieving tooth remineralization. These components were tested with silver particles and antibacterial monomers (DMADDM), and demonstrated antibacterial effects that were maintained for 6 months (Zhang, Li, et al. 2013).

Other monomers, such as metal methacrylates (i.e. zinc methacrylate) have recently been incorporated into these materials, and zinc showed strong antibacterial activity (Henn et al. 2011). In addition, plant extracts and essential oils isolated from medicinal plants have also shown biological activity *in vitro* (Peralta et al. 2013). Furthermore, butiá oil has been incorporated into adhesive systems and showed antimicrobial effect in the microcosm model (Peralta,et al. 2013). They may have a potential use in future commercial dental adhesive systems.

By means of this review it was possible to obtain a scientific and technological overview of the field of monomer antibacterial effectiveness. With regard to the technological protection of antibacterial adhesives, only nine patents (Hidekazu 1994, Silvio 1994, Imazato 1994, Jensen 1997, Kai 1997, Kazumitsu 1998a, Kazumitsu 1998b, Jjingwei 2012, Huakun 2012, ,) that developed and/or synthesized antibacterial monomers into adhesive systems were found. The patent document is an essential source of information for technological analysis, considering the wide variety of content available only in this type of document (Mazzolenia 2005, Sampat 2006). However, there are limitations to using patent data as an indicator of technological development: not all inventions meet the patentability standards and inventors can rely on secrecy or other appropriate means to protect their inventions (Ernst 2003, Choi and Park 2009). Furthermore, there is a time lag of at least 18 months between the first patent filing and the patent publication (Choi and Park 2009). Therefore, the most recent patents included in this study were deposited up to October 2012. Moreover, each patent office uses a different tool that allows the recovery of documents, which makes it very difficult to collect and find interesting information (Barroso et al. 2009, Da Rosa et al. 2014). Therefore, there is a need for obtaining licenses to software programs that facilitate technological monitoring by institutions and companies, such as Questel Orbit (Paris, France) or Vantage Point (Search Technology, Inc., Norcross, GA, USA). By combining

and analyzing scientific and technological information, the design of this study is able to provide strategic information to drive new projects. Furthermore, the development of new monomer antibacterial could be promising, since many dental materials may benefit from the addition of an antibacterial agent in their composition, and some involve technologies with the potential for technological protection, such as endodontic cements, pit and fissure sealants, denture adhesives, and pulp capping agents.

Moreover, one limitation on the development of this technology is that some monomers with QA are not miscible with dimethacrylate diluents such as triethylene glycol dimethacrylate (TEGDMA), and only a small amount can be incorporated into the adhesive, which limits the antibacterial effect (Antonucci et al. 2011, Liang et al. 2014).Thus**,** there are potential areas to be explored with antibacterial monomers for dentistry, and their use could have important implications for future more conservative dental treatments.

Conclusions chamar mais atenção falar das pespectivas futuras

Atraves desta revisão sistemarica nota-se que New antibacterial monomers are emerging rapidly and have shown a reduction in bacterial activity *in vitro* and in the short-term. Com o rastreamento tecnologico Available data demonstrated a potential future use for antibacterial monomers em sistemas adesivos como também para outros materiais odontológicos. A combinação de monômero e partículas antibacterianos parece ser uma boa opção para obter maior efeito antibacteriano e para evitar uma rápida lixiviação dessas partículas, o uso nanotubos, naoesferas ou nanofibras poderia melhorar a durabilidade desse efeito antibacteriano.; .

Acknowledgements

The authors would like to thank to CAPES for granting a scholarship to the first author and the teacher, Izabel Rubin Cocco for help in the design of chemical formulas (Fig. 4)

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Table 1. Search strategy in PubMed (MedLine).

Search	Terms
#4	Search #1 AND #2 AND #3
#3	Anti-Infective Agents OR Agents, Anti-Infective OR Anti Infective Agents OR Antiinfective Agents OR Agents, Antiinfective OR Microbicides OR Antimicrobial Agents OR Agents, Antimicrobial OR Anti-Microbial Agents OR Agents, Anti-Microbial OR Anti Microbial Agents OR anti-Bacterial Agents OR Agents, Anti-Bacterial OR Anti Bacterial Agents OR Antibacterial Agents OR Biofilm OR Bacterial Adhesion OR Dental Deposits or Adhesion, Bacterial OR Antibacterial activity
#2	Dental Bonding OR Bonding, Dental OR Dental Bonding, Chemically-Cured OR Chemically-Cured Dental Bonding OR Dental Bonding, Chemically Cured OR Dental Bonding, Self-Cured OR Dental Bonding, Self Cured OR Self-Cured Dental Bonding OR Chemical-Curing of Dental Adhesives OR Chemical Curing of Dental Adhesives OR Dental Bonding, Dual-Cure OR Dentin-Bonding Agents OR dental primer OR Dental Materials OR Materials, Dental OR Dental Material OR Material, Dental or dental resin or Dental Resins OR Resin, Dental OR Resins, Dental OR bonding interface OR adhesive
#1	Monomers OR monomers

As estratégias de buscas em outras bases de dados foram adaptadas.

Table 2. Demographic data and main results of studies.

Author	Year	Antibacterial agent	Antibacterial monomer	Microorganisms tested	Incorporation into primer or adhesive	Enamel, dentin, resin or other specimens	Methodologies antinbacterial	Main results
Imazato, S[87]	1997	QA	MDPB	S*treptococcus mutans, Actinomyces viscosus and Lactobacillus casei*	Primer	100 µl of each primer was added to 900 µl of bacterial suspension	Agar diffusion method	MDPB into dentin primer is beneficial for providing antibacterial activity after curing
Imazato, S[56]	1998	QA	MDPB	S*treptococcus mutans*	Resin experimental	Dental resin composite disc + resin monomer paste	Agar diffusion method and inhibitory effect	It was inhibited by contact with the surface of cured MDPB-MDP-containing resin, but the bactericidal effect was small
Imazato, S[88]	1998	QA	MDPB	S*treptococcus mutans, Actinomyces viscosus and Lactobacillus casei*	Primer	Adhesive system and composite disc	Bacterial growth (colonies counting)	Incorporation of antibacterial monomer MDPB into dentin primer is beneficial for providing antibacterial activity after curing.
Imazato, S[89]	2001	QA	MDPB	*Propionibacterium acnes, Eubacterium alactolyticum, Bifidobacterium bifidum, Peptostreptococcus asaccharolyticus, Lactobacillus acidophilus, Lactobacillus aracasei* ssp*, Lactobacillus plantarum, Lactobacillus*	Primer	Dentine blocks	MIC* and MBC* determination	The incorporation of MDPB into dentin primer could be beneficial for eliminating the residual bacteria in cavities

				salivarius ssp*, Lactobacillus brevis, Lactobacillus salivalius* ssp *and Lactobacillus fermentum*				
Imazato, S[90]	2002	QA	MDPB	*Streptococcus mutans* and *Lactobacillus casei*	Primer	Dentine blocks	Bacterial growth (colony counting)	In vitro, the MDPB was capable of killing bacteria within demineralized dentin
Imazato, S[63]	2003	QA	MDPB	*Streptococcus mutans*	Primer and Adhesive	Adhesive discs	Antibacterial activity	Adhesive resin with MDPB after curing has antibacterial activity and without influencing bond strength or curing performance
Kuramoto, A[91]	2005	QA	MDPB	*Streptococcus mutans*	Primer	Premolars	Anticariogenic effect	Experimental adhesive system can inhibit the progression of root-surface caries in vitro
Lobo, M.[92]	2005	QA	MDPB	*Streptococcus mutans*	Primer	Enamel and Dentine	Anticariogenic effect	All self-etching systems tested inhibit secondary caries in vitro and adhesive system containing MDPB and fluoride reduced glucan synthesis
Turkun[65]	2005	QA	MDPB	*Streptococcus mutans*	Primer	Paper disks, Dentin disks and Tooth cavity	Agar diffusion method/ bacterial growth (colony counting)	Clearfil Protect Bond® (Kuraray Dental Inc, Kurashiki, Japan) was the most effective antibacterial material and it was able to inactivate the bacteria in the cavity
Turkun[6]	2006	QA	MDPB	*Streptococcus mutans*	Primer	Paper disks and Tooth cavity	Agar diffusion method/	Clearfil Protect Bond® (Kuraray Dental Inc, Kurashiki, Japan)was

							Bacterial growth (colony counting)	able to inactivate the bacteria in the cavity more effectively than the tested cavity disinfectants
Feuerstein[11]	2007	QA	MDPB	*Streptococcus mutans*	Primer	Direct contact of the tested material	Agar diffusion method	All the tested adhesives showed immediate antibacterial effect on SM, but none had long-lasting antibacterial properties
Gondim[12]	2008	QA	MDPB	*Streptococcus mutans* and *Lactobacillus acidophilus*	Primer	Paper discs and Dentine discs	Agar diffusion method	MDPB incorporation has effect against cariogenic bacteria
Korkmaz, Y[58]	2008	QA	MDPB	*Streptococcus mutans, Lactobacillus casei* and *Streptococcus mutans*	Primer	Paper discs	Agar diffusion method	Clearfil Protect Bond® (Kuraray Dental Inc, Kurashiki, Japan) primer was found to be the most potent material against L. *acidophilus* and *L. casei*
Xiao, Y. H[61]	2009	QA	DMAE-CB	*Streptococcus mutans*	Adhesive system	Adhesive + composite specimens	Bacterial growth (colony counting)	Dental adhesive with strong and long-lasting bacteriostatic property without negatively influencing bonding ability
Li, F[93]	2009	QA	DMAE-CB	*Streptococcus mutans*	Adhesive system and Primer	Adhesive + composite specimens	Biofilm accumulation	The incorporation of DMAE-CB can provide the dental adhesive with contact antibacterial activity after polymerization by influencing the growth, adherence and membrane integrity of S. *mutans*
Giammanco[94]	2009	QA	MDPB	*Enterococcus faecalis*	Primer	Direct contact of the tested	Direct contact and Agar	The antibacterial effect of the Clearfil Protect Bond® (Kuraray

						materials	diffusion method	Dental Inc, Kurashiki, Japan) and Clearfil SE Bond® (Kuraray Dental Inc, Kurashiki, Japan) depend on direct contact and does not seem to be related to the diffusion of soluble components
Esteves, C. M[10]	2010	QA	MDPB	*Streptococcus mutans, Streptococcus oralis, Streptococcus cricetus* and *Streptococcus sobrinus*	Primer	Adhesive discs	Agar diffusion method	Clearfil Protect Bond® (Kuraray Dental Inc, Kurashiki, Japan) exhibited the most effective antibacterial activity against oral S. *mutans*
Antonucci, J.M[53]	2012	QA	IDMA-1, IDMA-2	*Streptococcus mutans*	Resin experimental	Monomer samples	Bacterial adherence	IDMA-1 increases the viscosity, DC and surface charge density 10% reduced bacterial growth
He, J. W[54]	2012	QA	DDMAI	*Streptococcus mutans*	Resin experimental	Resin experimental disks	Biofilm inhibition	DDMAI has no adverse effect conversion and flexural strength with 3% and 5% and has radiopacity and only 5% has antibacterial effect
Poggio, C[57]	2012	QA	MDPB	*Streptococcus mutans, Streptococcus sanguis and Streptococcus salivarius*	Primer	Sterilized paper disks with 10 µl of each adhesive system	Agar diffusion method	MDPB monomer added to an adhesive system enhances its antibacterial effect against S. *salivarius*, S. *sanguis* and S. *mutans*
Zhang, K.[73]	2012	QA	DMADDM, *NAg, *NACP				Microcosm model	DMADDM, NAg and NACP could impart three benefits: protecting dentin bond strength from degrading in long term water-ageing; potent

								antibacterial activity that was long-lasting (6 months); and remineralization
Polydorou, O[60]	2013	QA	MDPB	*Streptococcus mutans*	Primer	Unpolymerized and polymerized adhesive	Agar diffusion method	All materials exhibited certain antibacterial activity. The release of HEMA from all tested materials even after storing in human saliva increases the concerns about their toxicity. Their antibacterial effect does not seem to be due to the release of substances
He, J. W[95]	2013	QA	DDMAI	S*treptococcus mutans*	Resin dental	Resin formulation disk	Biofilm inhibition	DDMAI incorporation into resin system reduce mechanical properties and increased WS and WSL
Cheng, L[21]	2013	QA, NAg	DMAHM, DMADDM, DMADDM+ NAg	Human saliva, Total microorganisms, Total streptococcus and S*treptococcus mutans*	Primer and adhesive	Sterile paper disk	MIC* and MBC*, Microcosm model, Agar diffusion method	The new adhesive systems are promising to combat residual bacteria and inhibit secondary caries. The DMADDM plus Nag are more promising
He, J[55]	2013	QA	QAM+ BisGMA/TEGMA	S*treptococcus mutans*	Resin dental	Resin formulation disk	Biofilm accumation and bacteria viability (colony counts)	5% QAM has no adverse impact on DC an FS, but the alky chain length of QAM had influence on antibacterial activity
Zhang[64]	2013	QA, NAg	MDPB + *NAg	Human saliva – Total microorganisms, Total streptococcus and	Adhesive	Adhesive disks	Microcosm biofilm model	MDPB plus NAg in adhesive and in primer showed stronger antibacterial potency than MDPB

				Streptococcus mutans				alone, without compromising dentin bonding and cytotoxicity properties
Passariello[96]	2014	QA	MDPB, benzalkonium chloride	*Streptococcus gordonii, Streptococcus sanguinis , Streptococcus mutans* and *Lactobacillus acidophilus*	Primer	Disks of each test material	Agar diffusion method and biofilm accumulation (MTT)	Chlorhexidine (10%) and benzalkonium chloride (5%) appeared to be the most effective antimicrobial agents. In fact, they gave the material adequately long antibacterial activity without altering its clinical performance
Kim, S.R[97]	2014	QA	MDPB	*Streptococcus mutans*	Primer	Paper disks	Agar diffusion method	Among the self-etching systems considered, Clearfil SE Bond® (SE, Kuraray, Japan) primer exhibited the most effective antibacterial activity against *S. mutans*
Li, F [1]	2014	QA	DMAHDM	*Streptococcus mutans*	Primer and adhesive	Resin disks	Biofilm accumation and bacteria viability (colony counts) fluores	Increasing the charge density on bonding agent reduced *S. mutans* and CFU DMAHDM may be useful in other types of bonding agents, cements, sealants and composites to inhibit caries
Li, F[3]	2014	QA	DMADDM and *NAg	Human saliva – Total microorganism, Total streptococci and *Streptococcus mutans*	Primer and adhesive	Primer + adhesive + composite discs	Microcosm model	Antibacterial bonding agents containing novel DMADDM and NAg reduced the metabolic activity, CFU and lactic acid of microcosm biofilms on resin surfaces, even when pre-coated with salivary pellicles.

Imazato, S[46]	2004	QA	MDPB	*Streptococcus mutans*	Primer	Dogs teeth	Animal model	The results demonstrated that applying primer containing 5% MDPB was effective for killing bacteria in infected cavities of anterior teeth of beagle dogs, whereas, the LB primer showed a slight, but not significant reduction. Experimental primer containing MDPB could exhibit in vivo antibacterial effect, suggesting its possible clinical benefit
Uysal, T[47]	2010	QA	MDPB	Human saliva	Primer	First premolar - enamel	Animal model	The Clearfil Protect Bond® (Kuraray Dental Inc, Kurashiki, Japan) for bonding orthodontic brackets reducing enamel demineralization in vivo. This cariostatic effect was localized in the area around the brackets and was significant after 30 days
Da Silva[48]	2010	QA	MDPB	Human saliva	Primer	Third molars – enamel and dentin	In situ	Polymerized MDPB containing primer did not produce inhibition zones for dentin. Moreover, Clearfil Protect Bond® (Kuraray Dental Inc, Kurashiki, Japan) was incapable of preventing or reducing caries formation. For enamel, one-step self- etching

			adhesive system containing fluoride could inhibit caries formation

*MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; *NACP amorphous calcium phosphate; *NAg nanoparticle silver

Table 3. Patents data, antibacterial monomers and claims related to dental materials.

Patent	Country	Title	Year	Inventor	Antibacterial Agent	Claimed
CA2160182	Japan	Antimicrobial adhesive composition for dental use	1994	Imazato Satoshi[78]	MDPB	Dental adhesive systems
JP08134077	Japan	Antibacterial monomer and its synthesis	1994	Masuhara Hidekazu[80]	2-(meth) acryloyl aminophenylboric acid	Dental adhesive systems
IT94RM0172	Italia	Synthetic resin-based dental material composition with antiseptic properties	1994	Bandini Silvio[79]	Quaternary ammonium	Dental restoration and for the production of false teeth, bridges, dental prostheses
WO9836639	United State	Adhesive, antimicrobial and/or reparative dentin stimulating dental compositions and methods for forming and using such compositions	1997	Jensen Steven[76]	Organohalogens, antibiotics, alkali metal hydroxides, alkaline earth metal oxides and alkaline earth metal hydroxides	Adhesive antimicrobial dental compositions
WO9848766	United State	Antimicrobial dental materials containing 2,4,4'-trichloro-2'-hydroxydiphenyl ether	1997	Pflug Kai[75]	2,4,4'-trichloro-2'-hydroxydiphenyl ether	Dental adhesive systems
US20020035169	Japan	Bonding compositions for dental use	1998	Nakatsuka Kazumitsu[73]	Antibacterial polymerizable monomer	Dental adhesive systems
CA2280495	Japan	Bonding compositions for dental use	1998	Nakatsuka Kazumitsu[74]	Antibacterial polymerizable monomer; ethylenic unsaturated group; ammonium bases, pyridinium bases andphosphonium bases	Antibacterial adhesive compositions
CN102816089	China	Quaternary ammonium salt and carbamate structure containing antibacterial lmethylacrylate monomer, its preparation method and application there of	2012	He Jjingwei[81]	Quaternary ammonium salt and carbamate structure containing antibacterial methylacrylate monomer	Dental prostheticmaterials
WO2013119901	United State	Nanostructured antibacterial and remineralizing dental bonding agents and dental bonding systems	2012	Xu Huakun[82]	DMADDM, DMAHD, DMATDM, DMATTDM, DMAPDM	Dental adhesive systems

Figures

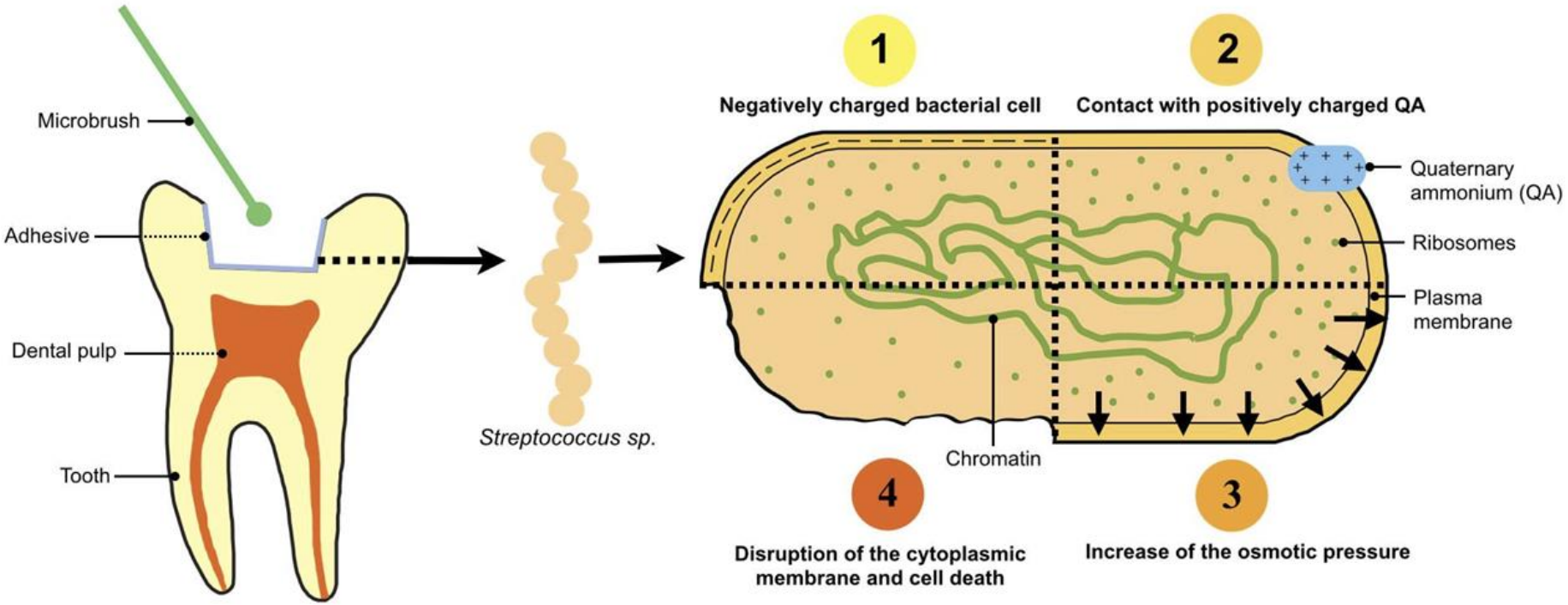

Figure 1. Mechanism of action of the quarternary ammonium (QA), main antibacterial substance incorporated into monomers.

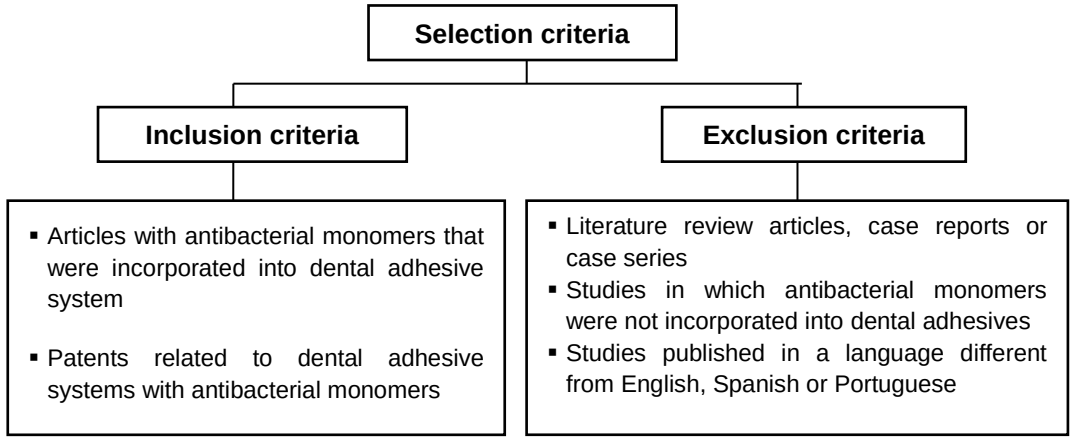

Figure 2. Selection criteria.

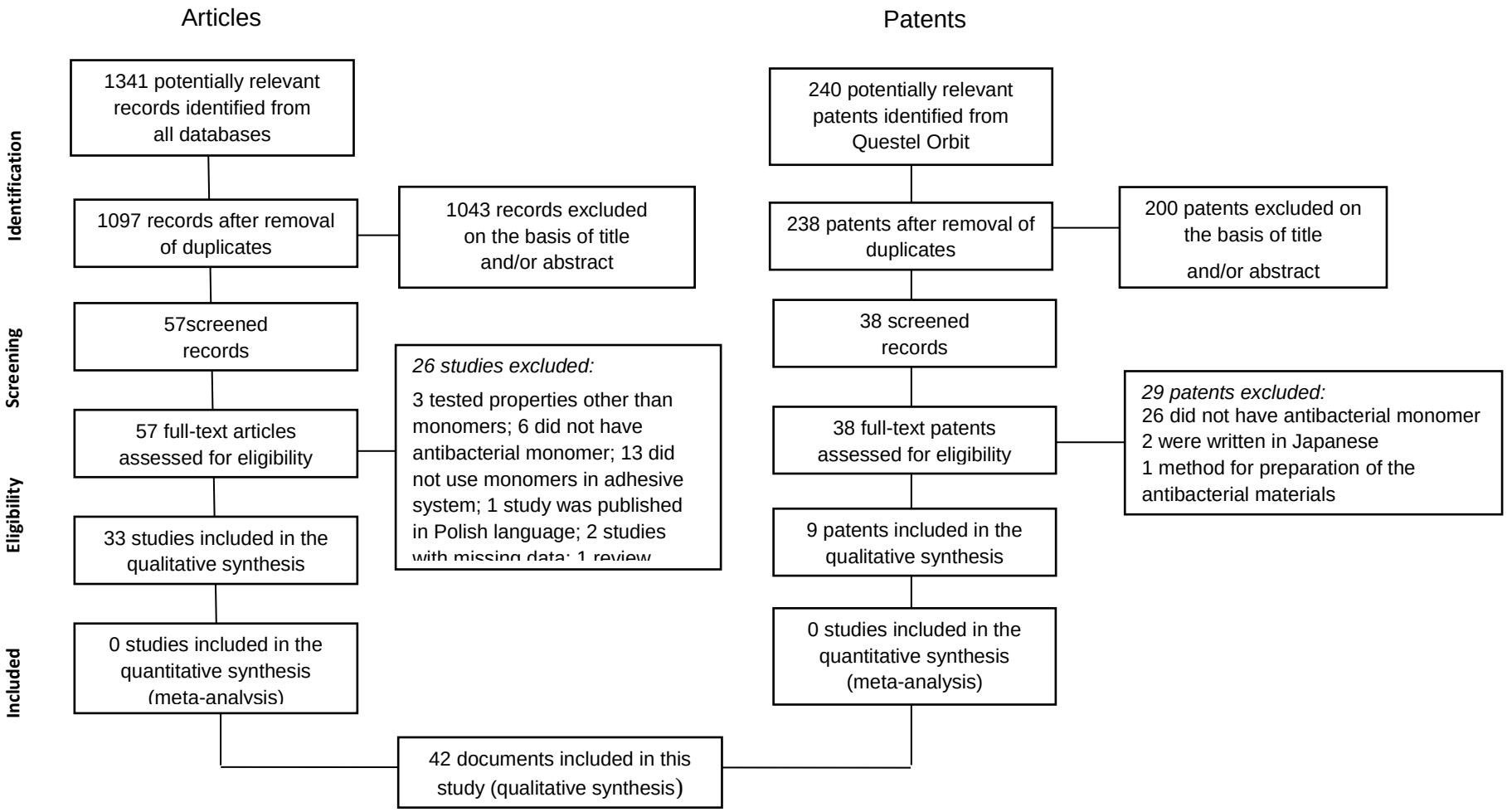

Figure 3. Search systematic review flowchart of articles and patents (adapted PRISMA Statement)[23]

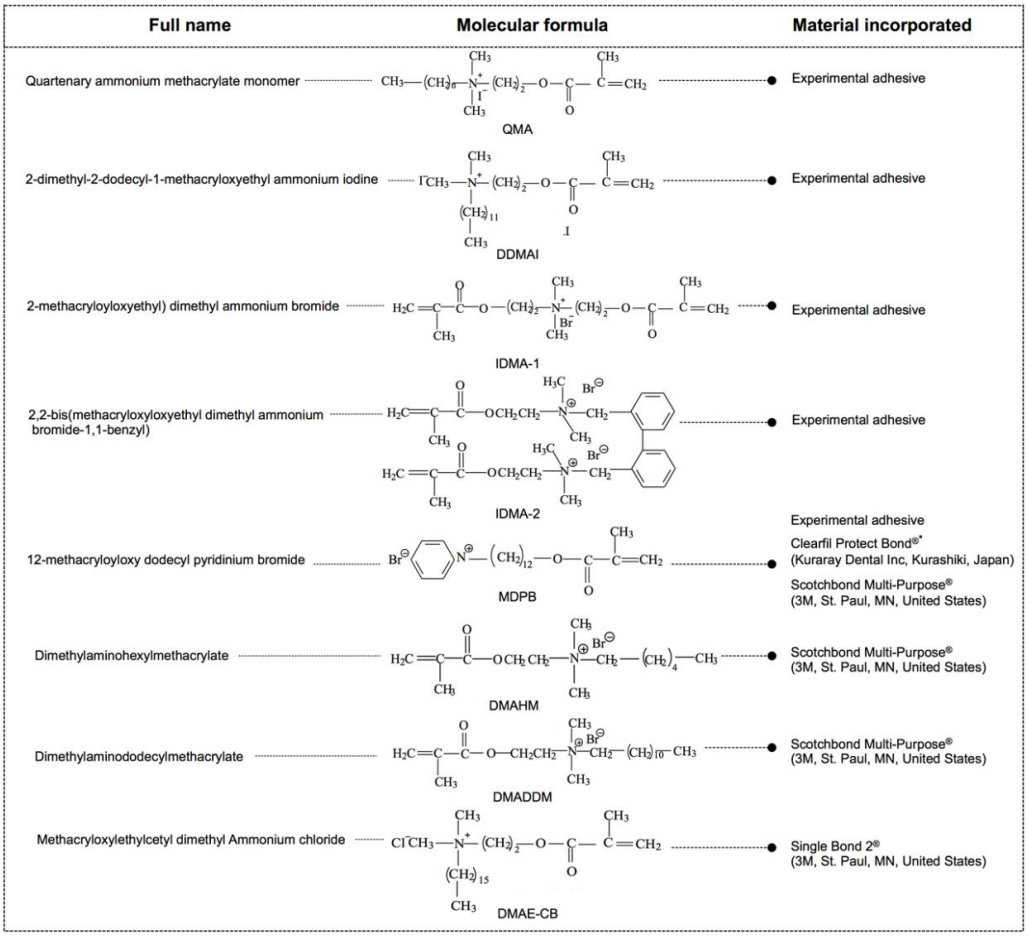

Figure 4. Molecular formula of antibacterial monomers incorporated into dental adhesives, used in the included studies.

*Unique antibacterial monomer commercially incorporated into adhesive system.

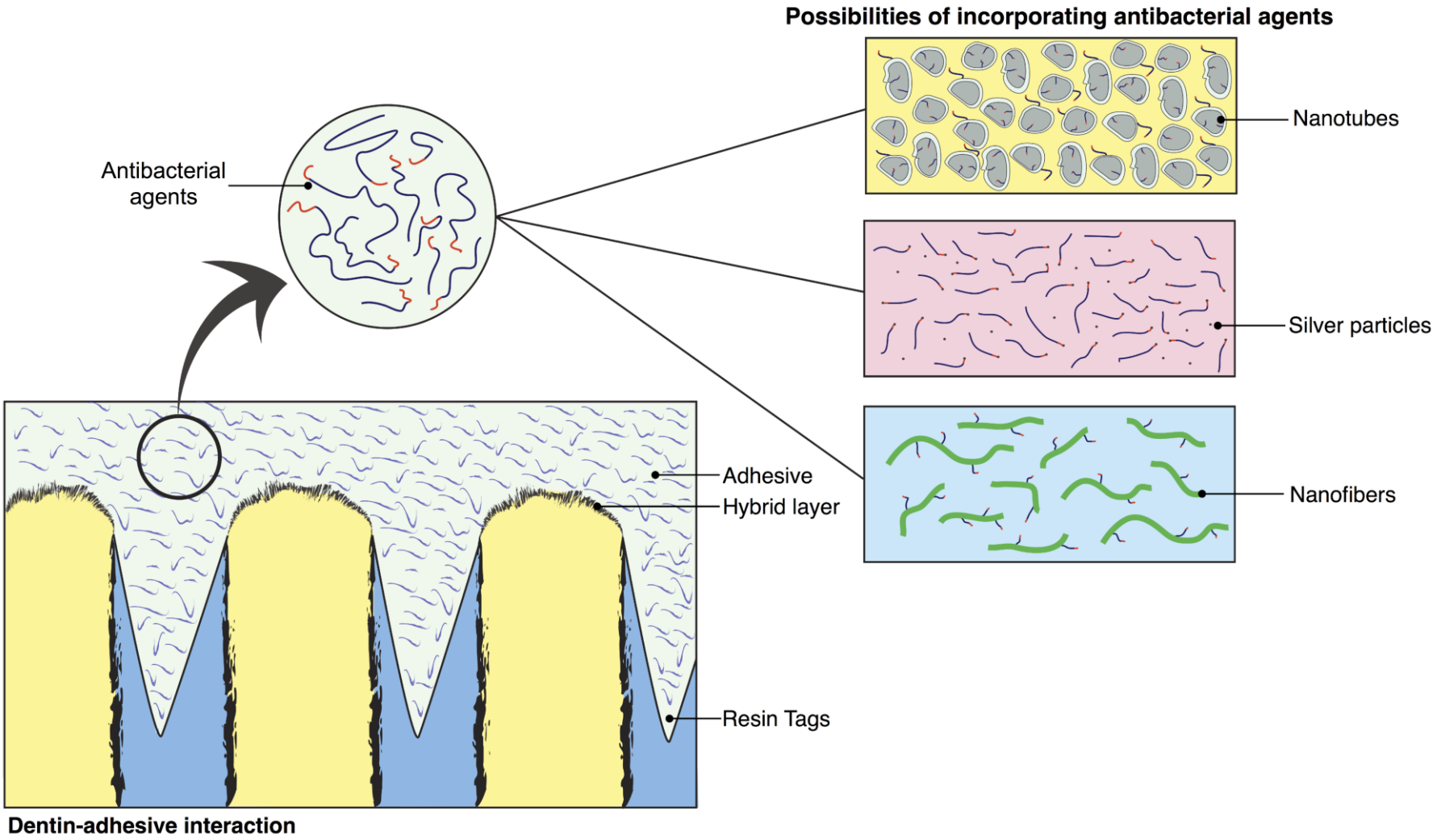

Figure 5. Future prospects of incorporating of antibacterial particles into products such as nanotubes and nanofiber.

Appendix REPENSAR

Appendix A. Risk of bias considering aspects reported in included studies.

	Blinding of Operator	Sample Size Calculation	Positive control	Negative control	More than one microorganism evaluated	Risk of Bias
Imazato et al.[98]	N	Y	N	Y	N	Medium
Imazato et al.[55]	N	N	N	N	N	High
Imazato et al.[99]	N	N	Y	N	Y	High
Imazato et al.[100]	N	N	Y	N	Y	High
Imazato et al. [101]	N	Y	Y	N	Y	Medium
Imazato et al.[63]	N	Y	Y	N	N	Medium
Imazato et al.[45]	N	Y	Y	N	Y	Medium
Kuramoto et al.[49]	N	N	Y	N	N	High
Lobo el al.[102]	N	Y	Y	N	N	Medium
Turkun et al.[65]	N	Y	Y	N	N	Medium
Turkun et al.[8]	N	Y	Y	Y	N	Medium
Feuerstein et al.[10]	N	Y	Y	Y	N	Medium
Gondim et al. [11]	N	Y	Y	Y	Y	Low
Korkmaz et al. [57]	N	Y	N	Y	N	Medium
Xiao et al. [60]	N	Y	Y	Y	N	Medium
Li et al.[50]	N	Y	Y	Y	N	Medium
Giammanco et al. [103]	N	Y	Y	Y	N	Medium
Esteves et al. [12]	N	Y	Y	N	Y	Medium
Uysal et al. [46]	N	Y	Y	N	Y	Medium
Da Silva et al. [48]	N	Y	N	N	Y	Medium
Antonucci et al. [52]	N	Y	N	Y	N	Medium

He et al. [53]	N	Y	N	N	N	High
Poggio et al. [56]	N	N	Y	Y	Y	Medium
Zhang et al. [73]	N	Y	N	Y	Y	Medium
Polydorou et al.[59]	N	Y	N	N	N	High
He et al. [54]	N	Y	N	Y	N	Medium
Cheng et al. [1]	N	N	Y	Y	N	Medium
He et al.[104]	N	Y	N	Y	N	Medium
Zhang et al.[64]	N	Y	Y	N	Y	Medium
Passarieloet al.[105]	N	Y	N	Y	Y	Medium
Kim et al. [106]	N	Y	Y	Y	N	Medium
Li et al.[3]	N	Y	N	Y	N	High
Li et al.[71]	N	N	N	Y	Y	Medium

Abbreviations: N, no; Y, yes.

Artigo 2*

*Artigo formatado conforme as instruções para autores do periódico Biofouling
http://www.tandfonline.com/loi/gbif20#.VMvNHdLF91Y
acesso em 30/01/2015

Novel dental adhesive systems containing metal methacrylates: antibacterial, cytotoxicity and bonding properties

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Abstract

The aim of this study was to evaluate the antimicrobial activity, degree of conversion (DC), microtensile bond strength (μTBS) and cytotoxicity of experimental self-etching adhesive systems after incorporation of silver (Ag) or tin (Sn) methacrylate. Seven groups were tested: Ag and Sn (0.5 mol%, 1 mol% and 2 mol%), Control Group (without metal methacrylate). The data were submitted to One-Way ANOVA or Kruskal-Wallis followed by Tukey's post hoc test ($p < 0.05$). Ag and Sn at concentrations of 1 % and 2 % were effective against *Lactobacillus* Total and *Streptococcus mutans. O*nly Ag 2 % showed statistically significant difference in DC and μTBS compared to other groups. The increase in metal methacrylate concentration was associated with a logarithmic decrease in cell viability (Ag, $R^2 = 0.99$; Sn, $R^2 = 0.99$; $p < 0.05$). The incorporation of Ag 1 % and Sn 1 % demonstrated antimicrobial effect without increasing cytotoxicity of adhesive resin.

Keywords: antibacterial monomer; adhesive system; mechanical properties; biological properties; in vitro

Introduction

The adhesive systems are composed of monomers and the most used are the methacrylates bisphenol glycidyl methacrylate (BisGMA) and the triethylene glycol dimethacrylate (TEGDMA) (He et al. 2012). About 20 years ago, a monomer with antibacterial action was synthesized, the MDPB (12-methacryloyloxydodecylpyridinium bromide), which is a quaternary ammonium monomer (Imazato and McCabe 1994). Nowadays, the MDPB is found commercially in a dental adhesive system (Imazato et al. 2006), the Clearfil Protect Bond™ (Kuraray Co. Ltd., Japan). This adhesive system contains 5 % MDPB in primer composition and has shown higher antibacterial activity when compared to other commercials primers (Feuerstein et al. 2007, Imazato, Kuramoto, Takahashi, Ebisu and Peters 2006). Moreover, it showed to reduce residual bacterial from caries cavity (Esteves et al. 2010, Poggio et al. 2012) and showed less enamel demineralization around brackets (Uysal et al. 2011). However, the durability of antibacterial action of this monomer remains unclear. Some evidence shows that the adhesive system Clearfil Protect Bond™ after photo-activation reduces significantly the antibacterial action (da Silva et al. 2010, Feuerstein, Matalon, Slutzky and Weiss 2007, Gondim et al. 2008), probably because there is a decrease of antibacterial component leached out from the cured adhesive (Turkun et al. 2006).

Therefore, new antibacterial agents have been investigated aiming prolong the antimicrobial activity. While particles can have action at long distance, due to the leaching of adhesive; monomers can act in direct contact with bacteria (Zhang, Li, Imazato, Cheng, Liu, Arola, Bai and Xu 2013). Many authors have incorporated particles such as silver (Ag) and antibacterial monomers to enhance and complement the antibacterial action (Zhang et al. 2013). Another alternative is research of monomers with potentially antibacterial metal groups. The synthesis of these monomers and mixture in adhesive resin can provide the inclusion of monomers in functionalized molecules that will crosslink in the polymeric matrix by photo-activation. Another advantage is the relatively low solubility of these molecules due its integration to the polymer.

Furthermore, metallic particles have also been used in dental adhesives in order to get antibacterial action, such as silver. Among metal ions, the tin can

be found in dentifrices and solutions, and has been used with fluoride in dental materials (Attramadal and Svatun 1984). To date, there are few published reports of dental materials containing metal methacrylates, including silver and zinc methacrylates (Yoshida et al. 2009, Henn et al. 2012). Silver and zinc methacrylate was already incorporated respectively in resin (Yoshida, Aoki and Yoshida 2009) and experimental adhesives system (Henn, de Carvalho, Ogliari, de Souza, Line, da Silva, Demarco, Etges and Piva 2012). Besides, these monomers have the disadvantage of their high cost (Yoshida, Aoki and Yoshida 2009).

Therefore, the purposes of this study were to: (1) develop adhesive system containing metal methacrylates com possíveis potenciais antibacterianos (silver and tin methacrylate) for the first time; (2) investigate the antibacterial activity, cell viability and mechanical properties. It was hypothesized that the addition of silver or tin methacrylate in adhesive resin would impact antibacterial function and would not compromise mechanical properties and cytotoxicity.

Materials and methods

This study was approved by the Ethics Committee of the Federal University of Pelotas, RS, Brazil (protocol #641.877, 07/05/2014).

Formulation of the experimental adhesive systems

Two-step self-etching adhesive systems were formulated. The experimental primer contained: 2-hydroxyethylmethacrylate – HEMA (Sigma-Aldrich, St. Louis, MO, USA), glycerol dimethacrylate phosphate - GDMA-P (is an equimolar mixture of glycerol dimethacrylate dihydrogen phosphate and glycerol tetramethacrylate hydrogen phosphate), distilled water and ethanol (Labsynth Ltda., Diadema, SP, Brazil) were added at mass concentrations of 30, 30, 20 or 20 %. The adhesive resin consisted of 25 % HEMA, 50 % bisphenol A diglycidyl methacrylate – BisGMA (Esstech Inc., Essington, PA, USA) and 25 % triethylene glycol dimethacrylate - TEGDMA (Esstech). A ternary photoinitiator system comprising 0.4 mol% camphorquinone - CQ, (Esstech), 1 mol% ethyl 4-dimethylaminobenzoate - EDAB (Fluka, Milwalkee,

WI, USA), and 1 mol% diphenyliodonium hexafluorophosphate - DPI (Sigma-Aldrich). All chemicals were used as received without further purification.

An antibacterial screen by incorporating metal powder in methacrylate resin adhesive was carried out. Zinc methacrylate, silver, dibutyltin, copper, iron, nickel, calcium hydrate, acid chromium salt, di-n-butildimetacrilato tin, zirconium dimethacrylate were tested at a concentration of 0.5, 1 and 2 mol% by the inhibition zone test. At these concentrations, only the silver and dibutyltin methacrylate had inhibition zone.

In addition, only two methacrylates (Aldrich Chemical Co., Milwaukee, WI, USA) were incorporated into the adhesive resin: silver methacrylate (Ag) and dibutyltin methacrylate (Sn), in three different molar concentrations: 0.5 %, 1 % and 2 %. A previous screening was performed to select the concentrations of these metal methacrylates to be assessed into the formulation of the experimental adhesives (unpublished data). Therefore, seven adhesive resin were formulated:

1. Control (without incorporation of metal methacrylate).
2. 0.5% Sn (adhesive resin with 0.5% tin methacrylate).
3. 1% Sn (adhesive resin with 1% tin methacrylate).
4. 2% Sn (adhesive resin with 2% tin methacrylate).
5. 0.5% Ag (adhesive resin with 0.5% silver methacrylate).
6. 1% Ag (adhesive resin with 1% silver methacrylate).
7. 2%Ag (adhesive resin with 2% silver methacrylate).

All experimental adhesive systems were subjected to the following tests:

Degree of C=C conversion (DC)

C=C converted into polymeric C–C was determined using Fourier transform infrared spectroscopy (Prestige 21 spectrometer Shimadzu Corporation, Kyoto, Japan), equipped with an attenuated total reflectance attachment incorporating a horizontal diamond crystal (PIKE Technologies, Madison, WI, USA). The LED (Radii® Curing Light, SDI, Bayswater, Victória, Austrália) was rigidly held in position, enabling standardization of the distance between the fiber tip and the top of the sample at 5 mm, and readings were carried out again for the polymer (n=3). Infrared analysis was performed at a

controlled room temperature of 23 ± 2 °C and 60 ± 5% relative humidity. A drop of each adhesive resin was placed in the total reflectance cell, and a preliminary reading for the uncured material (monomer). The adhesive resin was photoactivated for 20 s using the LED, and readings were carried out of the polymer (n=3). The spectra of the uncured and cured (after photo-activation for 20 s) adhesive resin were acquired between 1690 and 1575 cm^{-1} , averaging 24 scans at the 4 cm^{-1} resolution transmission mode, to provide a single spectrum. The extent of the unreacted aliphatic carbon double bonds (% C=C) was determined from the ratio of the absorbance intensities of the aliphatic C=C (peak height at 1637 cm^{-1}) to that of an aromatic C=C absorbance of the internal standard component (1608 cm^{-1}), both before and after curing the specimen. The baseline method used to determine peak height absorbance has been described previously (Ogliari et al. 2006). The DC was determined by subtracting the % C=C from 100%. Analyses were conducted in triplicate.

Microtensile bond strength (μTBS) to dentin

Seventy freshly extracted bovine incisors were obtained, cleaned of soft tissue, and inspected for the presence of fractures. Non-fractured teeth were stored in an aqueous solution of 0.5% chloramine-T for 7 days. The teeth were randomly assigned into seven groups according to the adhesive system tested. At least, 10 teeth were used per group. The buccal enamel was removed to expose the middle dentin layer. The exposed dentin surface was successively wet-ground with 400- and 600-grit SiC abrasive papers to create a standardized flat surface with consistent smear layer formation. After water-rinsing, the dentin substratum was dried with absorbent paper and then the experimental self-etching primer component was applied over prepared surfaces for 30 s and evaporated for 10 s, followed by the application of the adhesive resin that was light activated for 20 s using the LED previously described. A restoration was fabricated over the top of the cured bonding agent using an incremental technique with a resin composite material (N'Durance; Septodont, Confi-Dental Division, Louisville, CO, USA) and light-cured for 60 s. Specimens were then stored in distilled water at 37 °C for 24 h, after which they were sectioned perpendicularly to the bonding interfaces using a water-cooled, diamond saw at low speed (Isomet 1000, Buehler; Lake Bluff, IL, USA). This process was

reproduced after turning the cut sections 90°, resulting in beams of dentin bonded to composite with cross-sectional areas of 0.7 mm^2. At least, two beams per tooth were produced for the evaluation of μTBS immediately (n = 20 per group). Beam dimensions were precisely measured using a digital caliper (Mitutoyo, Tokyo, Japan), after which they were attached to the tensile testing device using a special cyanoacrylate glue (Super Bonder Gel, Henkel Loctite, São Paulo, SP, Brazil); the dentin portion was attached to a fixed platform, and the composite side was attached to the upper, movable crosshead. The attached specimen was subjected to tensile vertical loading in a mechanical testing machine (DL500; EMIC, São José dos Pinhais, PR, Brazil) at a crosshead speed of 0.5 mm min^{-1}, and the load recorded at specimen failure was recorded. Bond strength values (MPa) were calculated by dividing the maximal load at failure by the cross-sectional area of the bond interface.

Anti-biofouling testing

Biofilm preparation

The anti-biofouling testing followed the model proposed by Peralta (2013) with modifications (Peralta et al. 2013). The model consisted of microcosm biofilms grown in polypropylene 24-well microplates, where plaque-enriched human saliva was used as the inoculum (Peralta et al. 2013). Salivary flow was stimulated by chewing paraffin film (Parafilm 'M' ®, American National Can TM, Chicago, IL, USA) and 28 mL of fresh stimulated saliva was collected, in the morning, from a healthy human subject (female, age 24) who had not been under antibiotic therapy for at least 1 year, as well as abstained from oral hygiene for 24 h prior to the collection. One aliquot of the saliva was taken to determine the baseline microbial composition, measured in colony-forming units per unit volume (CFU ml^{-1}).

Seventy standardized discs were prepared of each adhesive resin in a mold (1 mm in diameter and 5 mm in thickness) and photo-activated for 60 s on each side by the LED's, described previously (n = 10). Then, the samples were sterilized by ultraviolet radiation. The discs were transferred into sterile wells (24-well tissue culture plate; TPP – Techno Plastic Products, Trasadingen, SU), and 0.4 ml of fresh and homogenized saliva were dispensed onto each disc. After storage for 1 h at 37 ± 1 °C, 1.8ml of the DMM solution with 1 % sucrose

was added. After incubation for 4 h with the enriched DMM solution, the discs were dip-washed for 10 s with sterile saline and transferred to a new plate with fresh DMM for 20 h. This procedure was repeated for 3 days. All plates were incubated at 37 °C in an environment of 5–10 % CO_2 (Anaerobac – Probac do Brasil products Bacteriological Ltda, Santa Cecília, SP, Brazil) in anaerobic jars (Probac do Brasil Produtos Bacteriológicos Ltda).

After the time allowed of biofilm growth, the discs containing the biofilms were washed three times with 0.9% NaCl and individually transferred to microcentrifuge tubes containing 1 ml of 0.9% NaCl. The biofilm was detached from the enamel surfaces and solubilized using vortex agitation for 30 s, followed by 30 s of sonication at 30 W (Sonicator DE S500, R2D091109 Brazil). The discs were then carefully removed from the suspension before aliquots of the suspension were used to determine bacterial viability and the dry weight of the biofilm.

Bacterial viability

One hundred microliters aliquots of the initial biofilm suspension were serially diluted in 0.9% NaCl up to 10^{-7}, after which 20 µl drops of each dilution were plated, in duplicate, on brain–heart infusion and blood agar, adjusted to pH 7.2, to obtain total microorganism counts. To determine the total aciduric counts, the pH of the brain–heart infusion was adjusted to 4.8 via the dropwise addition of HCl solution. Mitis salivarius agar was supplemented with 0.2 units of bacitracin ml^{-1} to grow Streptococcus mutans, while total lactobacilli counts were determined using Rogosa agar. The plates were incubated at 37 °C for 96 h under 5–10 % CO_2 (Anaerobac – Probac do Brasil produtos Bacteriológicos Ltda) in anaerobic jars (Probac do Brasil produtos Bacteriológicos Ltda). The number of CFU was counted in a stereomicroscope (x40) with external halogen illumination, and the results expressed as CFU mg^{-1} of biofilm dry weight. Counts for the selective plates were based on colony morphology and verified by Gram-stain and cell appearance using light microscopy.

Biofilm dry weight

To determine the biofilm dry weight, 200 µl aliquots of the initial biofilm suspension were transferred to pre-weighed microtubes and dehydrated with

ethanol solutions (75 and 99 %). The tubes were centrifuged, and the supernatants were discarded before the pellet was dried under a desiccator (P_2O_5) for 24 h and weighed (± 0.00001 g). The dry weight of the biofilm was determined by calculating the weight in the tube (initial weight - final weight) and in the original suspension (dry weight in 1 ml = dry weight in 200 µl x 5).

Cell culture

The cell culture medium was DMEM (Dulbeccos´s Modified Eagle Medium) supplemented with 10 % fetal bovine serum (FBS), 2 % L-glutamine, penicillin (100 U ml) and streptomycin (100 mg ml). Mouse fibroblasts of the L929 immortalized cell line were maintained in DMEM and incubated at 37 ºC in a humidified atmosphere of 5 % CO_2 until confluence.

Cytotoxicity Assay

The cytotoxicity test was performed following ISO 10993-5 (2009) (ISO10993-5 2009). Mouse fibroblasts L929 (20×10^3 $well^{-1}$) were maintained in DMEM medium in 96-well plates for 24 h. Specimens were made of each adhesive resin by a mold (5 mm diameter and 1 mm depth), and photo-activated by the LED's, described previously (n = 6). These specimens were placed in 24-well plates with 1 mL of DMEM at 37 ºC, pH 7.2. After 24 h, 200 µL of eludate of each group were transferred to the 96-well plates previously prepared and incubated for 24 h. WST-1 (Roche Applied Science, Germany) was applied to assess cell metabolic function by mitochondrial dehydrogenase activity, and the absorbance at 480 nm was measured via a microplate reader (SpectraMax M5; Molecular Devices, Sunnyvale, CA). Each assay was repeated at least twice.

Statistical analysis

The degree of conversion, microtensile bond strength, antibacterial activity and cell viability data were analyzed using the SigmaStat 3.5 (Systat Software In., San Jose, USA) statistical analysis software. One-way ANOVA and Tukey test was used to assess the degree of conversion. The data of microtensile bond strength and cytotoxicity were non-parametric and statistical

analysis was performed using Kruskal-Wallis followed by Tukey test. Correlation between monomer concentration and cell viability was assessed by non-linear regression (logarithmic). The CFU count data were non-normal and log transformed. Subsequently, statistical analyses were performed with the transformed data. A $\log_{10}$ transformation of each CFU count was performed to normalize the data before statistical evaluation due to the high range of bacterial numbers. Then, to determine viable bacteria counts, statistical analyses were performed using one-way ANOVA and the Fisher's least significance difference (LSD) post hoc test for pair-wise means comparisons.

Results

Table 1 shows the values of the degree of conversion and μTBS tests. The increase in metal methacrylate concentration is followed by a decrease in values of DC and μTBS. In degree of conversion, 2 % Ag was statistically different of control. In μTBS tests, the groups showed no statistically significant difference of control, only 0.5 % Sn was statistically different of the 2 % Ag.

Figure 1 shows the antibacterial effect of the experimental adhesive systems. In total microorganisms, the adhesives showed no statistically significant difference to control. The same occurred in acidurics bacteria, with the exception of adhesive containing 2 % Ag which demonstrated lower bacteria viability. For *Lactobacilli* and *Streptococcus mutans*, only 0.5 % Ag and 0.5 % Sn were similar to the control. The other groups showed lower bacteria viability, highlighting the 2 % Ag for *S. mutans* that showed null bacteria viability.

Figure 2 shows the percentage of cell viability assessed after 24h. The untreated group (cell control without eluate resin) was equal to 100%. The adhesive resin without metal methacrylate (control) were statistically similar to the untreated. The eluate with Ag 0.5 % showed 62% of cell viability, unlike the 1 % Ag and 2% Ag which revealed 21 % and 2.8 %, respectively. 0.5 % Sn demonstrated viability of 50 % and 1 % Sn and 2 % Sn showed 26 % and 10.2 % of viability, respectively. The viability of the fibroblasts to all adhesives with the addition of a metal methacrylate ranged from 2.8% to 62%.

Figure 3 depicts a non-linear regression plot with metal methacrylate as dependent variable, which showed that the increased incorporation of methacrylate into the adhesive resin was associated with a logarithmic

decrease in cell viability (Ag: $y = 81.888e^{-1.053x}$; $R^2 = 0.9932$ and Sn: $y = 171.32e^{-2.058x}$; $R^2 = 0.9995$; $p < 0.05$).

Discussion

The hypotheses tested were partially accepted by the data of the present study. The incorporation of metal methacrylates affected biological properties, but slightly influenced the mechanical properties. The silver and tin methacrylates (1%) allowed enhance the antibacterial activity of our experimental dental adhesive systems, and not altered the mechanical properties, indicating that had no negative influence on the curing behavior of Bis-GMA/HEMA-based adhesive resin.

Other studies show that the incorporation of antibacterial monomers, as 5% of MDPB, co-polymerized with other monomers did not affected the mechanical properties (Imazato, Kinomoto, Tarumi, Ebisu and Tay 2003). In our study, only the incorporation of 2 % silver methacrylate affected the degree of conversion and the bond strength, with alteration of adhesive coloration. Ag 2 % left the adhesive resin darker, which may have hindered the passage of light and avoided a more efficient polymerization (Turssi et al. 2005). Another study demonstrated that an addition of 0.5 % silver methacrylate turn out opaque the material and 0.1 % was the maximum recommended to obtain degrees of transparency (Yoshida, Aoki and Yoshida 2009).

Dental biofilm is a complex microbial community that is developed on the tooth surface. This biofilm covers dental materials, especially composites, and these have been reported to accumulate more biofilm than other materials like ionomers (Li et al. 2014, van de Sande et al. 2014). To verify the inhibition of biofilm it was performed a microcosm biofilm model, being a current trend once it offers the advantage of coming closer to the physical-chemical, microbiological and nutrient conditions, in addition to maintaining the complexity and heterogeneity of *in vivo* plaques (McBain 2009). The adhesive resin containing 2 % silver methacrylate showed antibacterial effect to the majority of bacteria tested, except for the total microorganisms. Silver ions has been shown to have antibacterial, antifungal and antiviral activity, being effective as an

antibacterial agent against a variety of microorganisms, and show low toxicity (Morones et al. 2005, Rai et al. 2009). It was suggested that Ag ions could act in the structure of the bacterial cell wall, altering the cell membrane, causing distortion and death. Additionally, the Ag may denature the DNA and RNA and inhibit bacterial replication (Castellano et al. 2007, Lansdown 2002, Liao et al. 2010). Furthermore, adhesive resins containing 1 % and 2 % tin methacrylate had similar values of antibacterial outcomes. The tin has been used together with fluoride in dental materials. *In vivo (Attramadal and Svatun 1984) e in vitro* (Svatun 1978, Svatun and Attramadal 1978) studies showed that solutions and dentifrices containing Sn inhibit the formation of dental plaque and probably reduce acid production of *Streptococcus mutans*. The monomer MDPB showed similar results, with increased concentration provided greater antibacterial activity (Imazato et al. 1997). In our study, the 0.5 % concentration of the adhesive resins (Ag and Sn) exerted a minimal antibacterial effect that was similar to control. We believe that, similar to MDPB, metal methacrylate could present antibacterial activity through direct contact with bacteria, because it is believed that the metal atoms should be located at one end of the molecule, not occurring leaching of ions.

Due the metal methacrylate reduce bacterial colonization, it was necessary to perform cytotoxicity experiments. The adhesive resins containing 1 % and 0.5 % of Ag and Sn methacrylate showed a small reduction of cell viability, and were statistically similar to cellular control. Organotin was used as antifouling paints for marine structures, however its use was prohibited to be toxic to the aquatic environment and to human health, and it is ranked among the national and international priority pollutants. It is important to emphasize that an increase in metal methacrylate concentration was followed by an increase of cytotoxicity effect. Another study of our group (Henn et al. 2011) showed a correlation between degree of conversion and levels of cytotoxicity. Additionally, the lowest average degree of conversion was found for 2 % Ag, which was also the most cytotoxic. It is believed that there is a higher cross-linking co-methacrylates monomers, and a lower output of low viscosity monomers (i.e. HEMA, TEGDMA, metal methacrylates) (Darmani et al. 2007, Emmler et al. 2008)**.**

Moreover, the specimens of this *in vitro* study were well polymerized, different to what happens in the mouth, where there are various factors such as humidity, which can prejudice polymerization of the adhesive system. Unlike MDPB, which the antibacterial agent is in primer, metal methacrylates were incorporated into bond, to avoid a higher release of monomer with polymerization of the bond. Besides, it is not possible to determine the clinical effect of our experimental adhesives, and future studies using in situ and in vivo model are needed.

Conclusions

It was possible to develop antibacterial adhesive systems with the incorporation of silver and tin methacrylate. The incorporation of 1 % Ag and 1 % Sn demonstrated antimicrobial effect without increasing the cytotoxicity of the adhesive resins. The increase of metal metacrylates concentration might present a dose-dependent detrimental effect on the cytotoxicity of the experimental resins to fibroblasts.

Acknowledgements

The authors would like to thank the Fundação de Amparo à Pesquisa do estado do Rio Grande do Sul (FAPERGS; PqG-Grant No. 2162-2551/13-5) for the financial support. We also give thanks to CAPES for granting a scholarship to the first author.

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Table 1. Degree of conversion (DC) and microtensile bond strength (μTBS**)** for each experimental two-step self-etching adhesive system

Dentin bonding agent	**DC (%)**	**μTBS (MPa)**
0.5% Sn	69.8 ±0.7[a]	29.9 ±2.4[a]
1% Sn	67.9 ±9.7[ab]	29 ±5[ab]
2% Sn	65.2 ±6.1[ab]	27.2 ±2.4[ab]
0.5% Ag	68.7 ±1.2[a]	28.8 ±2.6[ab]
1% Ag	69.7 ±4.7[a]	27.8 ±4.9)[ab]
2% Ag	55.6 ±3.1[b]	26.3 ±2.8[b]
Control	70.9 ±0.8[a]	29.7 ±4.2[ab]

Similar lower case letters in the columns indicate not significantly differences between the experimental materials ($p < 0.05$).

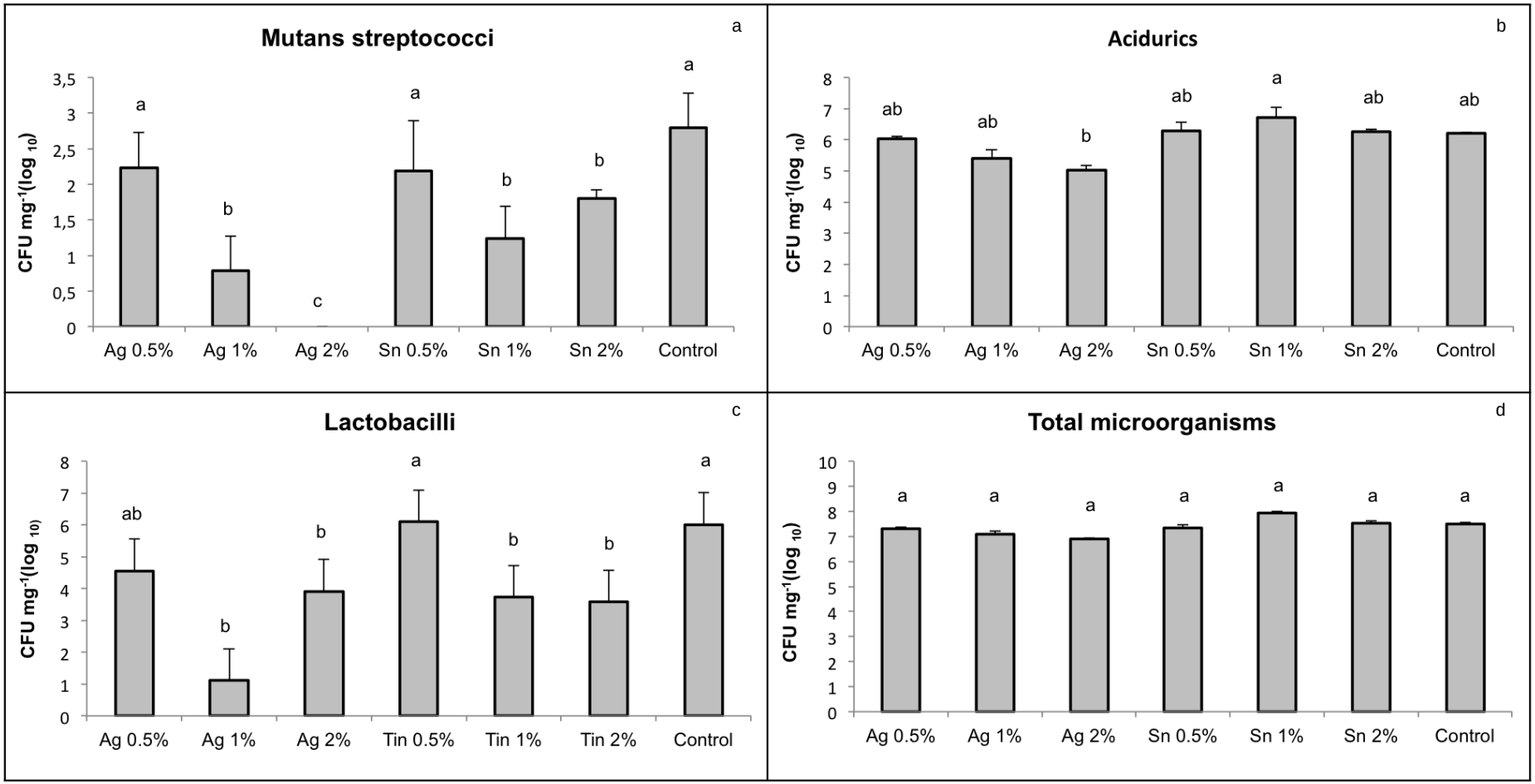

Figure 1. Mean viable bacteria (CFU cm^{-1} dry biofilm weight) in biofilms grown for 72 h (n = 10). The data were normalized by transforming by log10. Within a panel, group values that were identified using similar lower case letters were not significantly different ($p > 0.05$). A = *S. mutans*; B = aciduric bacteria; C = lactobacilli; D = total microorganisms.

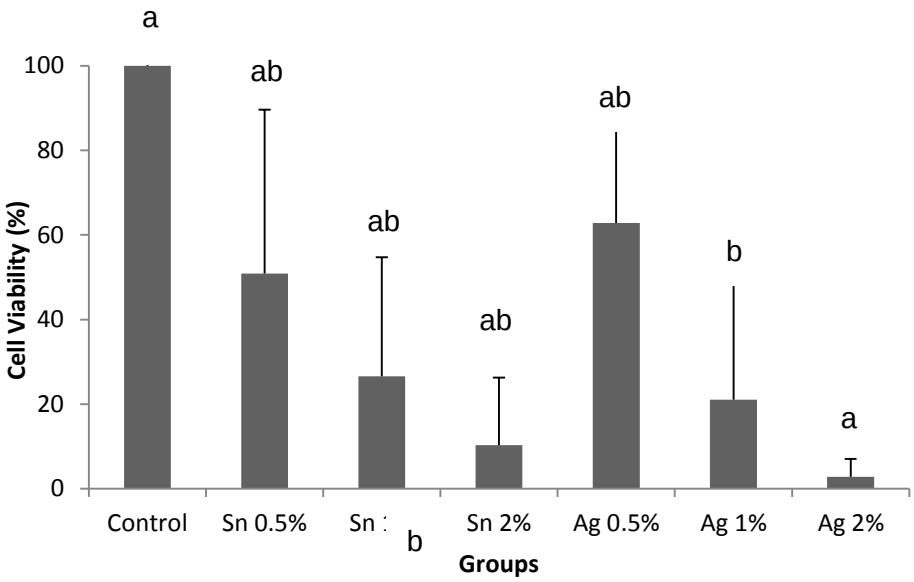

Figure 2. Cell viability and standard deviation (%) of adhesive resins with silver (Ag) and tin (Sn) at different concentrations. Control group: adhesive resin without metal methacrylate; untreated group: cell control without eluate resin. Different letters represent statistically significant differences between groups.

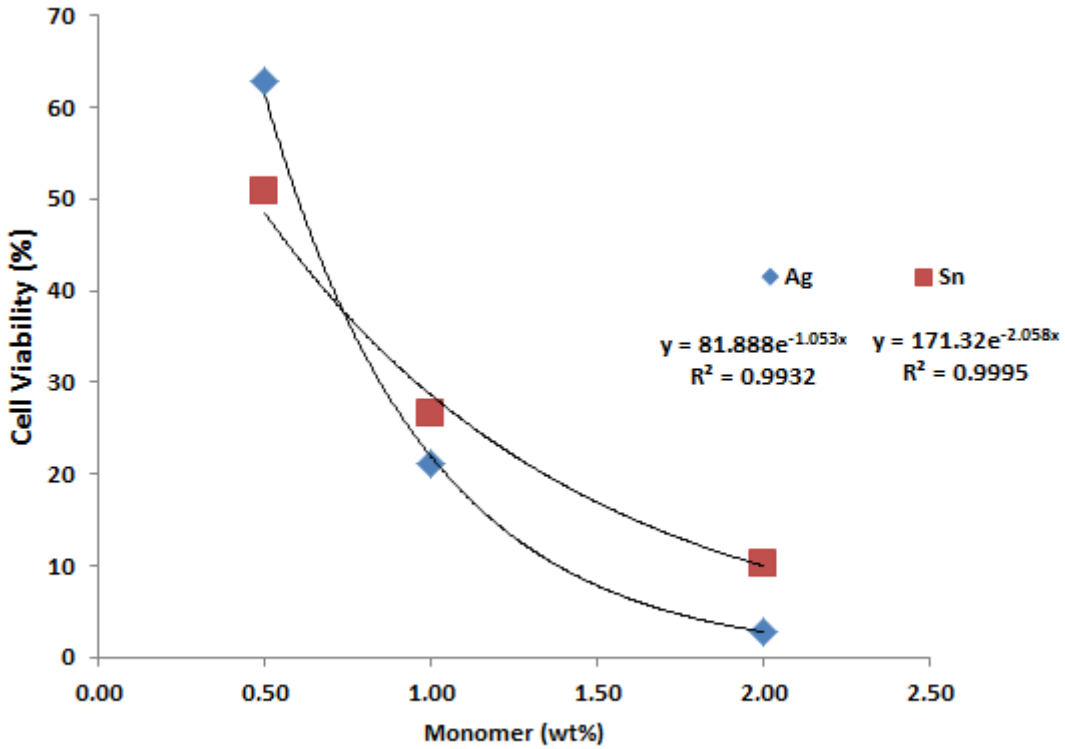

Figure 3. Non-linear regression plot with the metal monomer of the experimental self-etch adhesive as dependent variable. Model was significant thus showing a logarithmic decrease in cell viability associated with the increased concentration of metal methacrylate.

Considerações Finais

Dentro daquilo que foi encontrado na revisão sistemática, novos monômeros antibacterianos estão surgindo e sendo incorporados em muitos materiais odontológicos. Além disso, tem demonstrado efetividade na redução do biofilme bacteriano em muitos estudos *in vitro* e poucos clínicos.

Através do estudo laboratorial, conclui que as resinas adesivas testadas neste estudo, não afetaram as propriedades mecânicas, com exceção da resina adesiva contendo 2% de metacrilato de prata que teve leve redução no grau de conversão e na resistência de união.

Além disso, o uso de metacrilato de prata ou de metacrilato de estanho em concentração de 1 mol % em resinas adesivas pode ser uma opção para obtenção de ação antibacteriana sem alterar as propriedades mecânicas testadas e sem aumentar a citotoxicidade.

Apesar de serem necessários estudos clínicos para confirmar a efetividade desses materiais na prevenção e controle de patologias dentais, como a cárie, a revisão sistemática e o estudo laboratorial demonstraram que há evidência de atividade antibacteriana desses adesivos *in vitro*.

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Apêndice

Apêndice 1.

FACULDADE DE
ODONTOLOGIA DA
UNIVERSIDADE FEDERAL DE PELOTAS

PARECER CONSUBSTANCIADO DO CEP

Pesquisador: Alexandra Rubin Cocco
Título da Pesquisa: Caracterização físico-mecânica e atividade antibacteriana de resinas adesivas contendo grupamento metálico
Instituição Proponente: Faculdade de Odontologia da Universidade Federal de Pelotas/ FO-UFPel
Versão: 2
CAAE: 25775413.2.0000.5318

DADOS DO PARECER
Número do Parecer: 641.877
Data da Relatoria: 07/05/2014

Apresentação do Projeto: Mantido conforme parecer anterior
Objetivo da Pesquisa: Mantido conforme parecer anterior
Avaliação dos Riscos e Benefícios: Mantido conforme parecer anterior
Comentários e Considerações sobre a Pesquisa: Mantido conforme parecer anterior
Considerações sobre os Termos de apresentação obrigatória: Atendidos na íntegra. A especificação da fonte de financiamento prevê recursos além dos necessários à

execução da pesquisa. Porém, como o grupo do PPGO executa diversos projetos nesta linha, presume-se que esta verba contemple também outros projetos. Recomenda-se portanto a aprovação do projeto.

Recomendações: sem recomendações

Patrocinador Principal: Fundação de Amparo a Pesquisa do Estado do Rio Grande do Sul

Universidade Federal de Pelotas

Conclusões ou Pendências e Lista de Inadequações: Conforme admitido em reunião anterior do CEP(15/04/14), o parecer seria expedido ad-referendum caso os pesquisadores atendessem às pendências do parecer anterior, uma vez que referia-se apenas a uma coleta

de saliva. As pendências foram atendidas na íntegra e, dessa forma, recomendo a aprovação do projeto.

Situação do Parecer: Aprovado

Necessita Apreciação da CONEP: Não

Assinador por: Renato Waldemarin

(Coordenador)

Apêndice 2.

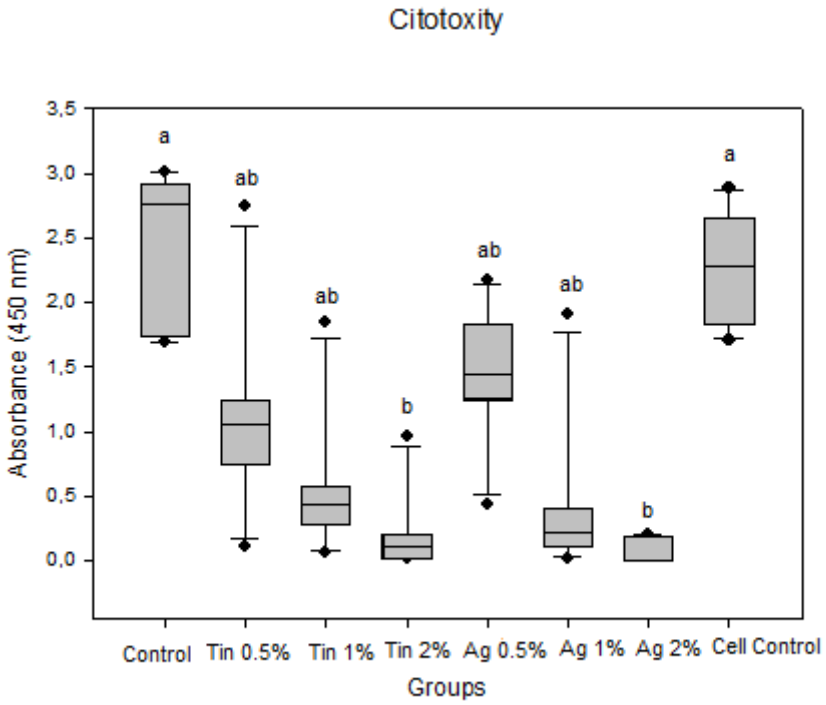

Gráfico complementar do teste de citotoxicidade.