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Dissertação

Caracterização de adesivos ortodônticos contendo diferentes agentes antimicrobianos

Carianne Mendes de Almeida

Pelotas, 2017

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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 Dentística.

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Resumo

DE ALMEIDA, Carianne Mendes. **Caracterização de adesivos ortodônticos contendo diferentes agentes antimicrobianos.** 2017. 109f. Dissertação (Mestrado em Odontologia). Programa de Pós-Graduação em Odontologia, Universidade Federal de Pelotas, 2017.

Neste trabalho, foi realizada uma revisão sistemática e meta-análise dos agentes antimicrobianos incorporados em sistemas adesivos ortodônticos e um estudo laboratorial a fim de caracterizar e incorporar nanopartículas de β-TCP dopadas com agentes antimicrobianos em uma matriz de um adesivo ortodôntico. Inicialmente, a revisão sistemática e meta-análise foram realizadas para avaliar a efetividade dos agentes antimicrobianos incorporados em sistemas de adesão ortodônticos, bem como seu futuro desenvolvimento. Uma busca em oito bases de dados foi realizada: MedLine (PubMed), Lilacs, Ibecs, Web of Science, Scopus, Scielo e Google acadêmico. Enquanto isso, um estudo laboratorial foi conduzido com o objetivo de avalir o efeito antimicrobiano, a inibição de cárie, a resistência de união por cisalhamento e as propriedades físico-químicas alcançadas pela adição de nanopartículas de β-TCP dopadas com agentes antimicrobianos a um adesivo ortodôntico. Na revisão sistemática e meta-análise, de um de total de 1320, 32 estudos estavam relacionados a sistemas de adesão ortodônticos com atividade antibacteriana e foram incluídos no estudo. Dez estudos foram excluídos da meta-análise por não apresentarem dados quantitativos de atividade antimicrobiana. Todos os estudos relataram a inclusão de agentes antimicrobianos em sistemas de adesão ortodônticos como uma estratégia de tratamento odontológico eficaz. Já no estudo laboratorial, os adesivos ortodônticos contendo nanopartículas de β-TCP dopadas com agentes antimicrobianos inibiram significativamente o crescimento bacteriano, mas também não resultaram em nenhum efeito sobre ΔS sob e em torno dos braquetes. Para a força de ligação, não foi observada diferença entre TB e YO. A utilização de $YO_{\beta\text{-}TCP@CHX}$ resultou em valores mais baixos de resistência de ligação, enquanto a adição de nanopartículas de β-TCP dopadas com outros agentes antimicrobianos não reduziu a resistência de ligação de YO. O tipo de adesivo ortodôntico não afetou dramaticamente o grau *in situ* de conversão, sorção de água e solubilidade em água. Apesar de serem necessários estudos clínicos para confirmar a efetividade desses materiais no controle antimicrobiano, a revisão sistemática e meta-análise e o estudo laboratorial demonstraram que há evidência, *in vitro*, de atividade antibacteriana sem prejudicar drasticamente as outras propriedades investigadas desses adesivos ortodônticos.

Palavras-chave: efeito antimicrobiano, adesivos, revisão sistemática.

Abstract

DE ALMEIDA, Carianne Mendes. Characterization of orthodontic adhesives containing different antimicrobial agents**.** 2017. 109f. Dissertação (Mestrado em Dentística). Programa de Pós- Graduação em Odontologia, Universidade Federal de Pelotas, 2017.

In this work, a systematic review and meta-analysis of antimicrobial agents incorporated in orthodontic adhesive and a laboratory study were carried out to incorporate and characterize β-TCP nanoparticles doped with antimicrobial agents in an orthodontic adhesive. Initially, the systematic review and meta-analysis were performed to evaluate the effectiveness of the antimicrobial agents incorporated in orthodontic adhesion systems, as well as their future development. A search in eight databases was performed: MedLine (PubMed), Lilacs, Ibecs, Web of Science, Scopus, Scielo and Google academic. Meanwhile, a laboratory study was conducted to evaluate the antimicrobial effect, caries inhibition, shear bond strength and physico-chemical properties achieved by the addition of anti-microbial doped nanoparticles with antimicrobial agents to an adhesive Orthodontic. In the systematic review and meta-analysis, out of a total of 1320, 32 studies were related to orthodontic adhesion systems with antibacterial activity and were included in the study. Ten studies were excluded from the meta-analysis because they did not present quantitative data of antimicrobial activity. All studies have reported the inclusion of antimicrobial agents in orthodontic adhesion systems as an effective dental treatment strategy. In the laboratory study, orthodontic adhesives containing β-TCP nanoparticles doped with antimicrobial agents significantly inhibited bacterial growth, but also did not result in any effect on ΔS under and around brackets. For the binding force, no difference was observed between TB and YO. Using $YO_{\beta\text{-TCP@CHX}}$ resulted in lowest values of bond strength, while addition of β-TCP nanoparticles doped with other antimicrobial agents did not reduce the bond strength of YO. The orthodontic adhesive type did not dramatically affect the in *situ* degree of conversion, water sorption, and water solubility. Although clinical studies are required to confirm the effectiveness of these materials in antimicrobial control, systematic review and meta-analysis and laboratory study have demonstrated that there is evidence, *in vitro*, of antibacterial activity without impairing the other investigated properties of these in vitro orthodontic adhesive.

Keywors: antibacterial effects, dental adhesive, Systematic Review

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Lista de Abreviaturas e Símbolos

β – TCP	Beta tri cálcio fosfato
g	Grama
mL	Mililitro
%	Porcento
ºC	Graus Celsius
@	Dopagem
µL	Microlitro
h	Hora
ΔS	Perda mineral integrada
µm	Micro metro
s	Segundos
CO_2	Gás carbônico
O_2	Oxigênio
N_2	Nitrogênio
H_2	Hidrogênio
mm	Milímetros
mm/min	Milímetro por minuto
UV	Radiação ultravioleta
mW	Megawatt
mW/cm	Megawatt por centímetro
LMW	Low Molecular Weigth
IAR	Índice adesivo remanescente
cm^{-1}	Centímetros $^{-1}$
GC	Grau de conversão
RU	Resistência de União

Mpa	Mega Pascal
M1	Massa constante 1
M2	Massa constante 2
M3	Massa constante 3
WS	Sorção
SL	Solubilidade
AFM	Microscopia de força atômica
RA	Rugosidade
H_2O	Água
BHI	Brain Heart Infusion
pH	Potencial hidrogeniônico
ppm	Parte por milhão

Sumário

1.Introdução

A presença de lesões de manchas branca ao redor dos braquetes tem sido uma das complicações mais frequentes e facilmente reconhecidas na prática clínica (NAPIMOGA et a., 2005, AL MUSALLAM et al., 2006). Este processo ocorre principalmente por alterações específicas no ambiente oral, como a retenção prolongada de placa bacteriana devido à superfícies irregulares dos aparelhos ortodônticos e condições de pH mais baixas produzidas por bactérias cariogênicas presentes na cavidade oral (NAPIMOGA et al., 2005, MITCHELL et al., 1992). O acúmulo de bactérias cariogênicas (PAPAIOANNOU et al., 2007), devido à dificuldade de higienização relacionada aos fios, braquetes e elásticos contribui para o desenvolvimento de lesões iniciais de cárie em um período relativamente curto de espaço de tempo. Considerando que o tratamento ortodôntico, em média, dure em torno de 3 anos, este é tempo suficiente para ocorrência de uma grande quantidade de lesões (MELO et al., 2014).

Abordagens preventivas como hábitos de higiene oral, regimes de flúor e controle dietético são geralmente realizadas para evitar o aumento do risco de cárie durante tratamentos ortodônticos fixos. No entanto, estratégias que não dependem a adesão do paciente podem ser mais eficazes no controle da desmineralização (BISHARA, 1996). Sistemas adesivos ortodônticos (Transbond Plus, Fuji ORTHO LC), por exemplo, contém na sua composição adição de flúor como alternativa para reduzir a desmineralização, no entanto, são considerados sistemas complementares e não substitutos na capacidade antimicrobiana (AHN 2010 e CALDEIRA 2013).

A utilização de sistemas adesivos ortodônticos contendo na suas composições agentes antimicrobianos pode ajudar a inibir a desmineralização do esmalte ao redor dos braquetes (BISHARA et al., 1996). Estudos recentes têm avaliado a efetividade desses sistemas contendo em sua formulação nanopartículas de prata, gluconato de clorexidina, cloreto de benzalcônio e triclosan (SEGHAL 2007). Outro composto que vem sendo utilizado em alguns materiais odontológicos com a função de promover a remineralização do esmalte é o beta tri cálcio fosfato (β-TCP), que, em contato com a saliva, promove a

remineralização dos tecidos (AHN et al., 2009). No entanto, não existem evidências na literatura em relação ao potencial antibacteriano e remineralizante de adesivos ortodônticos contendo nanopartículas β-TCP dopadas com agentes antimicrobianos.

Assim, o objetivo deste trabalho foi: (1) revisar sistematicamente a eficácia dos sistemas adesivos ortodônticos contendo diferentes agentes antimicrobianos, bem como os avanços e estudos futuros dos materiais a base de substâncias antimicrobianas; (2) e incorporar nanopartículas de β-TCP dopadas com substâncias antimicrobianas em uma matriz resinosa de um adesivo ortodôntico, bem como avaliar o efeito da adição destes componentes na resistência de união de braquetes submetidos ao desafio cariogênico, no desenvolvimento de lesões de cárie ao redor da interface de união e nas propriedades físico-químicas desse adesivo ortodôntico. As seguintes hipóteses testadas foram: (1) a incorporação de nanopartículas de β-TCP dopadas com agentes antimicrobianos em um adesivo ortodôntico não afetaria a adesão dos braquetes ao esmalte, (2) promoveria efeito inibitório na desmineralização do esmalte ao redor da interface de união e, (3) apresentaria desempenho físico-químico semelhante aos adesivos ortodônticos comerciais avaliados.

2. PROJETO DE PESQUISA

2.1 Caracterização do problema

A desmineralização do esmalte tem sido reconhecida comumente como um problema predominante e desafiador do tratamento ortodôntico (JULIEN, BUSCHANG, CAMPBELL, 2013). Estudos recentes indicaram que a maioria dos pacientes após terapia ortodôntica fixa tiveram lesões de mancha branca (CHAPMAN et al., 2010; TUFEKCI et al., 2011). Os aparelhos ortodônticos fixos podem conduzir a alterações específicas no ambiente oral, tais como pH ácido do local, acúmulo e retenção prolongada de placa bacteriana sobre a superfície do esmalte e formação de biofilme (SANTAMARIA et al., 2014; do NASCIMENTO et al., 2013). O desenvolvimento de lesões de cárie complica o tratamento ortodôntico, e aponta para a grande necessidade de controle do biofilme oral.

O acúmulo de um nível elevado de bactérias cariogênicas (PAPAIOANNOU et al., 2007), devido à dificuldade de higienização dos fios, braquetes e elásticos contribui para o desenvolvimento de lesões iniciais de cárie em um período relativamente curto de espaço de tempo, causando o aparecimento de lesões de manchas brancas. Essas manchas podem se desenvolver rapidamente, dentro de 4 semanas e progredir para cavitações. Considerando que o tratamento ortodôntico, em média, dure em torno de 3 anos, será tempo suficiente para ocorrência de uma grande quantidade de lesões (MELO et al., 2014).

Abordagens preventivas como hábitos de higiene oral, regimes de flúor e controle dietético são geralmente realizadas para evitar o aumento do risco de cárie durante tratamentos ortodônticos fixos (ENAIA, BOCK, RUF, 2011 e ZHANG 2015). Nesse contexto, a adesão do paciente é um fator limitante para obter um resultado positivo. No entanto, estratégias preventivas que não exigem a adesão do paciente podem ser mais eficazes no controle da desmineralização precoce.

Recentemente, cimentos de ionômero de vidro modificados por resina têm sido usados como adesivos ortodônticos devido a sua capacidade de liberação de flúor com função de inibição da desmineralização do esmalte (POOSTI, 2012). No entanto, notou-se resultados contraditórios, como a baixa resistência de união ao substrato dental resultando em altas taxas de falhas. Ainda, foi relatado que remanescentes do cimento de ionômero de vidro, ao redor dos suportes ortodônticos, pode ser um fator predisponente para a colonização e infecção de *S. mutans* quando comparados a resinas compostas (LIM et al., 2008). Além disso, estudos relataram que em ambiente de baixo pH apresentaram pouco efeito no processo de remineralização (DERKS et al., 2004 e BUYUKYILMAZ, T, OGAARD, B 1995).

Diante disso, uma alternativa sugerida é a incorporação de agentes antimicrobianos em adesivos ortodônticos a fim de torna-los resistentes ao acúmulo e retenção de placa bacteriana. A incorporação de nanopartículas de prata, por exemplo, possui uma grande vantagem como agente antimicrobiano sendo seletivamente tóxica para *estreptococos orais* sem comprometer as propriedades físicas do material (AHN, et al., 2009; POOSTI, 2012). A incorporação de clorexidina e triclosan, em quantidades diminutas, também demonstraram traços antimicrobianos para materiais dentários sem afetar significativamente suas propriedades físicas (SEGHAL 2007, KWON 2009). Imatazo et al., (1998) demonstraram que a adição de cloreto de benzalcônio, um amônio quaternário, também tem sido adicionado promovendo sucesso nas propriedades antibacterianas incluindo a manutenção do efeito após sua fotopolimerização. Outro composto que vem sendo utilizado em alguns materiais odontológicos com a função de estimular a deposição mineral e de promover a remineralização do esmalte é o beta tri cálcio fosfato. O β-TCP possui em sua composição íons cálcio e fosfato que em meio bucal, em contato com a saliva, promoveria remineralização dos tecidos.

Diversos estudos já sintetizaram o β-TCP e já avaliaram os agentes antimicrobianos a base de prata, triclosan, clorexidina e sal quaternário de amônio isolado, no entanto, nenhum deles avaliou a dopagem de nanopartículas de β-TCP com esses materiais incorporados em uma matriz resinosa de um adesivo ortodôntico.

O objetivo deste estudo será incorporar nanopartículas de β-TCP dopadas com agentes antimicrobianos em uma matriz resinosa de um adesivo ortodôntico, e avaliar seu potencial antibacteriano e remineralizante bem como caracterizar suas propriedades microbiológicas, mecânicas e físico-químicas.

2.2 Objetivos

2.2.1 Objetivo Geral

O objetivo deste estudo será avaliar o potencial antibacteriano e remineralizante de um adesivo ortodôntico contendo diferentes agentes antimicrobianos.

2.2.2 Objetivos Específicos

- Dopar as nanopartículas de β-TCP com agentes antimicrobianos em uma matriz resinosa de um adesivo ortodôntico;
- Avaliar a perda mineral dos cimentos ortodônticos comerciais e experimentais contendo agentes antimicrobianos;
- Caracterizar mecanicamente os cimentos ortodônticos comerciais e experimentais submetidos ao desafio cariogênico;
- Caracterizar fisicamente e quimicamente esses cimentos ortodônticos;

2.3 Metodologia

2.3.1 Materiais

Dentre os agentes antimicrobianos utilizados na composição dos grupos a serem avaliados estão o Triclosan 97% (TRI - Fagron; São Paulo, Brazil), Gluconato de clorexidina 50% (CHX - Neobrax; Barretos, Brasil), Cloreto de benzalcônio 50% (BAC - Alpha; Tianjin, China) que serão utilizados conforme recebidos. As nanopartículas de prata (AgNP) serão sintetizadas conforme descrito previamente por (Noremberg, et al, 2013) baseada na metodologia descrita por (Yim, 2004).

A obtenção do β-TCP será baseada na metodologia descrita por (Lee, 2013), através de uma síntese por precipitação química e está descrito por (Noremberg, et al, 2013) em seu trabalho de conclusão de curso.

2.3.2 Dopagem das nanopartículas de βTCP com agentes antimicrobianos

Soluções contendo os agentes antimicrobianos serão preparadas através da mistura de 30 ml H_2O + 2,5g AgNP, 30ml álcool etílico + 6g TRI, 50ml H_2O + 20ml CHX e 50 ml H_2O + 20 ml BAC afim de obter os agentes antimicrobianos na concentração de 20%. 2g de β-TCP serão adicionados através do método de imersão descrito previamente (Andrade, 2013), onde as soluções ficarão sob agitação constante por 3 horas e, após, a solução será levada à estufa à 50ºC por 24 horas.

2.3.3 Incorporação dasnanopartículas de β-TCP dopada com agentes antimicrobianos no cimento resinoso ortodôntico

As nanoparticulas de β-TCP dopadas com agentes antimicrobianos serão adicionadas em um adesivo ortodôntico comercial YStickerOrtho (YO) (YllerBiomaterials, Pelotas, Brasil) na concentração de 10%, em massa, formando os grupos experimentais: $YO_{\beta\text{-TCP@AgNP}}$, $YO_{\beta\text{-TCP@TRI}}$, $YO_{\beta\text{-TCP@CHX}}$ e $YO_{\beta\text{-TCP@BAC}}$. Como controle será utilizado $YO_{\beta\text{-TCP}}$, YO e Transbond XT (TB) (3M/Unitek, Monrovia, CA, USA).

2.3.4 Teste de contato direto

Cepas de Streptococcusmutans UA159 armazenadas a -80 ºC serão reativadas, sendo transferidos 100µl do inóculo bacteriano para um tubo estéril contendo 9 ml de LMW + 1ml de glicose, e incubados por 18 h em estufa de CO_2. Após este período, 10µl da mistura serão transferidos para uma placa contendo ágar sangue e se realizará um streak do caldo no ágar. A placa será incubada por 24h em estufa de CO_2 e a partir do crescimento registrado na placa, serão coletadas colônias isoladas, Estas serão transferidas para um tubo estéril com 9 ml de LMW + 1ml de glicose (starter) e incubadas por 18 h em estufa de CO_2.

Discos do adesivo ortodôntico com 6mm de diâmetro e 1mm de espessura (n=6), serão confeccionados com auxílio de uma matriz de silicone e fotoativados por 30 segundos. Sete grupos serão avaliados, incluindo os grupos do cimento ortodôntico comercial TB, YO, e grupos experimentais.

Os discos serão esterilizados por radiação UV e alocados individualmente nos poços das placas de cultura de 24 poços com 20µl de suspensão bacteriana, que posteriormente serão incubados a 37°C. Imediatamente será acrescentado 180 µl de TSB, para uma adequada homogeneização e será levado ao agitador por 5min e em seguida, realizada a diluição seriada, finalizando com o plaqueamento em meio TSA de 20µl de cada uma das diluições realizadas utilizando a técnica da gota. Finalmente as placas serão incubadas por 24h a 37°C. Após esse período será realizado a contagem das unidades formadoras de colônia (UFC).

2.3.5 Cimentação dos braquetes

Incisivos bovinos serão extraídos, limpos e armazenados em solução de cloramina T a 0,5%. Em seguida, discos (6 X 2 mm) serão confeccionados com auxílio de uma broca diamantada Sorensen, São Paulo,Brasil) sob refrigeração em uma furadeira Isomet Low Speed Saw (Buehler,Illinais, USA) e polidos com lixas de carbeto de silício granulação #320, #600, #1200 e #1500 por 90s. Para análise da rugosidade superficial (Ra), serão realizadas duas leituras nos discos de esmalte nas extremidades do espécime e uma leitura na região central com

auxílio de um rugosímetro digital (Mytutoyo SJ-310, Sul Americana Ltda, Japan) em um percurso de 3mm.

Para adesão dos braquetes a superfície dos discos de esmalte será condicionada com ácido fosfórico a 37% (Scotchbond; 3M ESPE, St. Paul, MN, EUA) por 15s, lavada com spray ar/água durante 60s e seca com jato de ar por 5s. O adesivo ortodôntico comercial YO, com e sem adição de β-TCP ou β-TCP@AM (β-TCP com os diferentes agentes antimicrobianos), será aplicado na base de braquetes planos de aço inoxidável (Edgewise Standard; Morelli, Sorocaba, SP) e posicionados no terço médio da superfície do disco de esmalte com auxílio de uma pinça ortodôntica de apreensão, e os excessos serão removidos com sonda exploradora. O adesivo será fotoativado por 20s nas faces mesial e distal do braquete, totalizando 40s. A polimerização será realizada utilizando LED Radii Cal (SDI; Bayswater, Australia) com irradiância 800 mW/cm^2. O TB será aplicado de acordo com as recomendações do fabricante. A superfície dos discos será condicionada com ácido fosfórico a 37% durante 15s, lavada com água e seca completamente com jato de ar. O adesivo será aplicado na região central do disco e friccionado durante 3s, seco e fotoativado durante 10s. Em seguida, uma fina camada do adesivo Transbond XT será aplicado na superfície do braquete e posicionado no terço médio da superfície. O adesivo será fotoativado por 20s nas faces mesial e distal do braquete, totalizando 40s.

2.3.6 Desafio cariogênico

A coleta de saliva estimulada será realizada por filme de parafina (Parafilm “M"®, EUA)) de um voluntário adulto, saudável e que não estiver em terapia antibiótica há 1 mês. O doador suspenderá a higiene oral no período de 24 horas previamente à coleta realizada no período matutino (em jejum). A saliva será depositada em um pote coletor graduado estéril e utilizada como inóculo.

Em seguida, a saliva será homogeinizada em em agitador Vortex (Biomixer QL-901, Biomol Equipamentos e Produtos Para Laboratórios, Ribeirão Preto, SP, Brasil) e será dispensada sobre os discos de esmalte com braquetes

cimentados na região central (n=8), em placas de 24 poços, em um volume de 400 µL por poço. Para simular os fluidos orais, após 1 hora em repouso na estufa a 37ºC será adicionado ao inóculo 1,8ml de meio DMM (com sacarose) em cada micropoço. O modelo usado será de regime semi-dinâmico, e a adição de DMM sem sacarose será efetuada em tempos de 6 horas e 12 horas, respectivamente. Após cada troca de meioas placas serão incubadas à temperatura de 37ºC e 5% de CO_2. Após cada período de desafio cariogênico durante 3 dias, os discos serão enxaguados através de imersão em 2mL de solução salina estéril, inseridos em uma nova placa contendo DMM, e novamente incubados. Os biofilmes serão formados individualmente sobre os discos de esmalte com o bráquete cimentado. As amostras serão suspensas em dispositivos de fio ortodôntico, em cada poço. As placas serão incubadas em condição atmosférica de anaerobiose (5-10% CO2 e menos que 1% O2) em jarras (Probac do Brasil produtos Bacteriológicos Ltda., Brasil) com geradores de anaerobiose (Anaerobac- Probac) mantidos em estufa a 37ºC. Os meios, DMM com e sem adição de sacarose, serão renovados diariamente. Após cada renovação, será realizada a leitura de pH (pHmetro, Brasil) do sobrenadante, individualmente em cada poço (FILOCHE; SOMA; SISSONS, 2007).Após 3 dias, os discos de esmalte com braquete serão removidos dos poços com pinça estéril e as bactérias não aderidas serão removidas gentilmente por lavagem em 2mL de solução salina estéril (THURNHEER et al. 2003). Os biofilmes serão removidos da superfície do disco com ajuda do sonicador. Para determinar a viabilidade celular será utilizado o peso seco do biofilme.

2.3.7 Resistência de união ao cisalhamento e análise do padrão de falha

Para o teste de resistência de união ao esmalte serão utilizados 84 discos preparados para o modelo de microcosmos (n=12) e utilizados após o desafio cariogênico. Os discos serão incluídos em canos de PVC com resina acrílica incolor ClassMold (Clássico, São Paulo, SP, Brasil). Uma fita de politetrafluoretileno será posicionada na face onde o braquete estará colado e em seguida, a amostra será posicionada e nivelada no centro do tubo, ficando a face vestibular exposta.

O teste de cisalhamento ao esmalte será realizado em máquina de ensaios mecânicos à velocidade de 1mm/min, utilizando atuador em forma de cinzel. As amostras serão montadas na máquina de ensaios com a interface braquete-esmalte paralelo ao cinzel. Uma carga compressiva será aplicada até desunião do braquete do esmalte. Os valores de resistência de união serão calculados em MPa considerando a área da base dos braquetes.

Realizado o teste de resistência de união ao cisalhamento, com uma lupa estereoscópica sob o aumento de 10×, será analisada a superfície onde o braquete será fixado, a fim de classificar o tipo de falha de acordo com o índice de adesivo remanescente (IAR), seguindo os seguintes escores:

Escore 0: nenhuma quantidade de material adesivo aderida ao dente;
Escore 1: menos da metade do material aderido ao dente;
Escore 2: mais da metade de material aderida ao dente;
Escore 3: todo material aderido ao dente.

2.3.8 Dureza

Após a leitura do índice de adesivo remanescente, as amostras serão seccionadas ao centro passando pela região de adesão do cimento entre o esmalte e o braquete através de um disco diamantado de alta concentração em cortadeira de precisão (Isometlowspeed, Bhueler, Lake Bluff, IL, EUA). Os fragmentos serão embutidos novamente em cilindros com resina acrílica e em seguida, a superfície interna de secção serão polidas com lixas de carbeto de silício (SiC) #320, #600, #1200 e #1500 durante tempo de 30 segundos. A fim de mensurar a perda mineral, as amostras serão submetidas ao teste de microdureza Knoop em um microdurômetro HMV-2 (FM-700, Future Tech Corp, Tóquio, Japão), acoplado a um edentador Knoop com aplicação de 25g de carga durante 5 segundos. Serão realizadas duas linhas de 8 endentações, sendo uma partindo do centro da interface de união e outra na margem desta interface. Em cada linha, as endentações serão feitas nas profundidades de 10, 20, 30, 40, 50, 100, 150 e 200 μm. A área integrada de desmineralização (ΔS) será calculada

pela subtração do perfil de dureza nas leituras marginais por aquelas obtidas no centro (esmalte hígido).

2.3.9 Confocal

Para o teste de microscopia confocal de varredura a laser foram utilizadas amostras (n=2) expostas anteriormente ao desafio cariogênico. Cada braquete será descolado da amostra e o remanescente adesivo será corado com brometo de etidio e laranja de acrinidina e em seguida lavado com PBS durante 1 minuto. O brometo de etidio é um corante verde que envolve microorganismos vivos e mortos. O laranja de acrinidina, em contraste, penetra apenas nas células mortas. Após, a vitalidade bacteriana do biofilme será visualizado por microscopia confocal de varredura a laser (TC5 SP8, Leica, Tokyo, Japan).

2.3.10 Grau de conversão

O grau de conversão de C=C (GC) dos cimentos ortodônticos será avaliado por meio de espectroscopia no infravermelho por transformada de Fourier (Prestige-21; Shimadzu, Tóquio, Japão), utilizando um cristal de diamante como dispositivo de refletância total atenuada. Serão dispensadas, sobre o cristal de diamante, amostras dos cimentos comerciais e experimentais em quantidade suficiente para cobrir a área do cristal (n=3). Uma varredura inicial do material não polimerizado (monômero) será realizada utilizando uma faixa espectral entre 1500 e 1800cm^{-1}, resolução de 4cm^{-1}. Será realizado fotoativação do adesivo ortodôntico por 20s utilizando um LED com irradiância de 1200mW/cm^2 (Radii Cal; SDI, Bayswater, Victoria, Austrália). Um suporte será acoplado para a fixação da unidade fotoativadora ao espectrofotômetro, permitindo a padronização de uma distância de 5 mm entre a extremidade da ponteira de fibra ótica e a amostra. O grau de conversão, por segundo, será calculado considerando a intensidade da vibração do tipo estiramento da dupla ligação carbono-carbono na frequência de 1635 cm^{-1}. O estiramento simétrico

do anel aromático em 1710 cm^{-1} das amostras polimerizadas e não polimerizadas será utilizado como padrão interno.

2.3.11 Sorção e Solubilidade

Sessenta espécimes (n=10) em forma de discos (6mm x 1 mm) serão preparados e fotoativados por 20 segundos, em ambas superfícies. Em seguida, os espécimes serão armazenados em local seco, com gel de sílica, em estufa a 37° C e pesados após intervalos de 24h, utilizando uma balança digital analítica (AUW22-D; Shimadzu; Quioto; Japão) com acurácia de 0,01 mg. Este ciclo será repetido até obter uma massa constante (m_1). Espessura e diâmetro também serão medidos de forma aleatória para calcular o volume da amostra. Os espécimes serão individualmente imersos em água destilada e armazenados a 37º C. Após 7 dias, o excesso de água da superfície dos espécimes será removida e a massa de cada espécime será mensurada novamente (m_2). Os espécimes serão novamente armazenados a seco à 37º C e pesados até obter uma massa constante (m_3). A sorção de água e solubilidade serão calculados como a porcentagem de ganho e perda de massa durante os ciclos de sorção e dessorção.

2.3.12 Microscopia de força atômica

Serão recortados, discos de esmalte bovino (n=10) de diâmetro 6mm x 1mm de espessura, para cada um dos 6 grupos, e neles colados braquetes ortodônticos na região central. Essas amostras serão utilizadas previamente no modelo de microcosmos, que após período de avaliação, serão lavadas em água destilada em ultrassom e secas a 37ºC por 24 horas, para análise em AFM. Os dados serão obtidos por equipamento de microscopia (SPM9500J3; Shimadzu, Tokyo, Japão), pelo modo de análise de contato. Um cantiléver de nitreto de silício, medindo 2 microns a uma velocidade de varredura de 1 Hz, se deslocará sobre a amostra por diferença piezoeléctrica. Serão realizadas 5 leituras por amostra em uma área demarcada de 5x5 microns, em diferentes posições ao redor do

bráquete. A rugosidade média (Ra) será calculada e as imagens serão obtidas em pixels de 256x256.

2.3.13 Análise estatística

Os dados de teste de contato direto, microcosmos, resistência de união ao cisalhamento, índice deadesivo remanescente, sorção e solubilidade e grau de conversão da camada híbrida serão submetidos àanálises estatísticas adequadas, utilizando o *software* SigmaStat v.3.5 (Systat Software Inc., EUA) considerando nível de significância de 0,001.

2.4. Aspectos éticos:

As análises microbiológicas que necessitarem de aprovação do comitê de ética, somente será realizada mediante aprovação do Comitê de Ética da Faculdade de Odontologia da Universidade Federal de Pelotas, para garantir a cientificidade da pesquisa e sua eticidade. O projeto será submetido ao comitê de ética local através da Plataforma Brasil.

2.5 Orçamento

Orçamento Item	**Quantidade**	**Preço Unitário**	**Total, R$**
Clorexidina	200mL	70,00	70,00
Triclosan	40g	35,00	35,00

Cloreto de Benzalcônio	200mL	19,50	19,50
Prata	40g	23,00	23,00
TCP	-	62,40	62,40
Bráquetes	26 caixas (260)	11,00	286,00
Canos de PVC	180	0,25	45,00
Resina acrílica	1	34,98	34,98
Cimento ortodôntico	12	150,00	1.800,00
Placas de cultura 24 poços	14	4,85	67,90
Caldo BHI	-	328,50	328,50
Meio Ágar BHI	-	261,90	261,90
Ponteiras Natural 1000-5000 uL	1pct (100)	21,70	21,70
Tubos eppendorfs	1pct (1.000)	29,30	29,30
Pipeta plástica 25mL	10	2,40	20,40
Pipeta com gravação	10	4,40	40,40
Placas Petri	27pct (270)	3,60	367,20
Ponteiras Amarelas	2pct (1.000)	8,40	16,80
Ponteiras Azuis	2pct (1.000)	26,00	52,00
Filtro Seringa	10	7,65	70,65
Triptona	-	91,95	91,95
Extrato de levedura	-	74,30	74,30
Fosfato de potássio dibásico	-	52,40	52,40
Placas de ágar sangue	2pct (20)	32,05	64,10
LMW	-	217,00	217,00
anaerobac	1 caixa	145,85	145,85
Placas de 96 poços	9	5,90	53,10
Despesas com impressão	8	10,00	80,00
		Total	**4.431,00**

2.6 Cronograma

CRONOGRAMA																									
Atividades	**2015**										**2016**												**2017**	
	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F
Revisão da Literatura																								
Redação do Projeto																								
Aquisição dos Materiais																								
Qualificação						28/ago																		
Testes Mecânicos																								
Testes Biologicos																								
Resultados e Análise de Estatística																								
Redação																								
Defesa																							**17/fev**	
Concluído						Em Andamento										Tarefa a Executar								

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Apêndice

Apêndice 1.

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE)

Convidamos o (a) Sr (a) para participar da Pesquisa — Caracterização de adesivos ortodônticos contendo diferentes agentes antimicrobianos sob a responsabilidade da pesquisadora Carianne Mendes de Almeida,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

Esta seção se refere a descrição das complementações e alterações que foram necessárias após aprovação do projeto de pesquisa, que explicam as diferenças entre o projeto inicial e os artigos a seguir apresentados.

O primeiro teste a ser realizado foi o teste de contato direto, com o propósito de comprovar a eficácia antimicrobiana através das zonas de inibição do crescimento bacteriano para cada grupo avaliado. Comprovando essa ação, a fim de complementar os resultados e decidimos fazer o ensaio de biofilme.

A ideia de realizar uma revisão sistemática surgiu com o propósito de ampliar o conhecimento na área de sistemas de adesão para ortodontia, focando na eficácia de novos agentes antimicrobianos, incorporados em materiais adesivos ortodônticos, inibirem a desmineralização ao redor dos braquetes.

O segundo teste realizado foi o modelo de microcosmos. Para a preparação das amostras, foram cortados discos de esmalte bovino com auxílio de uma broca diamantada e polidos com lixas de carbeto de silício. Para comprovar a lisura superficial do esmalte do dente bovino e não interferir nos resultados de biofilme, foi realizada a leitura da rugosidade superficial média (Ra) de cada corpo de prova. Em seguida, a face vestibular dos discos foi usada para cimentar os braquetes com os diferentes grupos avaliados.

Na etapa dos testes mecânicos, surgiu a possibilidade de mandarmos as análises do teste de grau de conversão para a Universidade Federal do Ceará (UFC), Fortaleza-Ceará. O grau de conversão testado foi através da camada híbrida, utilizando o microscópio MicroRaman. A técnica de Espectroscopia Raman permite avaliar com maior precisão a profundidade de polimerização e o grau de conversão dos materiais.

Devido aos prazos, a análise de Microscopia de Força Atômica deverá ser posteriormente realizada, bem como os resultados finais de dureza. Nesta primeira etapa da análise de dureza, ficou decidido fazer 6 corpos de prova para cada grupo testado e posteriormente serão finalizados.

Artigo 1

Efficacy of antimicrobial agents incorporation in orthodontic bonding systems: a systematic review and meta-analysis

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Short title. Antimicrobial agents in orthodontic bonding systems

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Abstract

This study aimed to investigate the effectiveness of orthodontic bonding systems containing different antimicrobial agents by a systematic review of the literature and meta-analysis. In addition, aimed to evaluate the influence of the incorporation of these agents in the adhesion properties. The following eight databases were screened: PubMed (Medline), Web of Science, Scopus, Lilacs, Ibecs, BBO, Scielo and Google Scholar using the search strategy developed for PubMed (MedLine). The inclusion criteria were articles that investigated the antimicrobial activity of experimental and / or commercial orthodontic materials containing different antimicrobial agents and their adhesion properties. After screening, 32 sicentific articles fulfiled all the criteria and were included. Ten studies were excluded from the meta-analysis because they did not present quantitative data of antimicrobial activity. Antimicrobial agents such as silver nanoparticles, benzalkonium chloride, chlorhexidine, triclosan, CPC, GCE, acid ursolic, DMADDM, DMAHDM, MPC, TAT, ZnO, OTi_2 have been found in papers discussing their incorporation into orthodontic adhesion system. All studies reported the inclusion of antimicrobial agents in adhesive systems as an effective dental treatment strategy. Antimicrobial agents were able to inhibit bacterial growth against *S. mutans*, *S. sobrinus* and *Lactobacillus*, without compromising their adhesive properties. Although there is evidence of antibacterial activity from *in vitro* studies, it still needs to be evaluated over longer periods and by *in vivo* models to evidence the longevity of this effect.

Keywords: antibacterial effects, dental adhesive, systematic review

Introduction

White spot lesions are frequently observed on the enamel surface of orthodontic patients (1, 2). The prevalence of this lesions varied from 59.2% to 68.8% in females and 55.0% to 61.9% in males (3, 4). The enamel demineralization occurs due to specific alterations in the oral environment, such as prolonged bacterial plaque retention in the irregular surfaces of orthodontic appliances (i.e. threads, brackets and elastics), and lower pH conditions produced by cariogenic bacteria (5, 6). This condition contributes to the development of early lesions of caries in a relatively short period of time. Considering that the orthodontic treatment is performed, on average, for approximately three years, it may be necessary to use strategies that minimize the appearance of caries lesions around the brackets (7).

Preventive approaches such as oral hygiene habits, fluoride regimens and dietary control are generally indicated to avoid increased risk of caries during fixed orthodontic treatments. However, strategies that do not depend on patient compliance may be more effective in controlling demineralization. Due to this some orthodontic bonding systems, as Transbond Plus (3M/UNITEK, Monrovia, CA, USA) e Fuji Ortho LC (GC Corporation, Tokyo, Japan) contain in their composition fluoride as an alternative to reduce demineralization. Nonetheless, they are considered complementary and non-substituting systems in antimicrobial capacity (8-10).

In order to improve the antimicrobial activity of orthodontic bonding systems, some studies have suggested the incorporation of antimicrobial agents into orthodontic bonding systems as prevention of demineralization through bactericidal and bacteriostatic action. Among the substances with potential for bacterial inhibition, it was reported that the MDPB (4), Glutaraldehyde silver nanoparticles (11-13) chlorhexidine, triclosan (14-16) or benzalkonium chloride (17, 18) have an antimicrobial effect. Studies have suggested that these antimicrobial agents are selectively toxic to oral streptococci, and their incorporation in orthodontic adhesive bonding systems help to prevent demineralization of the enamel without compromising its mechanical adhesion properties (11, 16, 19). However, some recent studies indicated a possible

reduction in the bond strength caused by some antimicrobial agents, which may compromise the orthodontic treatment (15, 16, 20)

The incorporation of antimicrobial agents in a resinous base of an orthodontic bonding system must be able to present antimicrobial effect, as well as to maintain their bonding capacity. Therefore, the objective of this systematic review was to evaluate the efficacy of orthodontic bonding systems containing different antimicrobial agents. Additionally, it was evaluated the influence of antimicrobial agent incorporation in the bonding properties of these materials. The hypotheses evaluated was that the incorporation of antimicrobial agents would improve antimicrobial activity of orthodontic bonding systems without affect bonding properties.

Materials and Methods

This systematic review is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA Statement)(21). The research question was: Does the incorporation of antimicrobial agents in orthodontic bonding systems effective in improving the antimicrobial activity without compromising the bonding properties?

Search strategies

The literature search was carried out by two independent reviewers until October 2016. The following eight databases were screened: PubMed (Medline), Web of Science, Scopus, Lilacs, Ibecs, BBO, Scielo and Google Scholar using the search strategy developed for PubMed (MedLine) (Table 1) and adapted for other databases. The references cited in the articles included were also checked. After the identification of articles in the databases, the articles were imported into Endnote X7 software (Thompson Reuters, Philadelphia, PA, USA) to remove duplicates.

Study selection

Two authors independently assessed the titles and abstracts of all of the documents. The studies were analyzed according to the selection criteria described in Table 2. Full copies of all of the potentially relevant studies were identified. Those appearing 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 were assessed independently and in duplicate by two authors. Any disagreement regarding the eligibility of the included studies was resolved through discussion and consensus or by a third reviewer. Only papers that fulfilled all of the eligibility criteria were included.

Data extraction

The data were extracted using a standardized form. If there was some information missing, the authors of the included papers were contacted via e-mail to retrieve any missing data. The following data were tabulated: demographic data, primary and secondary methodology (Table 3). The characteristics of the included studies, such as study (authors), publication year, country, primary outcomes and secondary outcomes (Table 4), and materials evaluated, control group, antimicrobial evaluation method, microorganism type, time points and main findings were also analyzed (Table 5).

Assessment of risk of bias

The methodological quality was assessed by the two reviewers. Studies were evaluated and classified according the following items: teeth randomization, sample size calculation, presence of control group, previous surface polishing and incomplete outcome data.

Statistical analysis

The analyses were performed using Review Manager Software version 5.2 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark). The global analysis was carried out using a random-effects model, and pooled-effect estimates were obtained by comparing the standardized mean

difference of each antimicrobial orthodontic bonding system compared with the respective control group (control). Different evaluation methods were considered in the global analysis, as agar diffusion test, biofilm assay and optical densities bacterial. For each method it was also performed a subgroup analysis regarding antimicrobial effect against *Streptococcus mutans* and *Streptococcus sobrinus*. Besides, for agar diffusion and biofilm a subgroup analysis considering only *Streptococcus sobrinus* was also be able to be performed.

In addition, for studies that presented the bond strength evaluation, the global analysis comparing the bond strength of orthodontic antimicrobial adhesives with the respective control was also carried out using a random-effects model. A p value < 0.05 was considered to be statistically significant. Multiple groups from the same study were analyzed according to Cochrane guidelines for combining groups (22). Statistical heterogeneity of the treatment effects among studies were assessed using the Cochran's Q test and the inconsistency I^2 test, in which values greater than 50% were considered to be indicative of substantial heterogeneity (23).

Results

Search strategy

Fig. 1 is a flowchart that summarizes the article selection process according to the PRISMA Statement (21). A total of 1320 potentially relevant records were identified from all of the databases. After the title and abstract examination, 1219 studies were excluded because they did not meet the eligibility criteria. Of the 101 studies retained for detailed review, 69 studies were excluded because they did not present antimicrobial action associated with the bond strength of the tested materials. A total of 32 studies fulfilled all the selection criteria and were included in the systematic review. Of the total of 32 articles, 10 were excluded from the meta-analysis because they did not present quantitative data of antimicrobial activity.

Descriptive analysis

The studies were published between 2002 and 2016. Twelve different types of antimicrobial agents incorporated into a resinous base of an orthodontic bonding system were evaluated. The Transbond XT was used as a control in 22 papers. All papers evaluated the effect of these materials against *Streptococcus mutans*, while 5 evaluated against *Streptococcus sobrinus*, and 8 against *L. casei, C. albicans, L. gasseri, S. sanguinis, S. gordoniu, esterococcus faecalis sanguis* and *acidophilus.* Besides, the studies evaluated the effect of incorporating chloride cetylpyridinuim (CPC), galla chinensis extract (GCE), oxide titanium (TiO_2,), triclosan, ursolic acid (1 study); dimethylaminododecyl methacrylate (DMADDM), dimethylaminohexadecyl methacrylate (DMAHDM,), 1,3,5-triacryloylhexahydro-1,3,5-triazine (TAT) (2 studies); benzalkonium chloride, zinc oxide, (3 studies); chlorexidine, methacryloyloxyethyl phosphorylcholine (MPC) (4 studies); silver nanoparticles (7 studies) in orthodontic bonding systems and 6 studies with only commercial orthodontic bonding systems. Also, the agar diffusion test was used to evaluate the antimicrobial activity in 15 studies, the optical densities bacterial in 2 studies, and biofilm in 17.

Risk of bias of included studies

Concerning the quality assessment (Fig. 2), no study reported teeth randomization or sample size calculation. The studies presented a low risk of bias in the following items: presence of control group and incomplete outcome data. Besides, half of the studies reported previous surfing polishing.

Meta-analysis

A meta-analysis was performed for 22 studies. The overall standardized mean difference for agar diffusion test (A), biofilm assay (B) and optical density bacterial (C) test was respectively 3.71 (95% CI 2.98 to 4.43) (Fig. 3), 0.41 (95% CI -0.05 to 0.86) and 6.78 (95% CI 4.78 to 8.77). In agar diffusion and optical

density the antimicrobial agent incorporation in orthodontic bonding systems showed higher antimicrobial activity than control group ($p < 0.05$). However, for biofilm the materials did not present antimicrobial activity ($p > 0.05$). Considerable heterogeneity was observed in all global analysis ($I^2 > 90\%$).

The subgroup analysis considering only *Streptococcus mutans,* the antimicrobial materials showed higher antimicrobial activity than control in all evaluation methods ($p < 0.05$). However, for *Streptococcus sobrinus* the antimicrobial orthodontic adhesives only showed higher antimicrobial activity than control in agar diffusion test ($p < 0.05$), while for biofilm both materials were similar ($p<0.05$).

Regarding bond strength, the global analysis showed antimicrobial orthodontic bonding systems were statistically similar than control, with an overall standardized mean difference equal to -0.32 (95% CI -0.74 to 0.10) (Fig 4). Considerable heterogeneity was also observed in this analysis ($I^2 = 87\%$).

Discussion

Through this systematic review and meta-analysis it was possible to demonstrate that the incorporation of antimicrobial agents in the orthodontic bonding systems had an antimicrobial effect for agar diffusion test, optical densities bacterial test and for the subgroup *Streptococcus mutans* in all evaluation methods, without affecting mechanical properties of these materials. The hypothesis tested was accepted, once orthodontic bonding systems with antimicrobial agents were able to reduce the antimicrobial activity without compromising their bonding properties. However, it is important to emphasize that in some studies an excess amount of antimicrobial agents impaired the mechanical properties of the materials. The excess of an antimicrobial agent incorporated in any adhesion system deteriorates the adhesive-enamel interface, due to the cyclic fatigue exerted during chewing, which induces microgaps at the interface. Microgaps can harbor biofilms and their production causes demineralization at the margins of the brackets directly affecting the adhesion

properties (24). Due to this, the determination of antimicrobial agent optimal concentration to improve antimicrobial activity without impair other properties is essential to the development of antimicrobial orthodontic bonding systems (Altmann 2015).

One of the agents that showed antibacterial effect was silver. The incorporation of these nanoparticles into two different orthodontics materals Transbonsd XT (3M / Unitek, Monrovia, CA, USA) and Fuji ORTHO LC (GC Corporation, Tokyo, Japan) showed that adhesion to cariogenic streptococcus was significantly lower after 24h in agar diffusion and biofilm assay. These materials with a silver concentration of 250ppm or 500ppm were able to prevent enamel demineralization (11, 19, 25). Although the detailed mechanism of the silver nanoparticle antimicrobial system is still not well determined, it has been suggested that the active oxygen formed by the catalytic action of silver causes structural damage to the bacteria (11, 21). As a result, the silver ion denatures proteins and enzymes of these bacteria leading to their inactivation. A study evaluating different concentrations of silver (500ppm, 800ppm and 1500ppm) in orthodontic bonding system, however, noted a decrease in bond strength at the concentration of 1500 ppm of AgNP (19). Besides, the incorporation of these antimicrobial agents reduced the degree of conversion (19) and surface roughness, important methodologies that correlate and are important for the final results of bacterial adhesion (11, 16, 17).

Another agent evaluated was benzalkonium chloride (BAC), which reported inhibition for *S. mutans* with continuous and constant antimicrobial activity over time in four studies, making it of potential clinical use. These studies evaluated BAC antimicrobial action at different times: 180, 240 and 270 days, in concentrations varying from 0.12% to 2.5%. The 0.25% and 0.75% concentrations exhibited antibacterial properties and low cytotoxicity (17). In addition, after 8 months of storage in distilled water, the materials retained antimicrobial properties at levels comparable to those observed in the test period. This consistency over time is important for orthodontic treatment that lasts on average 2 to 3 years (15). BAC is soluble in water, and a study that evaluated an orthodontic bonding system with BAC showed its solubility is directly related to the release of the antimicrobial agent and consequently in the microbiological

result (16). BAC is a quaternary ammonium that acts on the plasma membrane causing structural loss in the organization and integrity of microbial cells, in addition to other effects such as denaturation of proteins and enzymes (26).

Meanwhile, the use of chlorhexidine as an antimicrobial agent incorporated into orthodontic bonding systems has shown it could help to prevent demineralization of the enamel, probably due its ability to be stably immobilized and released in small amounts over the time and be partially soluble in water (13). The biofilm assay results, unlike BAC, showed that 1.0% to 1.5% chlorhexidine is the maximum and sufficient concentration to maintain antimicrobial activity against *Streptococcus mutans* and *Streptococcus sobrinus* without compromising the bond strength both for the enamel as for the dentin substrate (16, 27). Appropriate concentrations of chlorhexidine, ranging from 1.0% to 1.5% in orthodontic adhesion systems, have no adverse effect on the physical state of thermocycling properties and degree of conversion (13, 16).

Other antimicrobial agents such as DMAHDM and MPC have also been studied. One strategy that has been used is the combination of these different antimicrobial agents. DMAHDM and silver nanoparticles act on bactericidal properties and MPC reduces the protein coverage that acts as a substrate for binding of oral bacteria (17, 18, 28, 29). Methods of evaluation as Protein adsorption were determined using a micro bicinchoninic acid method. A dental plaque microcosm biofilm model was used to investigate metabolic activity. The addition of 3% MPC + 1.5% DMAHDM + 0.1% NAG substantially inhibited the biofilm viability and acid production without compromising the bond strength of the brackets to the enamel (29).

Also, the incorporation of a compound containing triazine at 15% and 20% concentrations in an experimental orthodontic bonding system resulted in lower degradation, higher bond strength and reduced the bacterial growth of *S. mutans* in the biofilm (30, 31). Zinc Oxide (ZnO) and Titanium Oxide (OTi_2) also have antimicrobial effects incorporated into orthodontic bonding systems Transbonsd XT (3M / Unitek, Monrovia, CA, USA) or Fuji ORTHO LC (GC Corporation, Tokyo, Japan). Zinc oxide serves as an activator of enzymes that may be toxic to microbes. At concentrations of 4, 6 and 16 ppm it was reported by agar diffusion

test, after 48h incubation, that this antimicrobial agent inhibits bacterial growth (20). Orthodontic composites containing 1% of TiO2 nanoparticles can have bacterial effects after 30 days by direct contact, without compromising the bond strength (32, 33).

Some commercial orthodontic bonding systems already contains antimicrobial agents, such as Transbonsd XT (3M / Unitek, Monrovia, CA, USA), Fuji ORTHO LC (GC Corporation, Tokyo, Japan), Clearfil Protect Bond (marca) and iBond. Transbond Plus and Fuji ORTHO LC contain fluoride in their composition and showed no adherence to *S. mutans* in the first 24 hours, that remained continuous after 60 days (34-36). Studies conclude that the duration of fluoride release is very short. The antimicrobial properties of fluoride are limited, and their release occurs mainly under brackets, where it is often inherent or can not avoid demineralization (15, 34, 37). On the other hand, Clearfil Protect Bond (CPB, Kuraray Medical Inc., Okayama, Japan) presents the monomer MDPB in its composition togheter with fluoride. MDPB 12-metacriloiloxidodecilpiridínio de Brometo is a quaternary ammonium with mainly bacteriostatic action, and presented a significant effect against enamel demineralization after 30 days of dilution (34, 35). Meanwhile, iBond Gluma Inside (iBond, Heraeus Kulzer GmbH) contains glucaraldehyde, which is iBond Gluma Inside (iBond, Heraeus Kulzer GmbH) contains glucaraldehyde, which is a desensitizer that helps reduce or eliminate bacterial levels on the tooth surface. The iBond showed antimicrobial activity against *S. mutans* and *Lactobacillus* after 48 hours of incubation (34).

Most studies have generally evaluated only bacterial activity for *streptococcus mutans*. However, *streptococcus mutans* and *streptococcus sobrinus* are the main microorganisms associated with dental caries. *Streptococcus sobrinus* is considered an isolated microorganism for patients highly susceptible to caries. Studies that tested the antimicrobial action of BAC incorporated in orthodontic bonding systems against *streptococcus sobrinus* did not present statistical differences when compared with the control group tested (15-17). One explanation for this bacterial action would be the high cariogenic potential of *S. sobrinus* compared to *S. mutans.* unlike BAC, showed that 1.0% to 1.5% chlorhexidine is the sufficient concentration to maintain antimicrobial

activity against *S. mutans* and *S. sobrinus* without compromising the bond strength both for the enamel. Only one study evaluated Lactobacilos. These microorganisms have acidogenic and acidic activity and are responsible for the progression of caries disease. Clearfil Protect Bond and iBond adhesives were able to inhibit bacterial growth for both S. mutans and L. gasseri in the agar diffusion test after 48 hours of incubation (34).

The main methodologies used for the antimicrobial action were the agar diffusion method and the biofilm assay. Agar diffusion test is an in vitro bacterial sensitivity assay against antimicrobial agents. However, it is a simpler susceptibility method, which acts on the surface of the samples. The current trend is the use of the microcosm model, since it offers the advantage of approaching physico-chemical, microbiological and nutritional conditions, in addition to maintaining the complexity and heterogeneity of the plaques in vivo (38). For the bond strength test it is difficult to determine maximum bond strength values given the wide range of numerical results and high variation in the test parameter. As caries is a mutifatorial disease, with several microorganisms involved, the microcosmos test turns out to be more reliable in the detection of bacterial inhibition. Orthodontic bonding systems should be capable of bonding the enamel surface with sufficiently strong bond strength to the masticatory forces without damaging the enamel during take-off. Therefore, union resistance studies should be interpreted with caution by the many variables involved (33) as type of bracket, type of test, type of teeth. It is also important to correlate the antibacterial potential of antimicrobial agents with cytotoxicity and other physico-chemical properties such as conversion degree, sorption and solubility, and pH.

The presence of antimicrobial agents in orthodontic bonding systems has been demonstrated through in vitro studies that may help reduce demineralization around the brackets. However, the antibacterial activity still needs to be evaluated over longer periods and by in vivo models to evidence the longevity of this effect. Therefore, the clinical efficacy of these materials needs to be studied, as no clinical study has yet been performed to confirm the antimicrobial effect of these substances. These advances in research may be important in the control of biofilm formation, since fixed orthodontic appliances

are the main sites of bacterial plaque retention due to their irregular surface prone to colonization by cariogenic bacteria.

Conclusion

Antimicrobial agents incorporated in orthodontic bonding systems were able to inhibit bacterial growth against *S. mutans, S. sobrinus* and *Lactobacillus sp.*, without compromising their adhesive properties. However, evidence of the results should be undertaken with caution, once clinical and long-term studies are still necessary to confirm the effectiveness of antibacterial orthodontic bonding systems in preventing caries disease.

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Table 1 - Search strategy used in PubMed (*MedLine*).

	Search Terms
#5	Search #1 AND #2 AND #3 AND #4

#4	Orthodontics OR Orthodontics
#3	Bracket, Orthodontic, Orthodontic Bracket OR Dental Braces OR Brace, Dental OR Orthodontic Braces OR Brace, Orthodontic OR
#2	Dental Cements OR Dental Cements OR Resin Cements OR Cement, Resin OR Resin Cement OR Cements, Resin OR Light-Curing of Dental Cements OR Dental Cements Light-Curing OR Light Curing of Dental Cements OR Orthodontic Adhesives OR Orthodontic Adhesives Cure OR Curing, Dental Cement OR Dental Cement Curing OR Cementation OR Dual-Curing of Resin Cements OR Orthodontic Cement OR orthodontic cements OR Orthodontic Composite OR Orthodontic Composites OR Dental Bonding OR Bonding, Dental OR Dental Bonding, Chemically-Cured OR Chemically-Cured OR 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	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, Antibacterial OR Anti Bacterial Agents OR Antibacterial Agents OR Biofilm OR Bacterial Adhesion OR Dental Deposits OR Adhesion, Bacterial OR Antibacterial activity

Table 2 - Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
▪ Antimicrobial activity in experimental orthodontic materials and / or commercial antimicrobial ▪ Antimicrobial bonding agents for orthodontics	▪ Literature reviews and systematic reviews ▪ Antimicrobial agents used as surface treatment ▪ Evaluation of bond strength to bands ▪ Only data of bond strength for antimicrobial materials

.

Table 3. Description of demographic data, primary methodology and secondary methodology.

Study (authors)	Year	Country	Primary outcomes	Secondary outcomes
Ahn(11)	2009	Korea	Antimicrobial evaluation and Bond strenght	Surface characteristics test, Bracket bonding test , ARI scores
Ahn(9)	2010	Korea	Antimicrobial evaluation	Surface roughness (mm), Surface fre energy (mJ/m)
Al Musallam(39)	2006	United States	Antimicrobial evaluation	-
Altmann(30)	2015	Brazil	Antimicrobial evaluation and Bond strenght	Degree of conversion and Knoop microhardness
Altmann(31)	2016	Brazil	Antimicrobial evaluation	Degree of conversion, Knoop microhardness, Bracket dislodgement, Mineral deposition
Caldeira(8)	2013	Brazil	Antimicrobial assay	Fluoride release analysis
Choi(25)	2012	Korea	Antimicrobial assay	-
Chung(13)	2016	Korea	Antimicrobil assay and Bond strenght	ARI scores
De Aquino(40)	2008	Brazil	Bond strength	ARI scores

Degrazia(19)	2016	Brazil	Antimicrobial assay and Bond strenght	Degree of conversion, Contact angle, Surface free energy
Jacobo(34)	2013	Spain	Antimicrobial assay	-
Jatania(20)	2014	India	Antimicrobial assay and Bond strenght	-
Kassis(35)	2010	Líbano	Antimicrobial assay	-
Know(27)	2009	Korea	Antimicrobial assay and Bond strenght	-
Lee(41)	2009	Korea	Antimicrobial assay	Surface roughness and surface free energy
Li(42)	2013	China	Antimicrobial assay and Bond strenght	-
Matalon(36)	2005	Israel	Antimicrobial assay	-
Melo(43)	2013	Brazil	Antimicrobial assay	Hardness
Melo(7)	2014	United States	Antimicrobial assay and Bond strenght	ARI scores
Mirhashemi(44)	2013	Iran	Antimicrobial assay	-
Othman(15)	2002	United States	Antimicrobial assay and Bond strenght	-
Poosti(32)	2012	Iran	Antimicrobial assay and Bond strenght	ARI scores

Saito(17)	2009	Japan	Antimicrobial assay	Cytotoxicity assay
Sehgal(16)	2007	Indian	Antimicrobial assay and Bond strenght	
Singh(45)	2013	Indian	Antimicrobial action	Shear bond strength, Diametral tensilde strength,
Spencer(46)	2009	France	Antimicrobial assay and Bond strenght	ARI scores
Wang(47)	2014	China	Antimicrobial assay and Bond strenght	-
Wang(48)	2016	China	Antimicrobial assay and Bond strenght	-
Zhang(28)	2015	China	Antimicrobial assay and Bond strenght	ARI scores
Zhang(29)	2016	China	Antimicrobial assay and Bond strenght	ARI scores
Zhang(49)	2016	China	Antimicrobial assay and Bond strenght	ARI scores, Calcium (Ca) and phosphate (P) ion release
Zhang(50)	2016	China	Antimicrobial assay and Bond strenght	ARI scores

Table 4 - Description antimicrobial evaluation

Study (authors)	Materials evaluated	Control group	Antimicrobial evaluation method	Microorganism type	Time points	Main findings
Ahn,2009(11)	Transbond XT with silica nanofillers + silver nanoparticles 0,250 and 500 ppm	Transbond XT	Agar diffusion test and Biofilm assay	*S. mutans* (ATCC25175), S. sobrinus (ATCC33478)	Agar 3,6,9,24 h, Biofilm 3,6 h	Silica nanofillers and silver nanoparticles helped to prevent enamel demineralization around their surfaces without compromising physical properties
Ahn,2010(9)	Enlight, Lightbond, Monolok2, Transbond Plus, Fuji ORTHO LC, Multi-cure	Transbond XT	Biofilm assay	*S. mutans* OMZ65, S. sobrinus 6715	Biofilm 3,6 h	That initial mutans adhesion was significantly influenced by surface free energy characteristics of adhesives
Al Musallam,2006(39)	Transbond XT with cetylpyridinium chloride-2.5%, 5.0%, and 10.0% (CPC)	Transbond XT	Agar diffusion test	*S. mutans* 10449	7,15,30,60,180 days	The incorporation of 2.5% Cetylpyridinium chloride in adhesive material imparted antimicrobial activity without altering diametral tensile strength

Altmann,2015(30)	Experimental orthodontic adhesive with 1,3,5-triacryloylhexahydro-1,3,5-triazine (TAT)	Experimental orthodontic adhesive	Biofim assay	*S.mutans* UA 159	24 h	Orthodontic adhesives containing (1,3,5-triacryloylhexahydro-1,3,5-triazine are promising antibacterial materials, especially those with 15% and 20%(1,3,5-triacryloylhexahydro-1,3,5-triazine
Altmann,2016(31)	Experimental orthodontic adhesive with TAT and phosphate invert glass containing 10 mol% of niobium pentoxid	Transbond XT	Biofilm assay	*S.mutans* UA 159	24 h	The orthodontic adhesive, with addition of 20% by weight) 1,3,5-tryacryloylhexahydro-1,3,5-triazine, exhibited antibacterial activity and was able to induce mineral deposition on its surface in vitro
Caldeira,2013(8)	Transbond Plus, Fuji ORTHO LC	Transbond XT	Biofilm assay	*S. mutans* (ATCC 25175), *L. casei* (ATCC 4646) and *C. albicans* (ATCC 10231),	24 h	Transbond XT Plus Color Change was the one that presented the best general behavior considering the evaluated aspects

Choi,2012(25)	Light Bond ,Blugloo Transbond XT ,Fuji ORTHO LC with silver nanoparticles	Light Bond ,Blugloo , Transbond XT , Fuji ORTHO LC without silver nanoparticles	Agar diffusion test and Optical densities bacterial	*S. mutans* 25175	Agar 24 h, Densities 3,6,9,12,24 h	The incorporation of silver nanoparticles into orthodontic adhesives inhibited cariogenic bacterial growth
Chung,2016(13)	Transbond XT primer with chlorhexidine and Transbond XT primer with ursolic acid	Transbond XT	Biofilm assay and Optical densities bacterial	*S. mutans* UA159	Biofilm 24 h, Densities 72 h	The incorporation of chlorhexidine into an orthodontic primer helped prevent demineralization of the enamel around the surfaces without compromising its physical properties
Degrazia,2016(19)	Transbond XT with silver nanoparticle 0,11%, 0,18% and 0,33%	Transbond XT	Agar diffusion assay and Growth inhibitory activity	*S. mutans* UA159	Agar=48 h, Inhibitory activity = 24 h	The addition of silver nanoparticle solutions to Transbond™ XT adhesive primer inhibited *S. mutans* growth
Jacobo,2013(34)	Clearfil Protect Bond ; Clearfil Self-etching Bond; Transbond Plus Self-Etching Prime; iBond	Transbond XT	Agar diffusion test	0.35 *L. gasseri*, 0.30 *S. mutans*	48 h	Clearfil Protect Bond has shown antimicrobial properties in vitro, the use of this self-etching adhesive contributed to reduce microbial decalcification, making the use of this self-etching adhesive an

						attractive option for bracket bonding
Jatania,2014(20)	Fuji ORTHO LC with 23,1% zinc oxide powder, Fuji ORTHO with 13% zinc oxide powder	Transbond XT	Agar diffusion test	*S. mutans*	48h	The incorporation of zinc oxide into a resin modified light cure Fuji Ortho, added antimicrobial property to the original compound
Kassis,2010(35)	Transbond Plus Self Etching Primer with Transbond XT and Clearfil Protect Bond with Kurasper	Transbond XT	Biofilm assay and Effect of adhesive type	*S. mutans* ATCC 25175	Biofilm 48 h, Adhesive type 90 min.	Transbond Plus increased antibacterial capacity due to the fluoride release pattern called. Clearfil Protect Bond limited the external action around brackets in an inhibitory bacteriostatic effect
Know,2009(27)	4-acryloyloxyethyl trimellitate anhydride and methyl methacrylate-tri-n-butylborane (4-META/MMA-TBB) resin with chlorhexidine digluconate (0.5%, 1.0%,1.5%, 2.0%, 2.5%, and 3.0%)	(4-META/MMA-TBB) resin with chlorhexidine digluconate (0%)	Agar diffusion test	*S. mutans* (ATCC 25175, KCTC 3065), *S. sobrinus* (KCTC 5134), *S. sanguinis* (KCTC 3287), *S. gordonii* (KCTC 3297), *Enterococcs faecalis*	24h	Incorporation of 1.0–1.5% digluconate into the 4-META resin was optimal in terms of antibacterial effects and bond strength to the tooth

				(ATCC 19433, KCTC 3206		
Lee,2009(41)	Lightbond, Transbond Plus and Fuji ORTHO LC	Transbond XT	Biofilm assay	*S. mutans* OMZ65*, S. sobrinus* 6715	3,6 h	Surface free energy characteristics play na important role in the initial mutans adhesion to orthodontic materials
Li,2013(42)	Fuji ORTHO LC with nanosilver 3% with the weight ratio of 1:99. 3:97. 5:95. 10:90 and 15:85	Fuji ORTHO LC	Agar diffusion test and Direct contact test	*S. mutans* (OD 0.6 at 650 nm).	Agar 48 h, Contact direct 1 day, 1, 2, 3, 4, 6, 8 weeks	Nanosilver contributed to decrease the demineralization rate around brackets without compromising bond strength
Matalon,2005(36)	Fuji ORTHO LC and Transbond Plus	Transbond XT	Agar diffusion test and Direct contact test	*S. mutans* 23571M	Agar 48 h, Contact direct 1 day, 1 week, 1 month	The reinforced glass ionomer cement possessed the most potent and long-lasting antibacterial activity
Melo,2013(43)	Fluoride microfilled composite, Orthodontic fill magic,and Flouride nanofilled composite orthocem	Natural Ortho (Non fluoride microfilled composite)	Biofilm assay and Concentrations of acid-soluble	*S. mutans* UA159	Biofilm 24 h, Concentration 3 h	Under the cariogenic exposure condition of this study, the fluoride-releasing nanofilled material had similar

						performance to fluoride-releasing microfilled materials
Melo,2014(7)	Transbond XT with 1.5%, 3%, 5% quaternary ammonium monomer dimethylaminododecyl methacrylate (DMADDM)	Transbond XT	Biofilm assay, MTT assay of metabolic activity, Lactic acid production and colony-forming unit (CFU) counts	*S. mutans*	48 h	The novel antibacterial orthodontic cement containing 3% quaternary ammonium monomer dimethylaminododecyl methacrylate inhibited oral biofilms without compromising the enamel bond strength, and was promising to reduce or eliminate demineralization in enamel around orthodontic brackets
Mirhashemi,2013(44)	Transbond XT with nanoparticle, chitosan and zinc oxide 1%,5% and 10%	Transbond XT	Agar diffusion test and Biofilm assay	*S. mutans* ATCC 25175, *S. sanguis* ATCC *10556* *L. acidophilus* ATCC 4356	Agar 48 h, Biofilm 24 h	A mixture of nanoparticle + chitosan and nanoparticle + zinc oxide has induced an antibacterial activity in resin composite; especially in 10% weight concentrations which was significantly

						higher than other groups
Othman,2002(15)	Reliance Phase II chemical cure composite with benzalkonium chloride 0.25%, 0.75%, 1.25%,1.75% and 2.5%	Reliance Phase II chemical cure composite	Agar diffusion test	*S. mutans* 10449, *S. sobrinus* B13	48 h	The incorporation of benzalkonium chloride in composite material added antimicrobial properties to the original compound without altering its mechanical properties
Poosti,2012(32)	Transbond XT with nanocomposite Titanium oxide (TiO_2)	Transbond XT	Biofilm assay	*S. mutans* ATCC 25175	48 h	The addition of titanium oxide nanoparticles to the orthodontic compound increased their antibacterial effects without compromising shear bonding strenght
Saito,2009(17)	Superbond C&B resin with benzalkonium chloride , 0.75%, 1.25%, 1.75%, 2.5%, and 5.0%	Superbond C&B resin with benzalkonium chloride 0,25%	Agar diffusion test	*S. mutans* 10449 and PS14, *S. sobrinus* 6715 and B13	48 h	The addition of benzalkonium chloride to resin confered an antibacterial effect .

Singh,2007(45)	Transbond XT with Chlorhexidine 0% and 2,5 %	Transbond XT	Antimicrobial action	*S. mutans*	48h, 6 days	Addition of chlorhexidine to the orthodontic composite resin enhanced its antimicrobial properties
Spencer,2009(46)	Fuji ORTHO LC with 13% and 23,1% zinc oxide	Transbond XT	Agar diffusion assay	*S. mutans*	48 h	The incorporation of zinc oxideinto Fuji Ortho LC added antimicrobial properties to the original compound without significantly altering the shear bond strength
Wang,2014(47)	Clearfil SE Bond with Dimethylaminododecyl methacrylate 2,5% and 5%(DMADDM)	Clearfil SE Bond	Biofilm assays and Lactic acid measurement	*S, mutans* ATCC 700610, UA159	Biofilm and Lactic acid, Confocal laser scanning microscopy, Scanning electron microscopy, 4,24,72 h	The bonding agents have the potential to control dental biofilms and combat tooth decay, and Dimethylaminododecyl methacrylate waspromising for use in a wide range of dental adhesive systems and restoratives
Wang,2016(48)	Fuji ORTHO LC with Galla chinensis extract	Fuji ORTHO LC	Agar diffusion test and Bacteria colonization susceptibility.	*S. mutans* (strain 25175)	48h	Galla chinensis extract containing adhesive cement exhibited a promising inhibitory

	0.1, 0.2, 0.4, and 0.8% (GCE)					effect on S. mutans growth and adhesion. Without compromising bond strength, adding GCE in adhesive cement was an attractive option for preventing white spot lesions
Zhang,2015(28)	Vitremer with 0.1% silver nanoparticles, Vitremer with 3% 2-methacryloyloxyethyl phosphorylcholine (MPC) and Vitremer with 0.1% silver nanoparticles and 3% (MPC)	Transbond XT and Vitremer	Biofilm assay, Protein adsorption, Live/dead assay, Lactic acid production and colony-forming	*S.mutans*	48 h	The novel antibacterial and protein-repellent Resin-modified glass ionomer cement with substantially-reduced biofilm acids was promising as an orthodontic cement to combat white spot lesions in enamel
Zhang, 2016(29)	Vitremer with 3% MPC, Vitremer with 1.5% DMAHDM, Vitremer with 0.1% silver nanoparticle, Vitremer with 3% MPC and 1.5% DMAHDM, Vitremer with 3% MPC and 0.1% silver nanoparticles, Vitremer with 1.5% DMAHDM and 0.1% silver	Transbond XT and Vitremer	Biofilm assay, Protein adsorption, Live/dead assay, Lactic acid production and colony-forming	*S.mutans*	48 hours	The novel bioactive cement was promising for orthodontic applications to hinder biofilms and plaque buildup and enamel demineralization.

	nanoparticle, Vitremer with 3% MPC, 1.5% DMAHDM and 0.1% silver nanoparticle					
Zhang 2016(49)	Vitremer with 3% MPC, 1.5% DMAHDM and 0.1% silver nanoparticle, Vitremer with 3% MPC, 1.5% DMAHDM, 0.1% silver nanoparticle, 10% NACP and 15% BisGMA-TEGDMA, Vitremer with 3% MPC, 1.5% DMAHDM, 0.1% silver nanoparticle, 15% NACP and 15% BisGMA-TEGDMA, Vitremer with 3% MPC, 1.5% DMAHDM, 0.1% silver nanoparticle, 20% NACP and 15% BisGMA-TEGDMA	Transbond XT and Vitremer	Biofilm assay, Protein adsorption, Live/dead assay, Lactic acid production and colony-forming	S.mutans	48 hours	A novel multifunctional orthodontic cement was developed with strong antibacterial and protein-repellent capabilities for preventing enamel demineralization
Zhang, 2016(50)	Vitremer with 1,5% ,3% and 5% MPC(- 2	Transbond XT and Vitremer	Biofilm assay, Protein adsorption, Live/dead assay, Lactic acid	*S.mutans*	48 hours	The 2 methacryloyloxyethyl phosphorylcholine -

methacryloyloxyethyl phosphorylcholine)	production and colony-forming	containing Resin-modified glass ionomer cement was promising to reduce biofilms and white spot lesions without compromising orthodontic bracket

Table 5 - Description bond strength evaluation

Study (authors)	Materials evaluated	Control group	Type of teeth	Number of teeth	Bond Strength evaluation method	Bracket type	Evaluation period
Ahn,2009(11)	Transbond XT with silica nanofillers + silver nanoparticles 0,250 and 500 ppm	Transbond XT	Human premolars	60	Stored in deionized Water for 24h at 37 ◦C. A universal testing machine (Instron 4465, Canton, MA, USA) with a crosshead speed of 1mm/min was used. An occlusogingival load was applied to the bracket using a chisel-edge plunger, the maximum load was recorded in kgf and then converted into MPa	Victory series, 3M,Unitek	24 h
Altmann,2015(30)	Experimental orthodontic adhesive with 1,3,5-triacryloylhexahydro-1,3,5-triazine (TAT).	Experimental orthodontic adhesive	Bovine incisors	48	Stored in distilled water at 4ºC for less than 3 months. A universal testing machine (EMIC DL200; Equipamentos de Ensaio Paraná, Brazil) using a knife-edged chisel	Roth Max, Morelli, Brazil	24 h

					with a crosshead speed of 1 mm per minute, megapascals.		
Chung,2016(13)	Transbond XT primer with chlorhexidine and Transbond XT primer with ursolic acid	Transbond XT	Bovine incisors	48	.A universal testing machine (Instron 4465, Canton, Mass) with acrosshead speed of 1mm/minute was used. Maximum loads were recorded in kgf and then converted into MPa	Artista series, Ortho-Direct, St Ann, Mo	24 h
De Aquino,2008(40)	Transbond XT/Clearfil Protect Bond and Fill Magic Orthodontic	Trasnbond XT/Clearfil Protec Bond	Bovine incisors	20	A universal testing machine Emic DL 20.000 (Emic®, São José dos Pinhais, PR, Brasil) with a crosshead speed of 1mm/min was used. Was recorded in kgf and then converted into MPa	Discovery, Dentaurum Ispringen, Germany	24 h
Degrazia,2016(19)	Transbond XT with silver nanoparticle 0,11; 0,18 and 0.33%	Transbond XT	Bovine incisors	84	After 24 h in distilled water, the teeth were positioned in a Universal Testing Machine Shimadzu	Dental Morelli, Sorocaba, SP, Brazil	24 h

					EZ-SX (Shimadzu Corp., São Paulo, SP, Brazil). The shear bond strength was tested using a chisel blade with crosshead speed of 0.5 mm/min and 500 N load cell.		
Jatania,2014(20)	Fuji ORTHO LC with 23,1% zinc oxide powder, Fuji ORTHO with 13% zinc oxide powder	Transbond XT	Human premolares	48	Stored in a solution of 0.2% (weight/volume) thymol. Universal testing machine having a capacity of 20,000 N	Gemini 3M Unitek Monrovia, California, USA	24 h
Kwon,2009(27)	(4-META/MMA-TBB) resin containing chlorhexidine digluconate (0.5%, 1.0%,1.5%, 2.0%, 2.5%, and 3.0%)	4-META/MMA-TBB) resin containing chlorhexidine digluconate (0%)	Human third molars	140	Immersed in water at 37 ºC for 24 h. Universal testing machine (Model 4202, Instron Inc., Canton, MA) at a crosshead speed of 1 mm/min	-	24 h
Li,2013(42)	Fuji ORTHO LC with nanosilver 3% with the weight ratio of 1:99. 3:97. 5:95. 10:90 and 15:85	Fuji ORTHO LC	Human premolars	160	Instron Universal testing instrument (8841,Instron Corp) using a looped	Masel Titan 9000TM Roth	24 h

					rectangular, stainless steel wire at a crosshead speed of 1 mm/min		
Melo,2014(7)	Transbond XT with 1.5% DMADDM, 3% DMADDM, 5% DMADDM (quaternary ammonium monomer dimethylaminododecyl methacrylate)	Transbond XT	Human first premolars	30	Incubated in distilled water at 37 °C for 24 h, Universal Testing Machine (MTS, Eden Prairie, MN), occlusogingival load (1 kN; speed = 0.5 mm/min) was applied to the bracket	Ormco, Glendora, California,USA	24 h
Othman,2002(15)	Reliance Phase II chemical cure composite (Reliance Orthodontic Products) with benzalkonium chloride 0.25%, 0.75%, 1.25%,1.75% and 2.5%	Reliance Phase II chemical cure composite (Reliance Orthodontic Products)	Bovine incisors	32	Incubated in distilled water at 37 °C for 24 h. The universal testing machine	Traction hooks	24 h
Poosti,2012(32)	Transbond XT with nanocomposite Titanium oxide (TiO_2)	Transbond XT	Human first premolars	30	Immersed in deionized water for 24 hours at 37°C. Bond strength was determined by a universal testing machine (Zwick, GMbH, Vlm, Germany), the bracket base (MPa = N/mm2)	Dentaurum, Ispringen, Germany	24 h
Seghal,2007(16)	Composite with benzalkonium chloride , Composite with Triclosan	Composite United Bonding	Human premolars	80	Storage in distilled water, 25 days, Universal	TP Orthodontics,	24 h

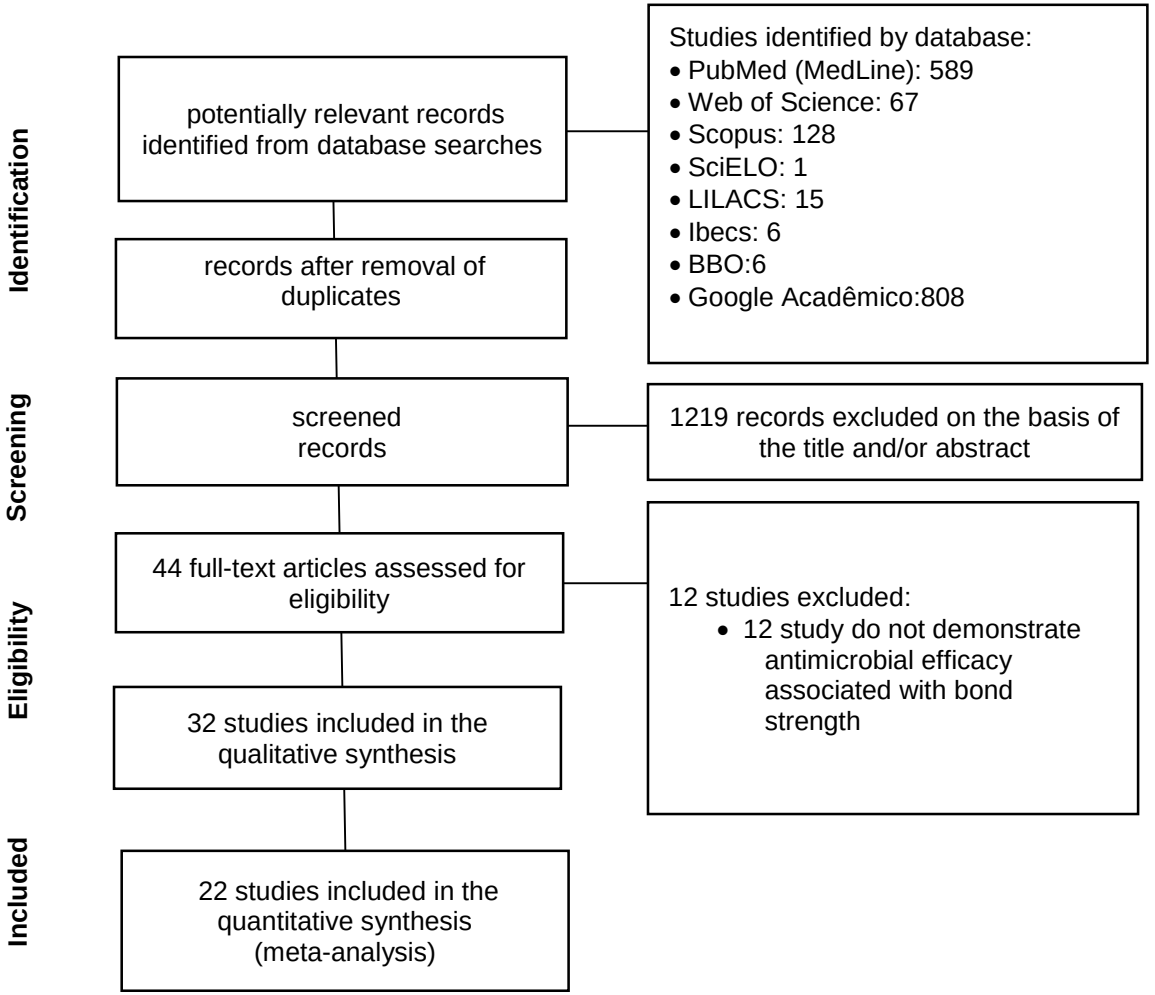
	and Composite with Chlorhexidine	Adhesive(3M Unitek)			testing machine (AG-I series AUTOGRAPH, Shimadzu, Kyoto, Japan) having a capacity of 1 ton (1000 kgf)	LaPorte, Indian	
Singh,2013(45)	Transbond XT with Chlorhexidine 0% and 2,5 %	Transbond XT	Human premolars	60	Measurement was performed using a column-type motorized constant loading machine delivering a perpendicular force to the bracket by a flat end steel rod from the machine, producing a shear force at the bracket-tooth interface. Each specimen was tested at a cross-head speed of 1 mm/min	-	24 h
Spencer,2009(46)	Fuji ORTHO LC with 13% and 23,1% zinc oxide	Transbond XT	Deciduous bovine incisors	60	Stored in water at 37ºC for at least 24 hours, A screw-driven universal test machine (model 5567, Instron, Norwood, Mass), with a crosshead speed of	Strite Industries, Cambridge, Ontario, Canada	24 h

					1 mm/min		
Wang,2014(47)	Clearfil SE Bond with Dimethylaminododecyl methacrylate (DMADDM) 2,5% and 5%	Clearfil SE Bond	Human third molars	30	Storage in deionized water at 37 °C for 24 h, Universal Testing Machine (MTS, Eden Prairie, MN, USA) at a cross-head speed of 1 mm/min	-	24 h
Wang 2016(48)	Fuji ORTHO LC with Galla chinensis extract 0.1, 0.2, 0.4, and 0.8% (GCE)	Fuji ORTHO LC	Human premolars	50	Stored in a 10% formalin solution at room temperature. Universal testing machine (MTS, Eden Prairie, USA) was used, crosshead speed of 0.5 mm/min	Gemini, 3M Unitek, Monrovia, USA	24 h
Zhang,2015(28)	Vitremer with 0.1% silver nanoparticles, Vitremer with 3% 2-methacryloyloxyethyl phosphorylcholine (MPC) and Vitremer with 0.1% silver nanoparticles and 3% (MPC)	Transbond XT and Vitremer	Human first premolars	100	Stored in distilled water at 37 ºC for 1 day, Universal testing machine (MTS, Eden Prairie, MN), An occlusal-gingival load (speed = 0.5 mm/min) was applied to the bracket	Ormco 2000, Sybron Dental, Orange, California, USA	24 h
Zhang, 2016(29)	Vitremer with 3% MPC, Vitremer with 1.5% DMAHDM, Vitremer with 0.1% silver nanoparticle, Vitremer with 3% MPC and 1.5% DMAHDM, Vitremer	Transbond XT and Vitremer	Human first premolars	180	Stored in distilled water at 37 ºC for 1 day, Universal testing machine (MTS, Eden Prairie, MN), An occlusal-gingival load (speed = 0.5	Ormco Series 2000, Sybron Dental, Orange,	24 h

	with 3% MPC and 0.1% silver nanoparticles, Vitremer with 1.5% DMAHDM and 0.1% silver nanoparticle, Vitremer with 3% MPC, 1.5% DMAHDM and 0.1% silver nanoparticle				mm/min) was applied to the bracket	California, USA	
Zhang, 2016(49)	Vitremer with 3% MPC, 1.5% DMAHDM and 0.1% silver nanoparticle, Vitremer with 3% MPC, 1.5% DMAHDM, 0.1% silver nanoparticle, 10% NACP and 15% BisGMA-TEGDMA, Vitremer with 3% MPC, 1.5% DMAHDM, 0.1% silver nanoparticle, 15% NACP and 15% BisGMA-TEGDMA, Vitremer with 3% MPC, 1.5% DMAHDM, 0.1% silver nanoparticle, 20% NACP and 15% BisGMA-TEGDMA	Transbond XT and Vitremer	Human first premolars	180	Stored in distilled water at 37 ºC for 1 day, Universal testing machine (MTS, Eden Prairie, MN), An occlusal-gingival load (speed = 0.5 mm/min) was applied to the bracket	Ormco Series 2000, Sybron Dental, Orange, California, USA	24 h

Zhang, 2016(50)	Vitremer with 1,5% ,3% and 5% MPC(- 2 methacryloyloxyethyl phosphorylcholine)	Transbond XT and Vitremer	Human first premolars	100	Stored in distilled water at 37 ºC for 1 day, Universal testing machine (MTS, Eden Prairie, MN). An occlusal load (speed = 0.5 mm/min) was applied to the bracket	Ormco 2000, Sybron Dental, Orange, California, USA	24 h

Figure 1. Systematic review flowchart according PRISMA Statement.


Identification
Screening
Eligibility
Included
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• Web of Science: 67
• Scopus: 128
• SciELO: 1
• LILACS: 15
• Ibecs: 6
• BBO:6
• Google Acadêmico:808
records after removal of duplicates
screened records
1219 records excluded on the basis of the title and/or abstract
44 full-text articles assessed for eligibility
12 studies excluded:
• 12 study do not demonstrate antimicrobial efficacy associated with bond strength
32 studies included in the qualitative synthesis
22 studies included in the quantitative synthesis (meta-analysis)

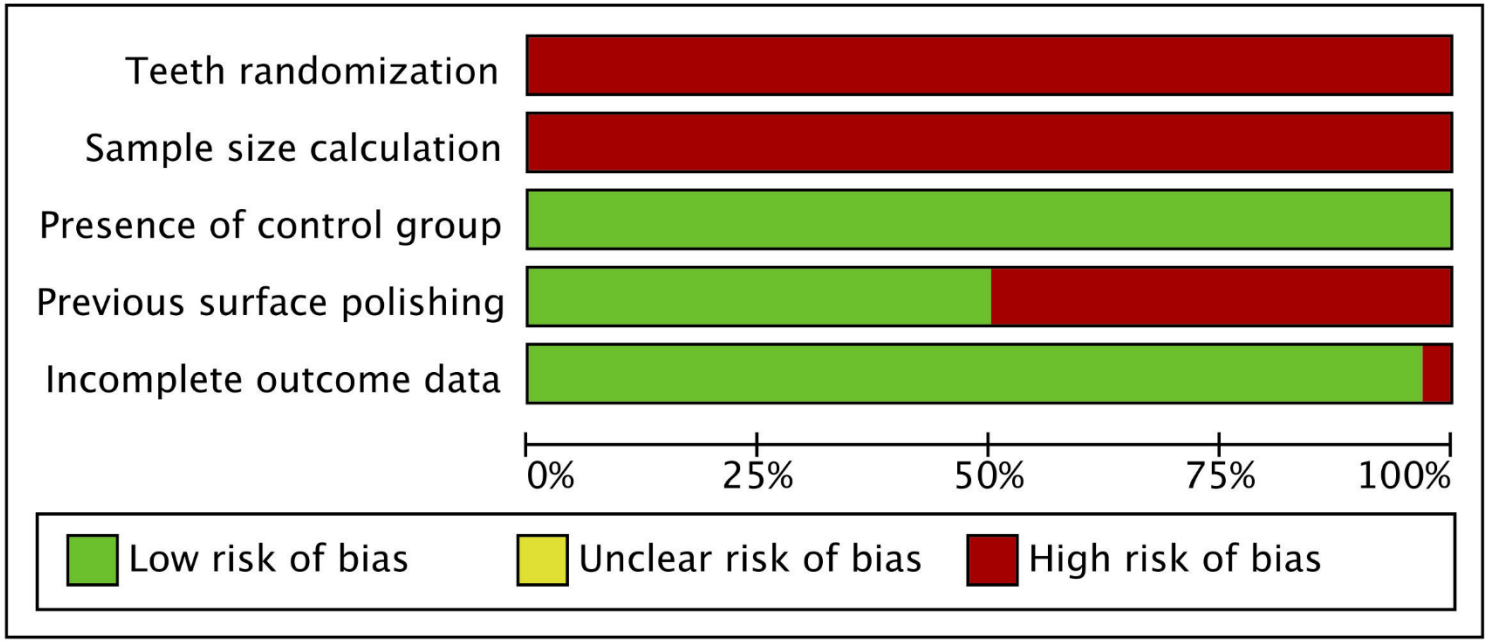

Figure 2. Review authors' judgements about each risk of bias item presented as percentages across all included studies.

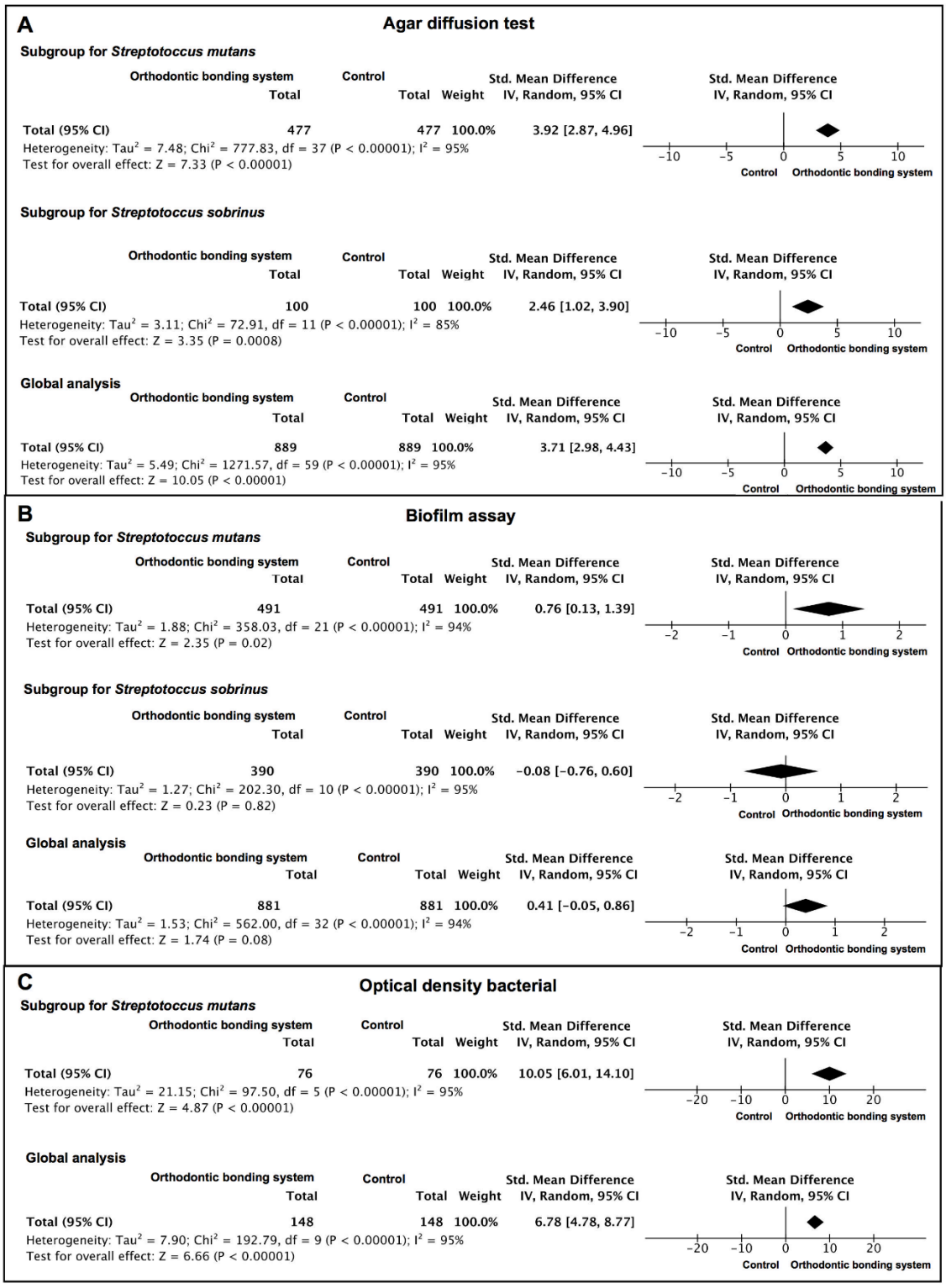

Figure 3. Standardized mean difference of antimicrobial orthodontic bonding systems when compared with conventional materials for agar diffusion test (A), biofilm assay (B) and optical density bacterial (C). Agar diffusion test and optical density the antimicrobial agent incorporation in orthodontic adhesive systems showed higher antimicrobial activity than control group ($p < 0.05$). For biofilm the materials did not present antimicrobial activity ($p > 0.05$).

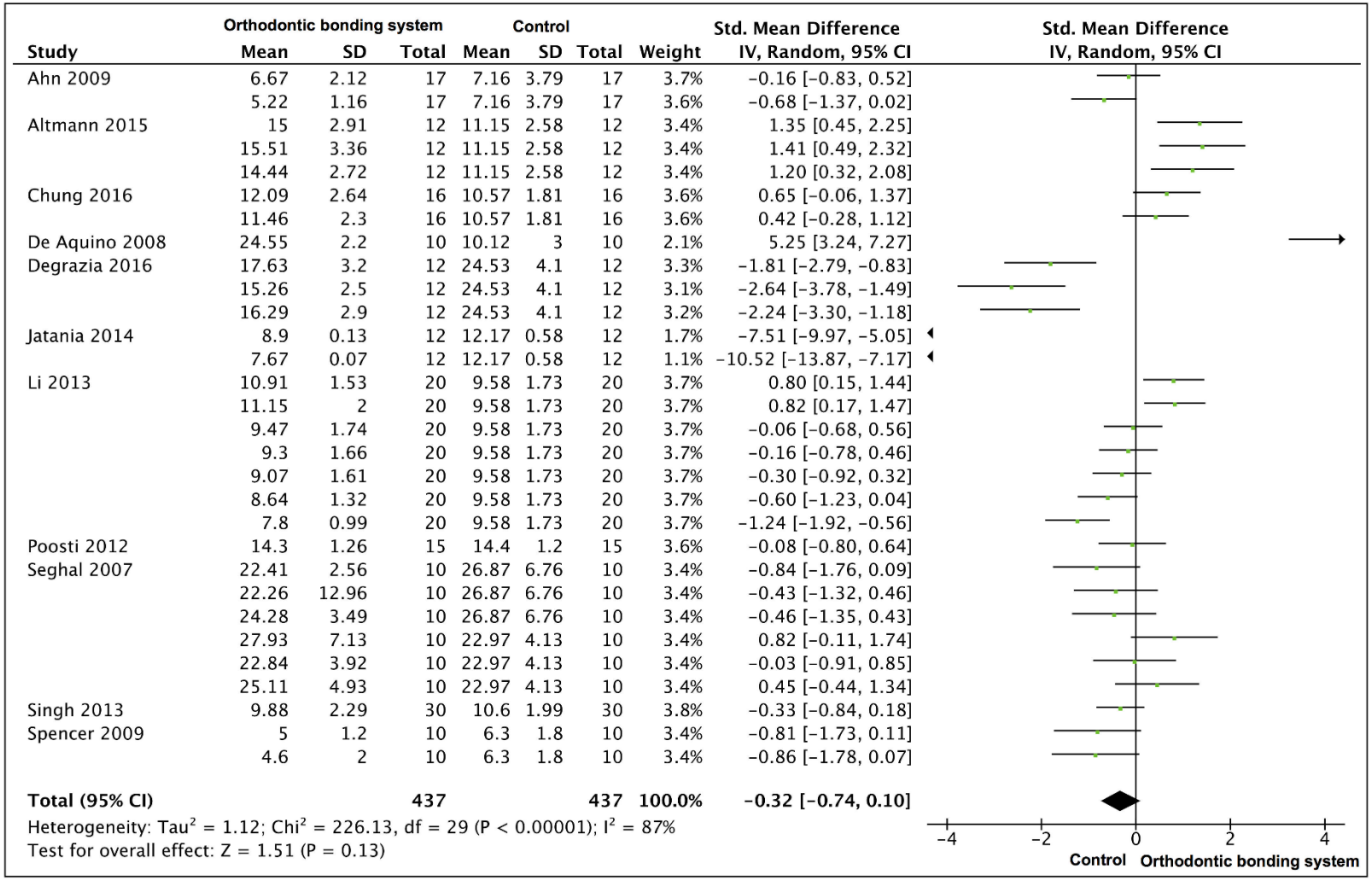

Figure 4. Global analysis of orthodontic antimicrobial bonding systems when compared with conventional materials for bond strength. No statistically significant differences among groups (p>0.05).

Artigo 2

β-TCP nanoparticles doped with antimicrobial agents as an orthodontic adhesive component.

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ABSTRACT

Objective. This study evaluated the antimicrobial effect, caries inhibition, shear bond strength, and physical-chemical properties reached by addition of β-TCP nanoparticles doped with antimicrobial agents to an orthodontic adhesive bonding brackets.
Methods. Experimental orthodontic adhesive were formulated with addition of 10% of β-TCP nanoparticles doped with antimicrobial agents to YSticker Ortho (YO - Yller Biomaterials) and used to bonding brackets to enamel of bovine incisors (n=17). As control were used β-TCP nanoparticles without doped antimicrobial agents $YO_{\beta\text{-TCP}}$ and commercial orthodontic bonding adhesives YO and Transbond XT (TB - 3M/Unitek). The antimicrobial effects of materials were assessed by modified direct contact test using *Streptococcus mutans*, confocal microscopy, and biofilm from microcosm model was cultivated in specimens under cariogenic challenge for 3 days. Brackets were submitted to shear bond strength test followed by failure mode analysis. The fractured specimens were transversally sectioned and the internal hardness of enamel was measured around the brackets to calculate the integrated mineral loss (ΔS). In addition *in situ* degree of conversion, water sorption and solubility were evaluated. Statistical comparisons were conducted at a 5% significance level.
Results. Orthodontic adhesives containing β-TCP nanoparticles doped with antimicrobial agents significantly inhibited bacterial growth, but also resulted in no effect on ΔS under and around brackets. For bond strength, no difference was observed between TB and YO. Using $YO_{\beta\text{-TCP@CHX}}$ resulted in lowest values of bond strength, while addition of β-TCP nanoparticles doped with other antimicrobial agents did not reduce the bond strength of YO. The orthodontic adhesive type did not dramatically affect the in *situ* degree of conversion, water sorption, and water solubility.
Significance. β-TCP nanoparticles doped with antimicrobial agents addition could be used to produce orthodontic adhesive with enhanced inhibition of bacterial growth without harming the other properties investigated.

Keywords: Adhesive; Antibacterial activity; Dental Caries; Orthodontic Brackets.

1 Introduction

During the orthodontic treatment, one of the most common complications and more easily acknowledged in the clinical practice is the emergence of white spot injuries around the brackets which occurs due to a higher accumulation of biofilm in this area[1,2]. This process occurs mainly by specific changes in the oral environment, such as the prolonged retention of bacterial plaque due to the irregular surfaces of the orthodontic appliances and lower pH conditions produced by cariogenic bacteria present in the oral cavity[1,3]. The high accumulation of bacteria associated to a cariogenic diet contributes for the development of initial white spot injuries in a relatively short period of time[1,2]. These white spot injuries can develop quickly and lead to cavitations within four weeks. Considering that the orthodontic treatment is carried out for, usually, a period of three years, it is necessary to make use of strategies which can minimize the emergence of caries lesions around the brackets[4].

Preventive approaches, such as oral hygiene instructions, fluoride therapy and dietary control are carried out to avoid the emergence of caries lesions during the orthodontic treatment. However, strategies which do not depend on the patient's compliance may be more efficient in the control of early teeth demineralization[5]. The fluoride releaser orthodontic adhesive used for brackets bonding, such as Transbond Plus (3M, Unitek, Brazil) and Fuji Ortho are an alternative to reduce demineralization around the brackets, but are considered complementary and non-substitutes systems in the antimicrobial capacity under lower pH conditions produced by cariogenic bacteria of the oral cavity[6-9]. Besides this, several studies[10-16] assessed the incorporation of microbial agents in resin materials to prevent the enamel demineralization through the bactericidal and bacteriostatic action.

Orthodontic adhesive resin containing containing in their formula silver nanoparticles[10-12], chlorexidine, triclosan[13,14] or benzalkonium chloride[15,16] present antimicrobial effect, being selectively toxic for *estreptococos orais*. Ahn 2009, Degrazia 2016, Atbaya 2013, suggest that the addition of these antimicrobial agents help to prevent the enamel demineralization without compromising its physical properties[10,17,18]. Another potential component to be added in dental materials is the beta tricalcium phosphate (β-TCP) which has

ions calcium and phosphorus in its chemical composition which in oral environment, in contact with the saliva, promote the enamel remineralization[10]. However, there is no evidence in literature regarding the antibacterial and remineralizing potential of orthodontic adhesive resins containing β-TCP nanoparticles doped with antimicrobial agents. Therefore, the purpose of this study was to evaluate the antimicrobial effect, caries inhibition, shear bond strength, and physical-chemical properties reached by addition of β-TCP nanoparticles doped with antimicrobial agents to an orthodontic adhesive bonding brackets. The study hypothesis was that the addition β-TCP nanoparticles doped with antimicrobial agents would present antimicrobial effect and would reduce the extension of carious lesions around orthodontic brackets without affect its bond strength to enamel and other properties investigated.

2 Materials and Methods

2.1 Materials

Antimicrobial agents Triclosan 97% (TRI - Fagron; São Paulo, Brazil), chlorhexidine gluconate 50% (CHX - Neobrax, Barretos, Brazil), and benzalkonium chloride 50% (BAC - Alpha, Tianjin, China) were used as received. Silver nanoparticles (AgNP) and β-TCP were synthesized by Noremberg, et al, 2013 and used as received.

2.2 Doping of β-TCP nanoparticles with antimicrobial agents

Solutions containing 20 % of antimicrobial agents were prepared by mixing 30 ml H_2O + 2,5g AgNP, 30ml ethyl alcohol + 6g TRI, 50ml H_2O + 20ml CHX, and 50 ml H_2O + 20 ml BAC. 2g of β-TCP were added by the previously described immersion method[19], where the solutions were under constant stirring for 3 hours and then the solution was brought to the oven at 50 ° C for 7 days.

2.3 Orthodontic bonding agents evaluated

β-TCP nanoparticles doped with antimicrobial agents were added in a commercial orthodontic bonding adhesive YSticker Ortho (YO - Yller

Biomaterials, Pelotas, Brazil) in the concentration of 10%, by mass, resulting the experimental groups: $YO_{\beta\text{-}TCP@AgNP}$, $YO_{\beta\text{-}TCP@TRI}$, $YO_{\beta\text{-}TCP@CHX}$ e $YO_{\beta\text{-}TCP@BAC}$. As control were used β-TCP nanoparticles without doped antimicrobial agents $YO_{\beta\text{-}TCP}$ and commercial orthodontic bonding adhesives YO and Transbond XT (TB - 3M/Unitek, Monrovia,CA, USA). The commercial orthodontic bonding adhesives tested in this study are described in the table 1.

2.4 *Modified Contact Direct Test*

Strains of *Streptococcus mutans* UA159 stored at - 80°C were reactivated, transferring 100 µl of the bacterial inoculum to a sterile tube containing 9 ml of LMW + 1 ml of glucose, and incubated for 18 h in anaerobic atmosphere. After this period, 10µl of the mixture was transferred to a plate containing blood agar and a broth streak was performed on the agar. The plate was incubated for 24 h in anaerobiosis. The growth isolated colonies were collected. These were transferred to a sterile tube with 9 ml of LMW + 1 ml of glucose (starter) and incubated for 18 h in CO_2 oven.

Discs 6mm diameter and 1mm (n = 6) were made using a silicon matrix and and light cured for 40 seconds on both surfaces. After, they were sterilized by ultraviolet light, during 30 minutes on each side of the sample. In each well and the material was inoculated with 20µl of *Streptococcus mutans* UA159 suspension. The discs were incubated for 1 and 24 h at 37ºC in anaerobic atmosphere. Microplate wells containing the same volume of bacterial suspension without test discs were also incubated as controls.

After, 180 µL of culture medium Brain and Heart Infusion Broth – BHI, supplemented with 10% sucrose was added in each well and shaked (Guangzhou Mecan Trading Co., Ltd., China) for 10 min. One hundred microliters were removed 100 µL of the bacterial suspension from each well was transferred to saline for carrying out serial dilutions, thus obtaining 10x dilutions (1), 100x (2), 1000x (3) and 10000x (4). Samples were plated on BHI agar, dividing them into 5 parts, they were placed in 3 aliquots (20 uL) of each dilution. The plates were incubated at 37 ° C in anaerobic atmosphere for 24h. The test was made in triplicate. Beyond the period of incubation, the Colony Forming Units (CFU/mL) was counted.

2.5 *Brackets bonding*

Bovine incisors were extracted, cleaned and stored in 0.5% chloramine T solution. Enamel disks (6 x 2 mm) were obtained using a diamond drill bit (KG Sorensen, São Paulo, Brazil) under refrigeration in a drill Isomet Low Speed Saw (Buehler, Illinais, USA) and polished with 320-, 600-, 1200- and 1500-grit SiC papers for 90 s. Surface roughness (Ra) of all enamel disks were measured by a needle probe (Mitutoyo SJ-310, Sul Americana Ltda, Japan).

The enamel was acid etched with phosphoric acid at 37% (Scotchbond; 3M ESPE, St. Paul, MN, USA) for 15 s. Afterwards, the acid was removed with water-stream by the same etching time and the enamel was air-dried. Experimental orthodontic bonding adhesives and YO was applied to the base of the brackets (Edgewise Standard; Morelli, Sorocaba, Brazil), which were hand-pressed to the center of the buccal face followed by material excess removal.

The enamel was acid etched with phosphoric acid at 37% for 20 s. Afterwards, the acid was removed with water-stream by the same etching time and the enamel was air-dried. The TB adhesive (3M/Unitek, Monrovia, CA, USA) was applied onto the etched enamel and light-cured by 20 s. TB paste was applied to the base of the brackets, which were hand-pressed to the center of the buccal face followed by cement excess removal.

Orthodontic bonding adhesives were light-cured for 20 s in mesial and distal side of the bracket, totalizing 40 s. All light-activations were performed using a LED-based light-curing unit Radii Cal (SDI, Bayswater, Victoria, Australia) with irradiance around 800 mW/cm^2.

2.6 Cariogenic challenge

This study was approved by the local Research Ethics Committee (CAAE 57403816.2.0000.5318) The enamel discs allocated to cariogenic challenge were maintained in distillated water for 24 h after the bonding of brackets (n=17). Saliva was collected from a healthy volunteer who had not been under antibiotic therapy for at least 1 year.

The volunteer abstained from oral hygiene for 24 h and food for 2 h prior to collection of 20 mL of saliva stimulated by paraffin film (Parafilm "M" ®, American National CanTM, Chicago, USA). The collected saliva inserted into a

sterile graduated tube and immediately homogenized in Vortex mixer (Biomixer QL-901, Biomol Equipments and Products for Laboratories, Ribeirão Preto, Brazil).

The enamel disks (sterilized by gamma radiation at 4.08 KGy, Theratronics, Eldorado 78, Best Theratronic LTDA, Ottawa, ON, Canada) were placed in 24 wells-plates followed by inoculation of 400 µL of saliva. After 1 h, the saliva was removed from the bottom of the wells and replaced by 1.8 mL of defined medium enriched with mucin (DMM) containing 1 % sucrose. Then, the samples were incubated in anaerobic atmospheric condition (80% N2, 10% CO_2 and 10% H_2) at 37 ° C. After 6 hours, the samples were dip-washed for 5s in sterile saline and inserted into new plates containing 1.8 ml of DMM without sucrose and incubated for 18 h at the same condition. The biofilms were cultivated for 3 days in this alternating sucrose regimen, and the medium was replaced twice per day. Unbound bacteria were gently removed by washing with 2 ml of sterile saline[20] and biofilms were carefully removed from discs surface by sonication for 30 s at 30W (Sonicator DE S500, R2D091109 Brazil). Bacterial suspension from each well was transferred to saline for carrying out serial dilutions, thus obtaining 10x dilutions (1), 100x (2), 1000x (3) and 10000x (4). To determine cell viability (n=8), the dry weight of the biofilm. Beyond the period of incubation, the Colony Forming Units (CFU/cm^{-2}) was counted.

2.7 *Confocal microscopy*

The vitality of the bacterial biofilm was visualized by confocal laser scanning microscopy (TC5 SP8, Leica, Tokyo, Japan). After cariogenic challenge, enamel discs (n = 2) were detached and the remaining adhesive stained with ethidium bromide and acrinidine orange. Ethidium bromide is a green dye that involves live microorganisms and the orange of acrinidine, in contrast, penetrates only the dead cells.

2.8 *Shear bond strength and failure mode*

After cariogenic challenge, all samples (n=12) were included in acrylic resin Class Mold (Clássico, São Paulo, Brasil) cylinder. The cylinders were positioned on a shear device with the knife-edged chisel placed at the enamel-bracket interface in an mechanical machine (EMIC DL 500, São Jose dos Pinhais, Brazil). A

compressive load was applied at a 0.5 mm/min crosshead speed until failure of the bonding. Bond strength values were calculated in MPa considering the bracket base area. After the test, the teeth surfaces were observed in a stereomicroscope, at ×40 magnification, to determine the failure mode: Type I – No adhesive covering the enamel; Type II – less than 50% of adhesive covering the enamel; Type III – more than 50% of adhesive covering the enamel; Score IV – 100% of adhesive covering the enamel.

2.9 *Hardness measurements*

After the shear test, the enamel discs (n=6) were longitudinally sectioned through their center with a water-cooled diamond saw Isomet Low Speed Saw (Buehler, Illinais, USA). One half of the disc was embedded in acrylic resin and gently sequentially polished with 320-, 600-, 1200-, and 1500-grit Sic sandpaper. Knoop hardness measurements were carried out using a micro-hardness tester HMV-2 (FM-700, Future Tech Corp, Tokyo, Japan) under load of 25 g and dwell time of 5 s. Two rows of eight indentations were made on each side of the specimen. The first row was performed in the center of enamel located below of bracket, while the other started on margin of the bonding interface. The indentations were made for both columns at depths of 10, 20, 30, 40, 50, 100, 150, and 200 μm from the surface of the specimen. The ∆S was calculated by subtracting the hardness profile in the marginal readings obtained for those in the center (sound dentin). Figure 1 shown schematic representation of cariogenic challenge, brackets submitted to shear bond strength test followed by transversally sectioned and the internal hardness of enamel was measured around the brackets to calculate the integrated mineral loss (∆S).

2.10 In situ degree of conversion (DC) within adhesive/hybrid layers

After the cariogenic challenge, the samples (n=3) were longitudinally sectioned through their center with a water-cooled low sped diamond saw (Isomet 100, Buehler, Lake Bluff, USA) into slice with cross-sectional area of approximately 1,5 mm^2 The sticks were wet polished using 1500- and 2000-grit SiC sandpaper. The specimens were ultrasonically cleaned for 10 min and positioned into micro-Raman equipment (Senterra spectrophotometer Bruker, Ettlingen, Baden-Württemberg, Germany), which was first calibrated for zero and

then for coefficient values using a silicon sample. The samples were analyzed using a 20 mW Neon laser with 532 nm wavelength, 3 μm spatial resolution, 5 cm^{-1} spectral resolution, 30 sec accumulation time with 5 co-additions and 100× magnification (Olympus Microscope, London, UK) with a beam diameter of 1 μm. Spectra were obtained at the enamel-orthodontic bonding adhesives interface at three random sites per bonded slice. Post-processing of the spectrum was performed using Opus Spectroscopy Software version 6.5. The ratio of the double bond content of monomer to polymer in the orthodontic adhesive adhesives was calculated according to the following formula: DC (%) = (1 - [R cured / R uncured)) x 100, where "R" is the ratio of aliphatic and aromatic peaks intensity at 1636 cm^{-1} and 1608 cm^{-1} in cured and uncured material.

2.11 Water *Sorption and Solubility*

Seventy disc (6 x 1mm) specimens (n = 10) were prepared using a silicon matrix and light cured for 40 seconds on both surfaces. The samples were then stored in a dry place with silica gel in an oven at 37 ° C and weighed after 7 days intervals using an analytical digital scale (AUW22-D, Shimadzu, Kyoto, Japan) with accuracy of 0,01 mg. This cycle was repeated until a constant mass (m_1) was obtained. Thickness and diameter were also measured at random to calculate sample volume. The specimens were individually immersed in distilled water and stored at 37 ° C. After 7 days, the excess water from the surface of the specimens was removed and the mass of each specimen measured (m_2). The specimens were again stored dry at 37 ° C and weighed until a constant mass (m_3) was obtained. The water sorption (WS) and solubility (SL) were calculated as the percentage of gain and mass loss during the sorption and desorption cycles.

2.12 Statistical analysis

Data from ΔS under and around brackets were individually submitted to two-way Analysis of Variance (ANOVA). Data from other variable were individually submitted to one-way ANOVA. When data failed normality or equal of variance tests, data were analyzed using ANOVA on Ranks. All pairwise multiple comparison procedures were carried out using the *Tukey's* post hoc test. A 5% significance level was set for all analyses.

3 Results

The results of antimicrobial activity of orthodontics bonding adhesives are shown in Figure 2. The orthodontic bonding agent (p=0.005) and time (p=0.005) affect the antimicrobial activity in modified contact direct test. After 1 h, the orthodontics bonding adhesives YO with or without β-TCP nanoparticles doped antimicrobial adhesives showed antimicrobial effect higher than bacterial control. TB, $YO_{\beta\text{-TCP}}$, and $YO_{\beta\text{-TCP@AgNP}}$, in contrast, showed less antimicrobial effect being similar to bacterial control. After 24 h, $YO_{\beta\text{-TCP}}$, $YO_{\beta\text{-TCP@TRI}}$, and $YO_{\beta\text{-TCP@BAC}}$ were both similar and showed antimicrobial effect higher than bacterial control, while $YO_{\beta\text{-TCP@CHX}}$ totally inhibited bacterial growth. TB, YO and $YO_{\beta\text{-TCP@AgNP}}$, in contrast, showed less antimicrobial effect being similar to bacterial control. Only $YO_{\beta\text{-TCP@CHX}}$ increased the antimicrobial activity from 1h to 24 h. The results of biofilm development were shown in Figure 3 (A-D).Biofilm development for total microorganisms (p=0.007), mutans streptococci (p=0.003) and lactobacilli (p≤0.001) were significantly affected by orthodontic bonding adhesives, except for aciduric bacteria (p=0.270). For total microorganisms (Figure 3A), TB and YO were both similar bacterial grow to YO with β-TCP nanoparticles doped antimicrobial agents, except for $YO_{\beta\text{-TCP}}$ (p≤0.022). For mutans streptococci (Figure 3C), YO with or without β-TCP nanoparticles doped antimicrobial agents were both similar bacterial grow (p≥0.055), while YO, $YO_{\beta\text{-TCP}}$, and $YO_{\beta\text{-TCP@BAC}}$ were more potent bacterial grow inhibitor than TB (p≤0.044). For lactobacilli, TB showed highest bacterial grows (p≤0.02), while $YO_{\beta\text{-TCP}}$, $YO_{\beta\text{-TCP@AgNP}}$, $YO_{\beta TCP@CHX}$, and $YO_{\beta TCP@BAC}$ were both similar and more bacterial grow inhibitor. Representative images of cell viability (green pixels: viable cells; red pixels: death cells) from biofilm acquired by confocal microscopy are shown in Figure 4.

The results of demineralization measurements after cariogenic challenge are shown in Table 1. The orthodontic bonding adhesives type (p = 0.970) not affected the demineralization under and around the orthodontic bracket. The local of hardness measurement affected (p = 0.046) the values of demineralization, while an interaction between these factors was not observed (p = 0.580). Only $YO_{\beta TCP@CHX}$ showed significantly lower hardness under bracket than around bracket.

The results of *in situ* degree of conversion, water sorption, water solubility, bond strength and failure mode analysis are shown in Table 2. No difference was observed between orthodontic bonding adhesives, except for $YO_{\beta\text{-TCP@TRI}}$ that presented significantly higher degree of conversion ($p < 0.001$). The YO showed significantly lower water sorption than the TB and similar than the $YO_{\beta TC@AgNP}$ and $YO_{\beta TCP@TRI}$. The addition of β-TCP nanoparticles doped with antimicrobial agents resulted in a significant water sorption increase for $YO_{\beta\text{-TCP}}$, $YO_{\beta\text{-TCP@CHX}}$ and $YO_{\beta\text{-TCP@BAC}}$ than the YO. There is not a statistically significant difference between orthodontic bonding adhesives, except for $YO_{\beta TCP@AgNP}$ that shown significantly higher water solubility than YO. For bond strength, no difference was observed between commercial orthodontic bonding agent TB and YO, $YO_{\beta\text{-TCP}}$, $YO_{\beta\text{-TCP@TRI}}$, and $YO_{\beta TCP@BAC}$. Using $YO_{\beta\text{-TCP@CHX}}$ resulted in lowest values of bond strength, while addition of β-TCP nanoparticles doped with other antimicrobial agents did not affect the bond strength of YO. Addition of β-TCP nanoparticles doped with antimicrobial agents to YO did not altered the distribution of failure mode (score II failure), while using $YO_{\beta\text{-TCP@CHX}}$ resulted in highest amount of score I failure.

4 Discussion

The results of the present study showed that the addition of β-TCP nanoparticles doped with antimicrobial agents in orthodontic adhesive, although did not affecting the demineralization pattern under and around the bracket, can act to inhibit bacterial growth without impairing the material properties. Orthodontic adhesive with antimicrobial and / or remineralizing potential can prevent the appearance of white spot lesions around the brackets of patients undergoing orthodontic treatment because of greater difficulty in removing the biofilm from this site[21,22]. In *in vitro* studies, antimicrobial activity can be assessed through the modified direct contact test or through models using complex multi-species biofilms from natural dental plaques[23] or saliva, which are also capable of reproducing the development of carious lesions[24].

Orthodontic adhesive with addition of β-TCP nanoparticles doped with antimicrobial agents had a higher antimicrobial effect than the bacterial control in the modified direct contact test, except for $YO_{\beta\text{-TCP@AgNP}}$. While commercial

orthodontic adhesive resin TB did not inhibit bacterial grow of *mutans Streptococci*, Being similar to bacterial control. Modified direct contact test is a quantitative and reproducible method that simulates contact of specific microorganisms with tested material by measuring its bactericidal effect[25]. In this test the bacteria are more susceptible to decease because they are not organized in the biofilm form. In addition, orthodontic adhesive is not bonded to bracket, presenting a larger contact surface for antimicrobial inhibition. Microcosm model is a multi-species ecosystem that mimics naturally formed biofilms using as human saliva inoculum[26,27]. It has the advantage of simulating the physical, chemical, microbiological and nutritional bacterial conditions, besides maintaining the complexity and heterogeneity of *in vivo* supragingival plaque, with semi continuous sucrose supplementation[28]. This study used an in vitro microcosm dental biofilm model, with a semi-continuous sucrose exposure and human saliva as inoculum. Biofilm development for total microorganisms, *mutans streptococci and lactobacilli* were significantly affected by orthodontic bonding adhesives, except for aciduric bacteria.

The antimicrobial effect of β-TCP nanoparticles doped with antimicrobial agents, is in line with previously studies that showed orthodontic adhesive containing, chlorexidine, triclosan[13,14] or benzalkonium chloride[15,16] present antimicrobial effect. CHX added in orthodontic adhesive resin can have antimicrobial effect by direct contact with CHX on the bottom of the plaque[29], or CHX can diffuse into environment inactivating bacteria by positive charge at each end of CHX which readily binding to negatively charged sites of the cell wall from microorganism[30]. BAC, as a quaternary ammonium, acts on plasma membrane causing structural loss in the organization and integrity of microbial cells, in addition to other effects such as denaturation of proteins and enzymes and, therefore, it is effective in the bacterial inhibition of microorganisms such as S. mutans and lactobacillus[31]. While TRI acts on the cytoplasmic cell membrane of the microorganism, promoting the general disorganization of the cell membrane and the specific inhibition of membrane enzymes, causing bacterial lysis[32]. Previous studies using silver nanoparticles[10,33] demonstrated reduction on growing and accumulation of Streptococcus mutans by addition of 250ppm or 500ppm of that filler to commercial adhesives. However, in the present study, the addition of β-TCP@AgNP was not able to act as bacterial grow inhibitor. It is

speculated that the rason for unsatisfactory antimicrobial effect might be linked to the reaction that occurred between β-TCP nanoparticles and silver nanoparticles. Ca^{+} and P^{-} ion released by β-TCP can neutralize the Ag ions and thus, inhibit their bactericidal function. These effectts, however, should be addressed in future studies.

Caries process observed under clinical conditions was reproduced by microcosm model. Demineralization under and around the bracket was obtained by hardness measurements after the cariogenic challenge. In this study the type of orthodontic adhesive did not affect the degree of demineralization under and around the bracket. The microcosm model results in caries-like lesions and allows to control the extension of lesion depends on the incubation time[34]. Previous studies using biofilms cultivated for 5 days demonstrated reduction on mineral loss around bracket by addition of to orthodontic bonding system.[34] However, in the present study, the biofilms cultivated for 3 days was not effectiveness on carious lesion inhibition. Microorganisms were able to recover between the periods of demineralization and remineralization without altering the hardness values of the groups evaluated[24]. In addition, β-TCP acts on dental substrate remineralization through mineral deposition of calcium phosphate[35].

β-TCP nanoparticles doped with antimicrobial agents addition did not impair physico-chemical properties of orthodontic adhesive. The results of *in situ* degree of conversion showed that no difference was observed between comercial orthodontic adhesive system TB, YO and, YO with or without β-TCP nanoparticles doped antimicrobial, except for $YO_{\beta\text{-TCP@TRI}}$ that presented significantly higher degree of conversion. Higher degree of conversion is related to better mechanical properties[36], monomer crosslink[37] and less unreacted monomer leaching[38]. The addition of β-TCP nanoparticles doped with antimicrobial agents resulted in a significant increase in water sorption of $YO_{\beta\text{-TCP}}$, $YO_{\beta\text{-TCP@CHX}}$, and $YO_{\beta\text{-TCP@BAC}}$. This effect could be related to the results of higher antimicrobial activity. Water solubility, in contrast, there is not a statistically significant difference between orthodontic bonding adhesives, except for $YO_{\beta TCP@AgNP}$ that shown significantly higher water solubility than YO.

For bond strength, no difference was observed between commercial orthodontic bonding agent TB and YO, and both with $YO_{\beta\text{-TCP}}$, $YO_{\beta\text{-TCP@TRI}}$, and $YO_{\beta TCP@BAC}$. These results is in according to previous studies that showing shear

bond strength values around 8-9 Mpa[10,33,39,40]. Orthodontic adhesives evaluated require enamel surface etching with phosphoric acid prior to bonding, they presented the same pattern of demineralization and micromechanical interlocking[41]; however YO has advantage of smaller step numbers. An orthodontic adhesive can exhibit a bond strength resistant to masticatory loads without damaging the enamel during de-bonding[42].

The results of present study showed that, in general, $YO_{\beta\text{-TCP@TRI}}$, $YO_{\beta\text{-TCP@CHX}}$, $YO_{\beta\text{-TCP@BAC}}$ can be a viable strategy in controlling antimicrobial activity without compromising the material properties. The addition of bioactive materials in dental materials, such as β-TCP, has been studied because they can promote remineralization and inhibit the caries process. Ca and P ions release induces remineralization at a low cariogenic pH when necessary to inhibit demineralization[43,44]. Furthermore, in bonding systems, bond stability is limited by the hybrid layer degradation, while Ca and P ions release can be highly beneficial in remineralization, protecting exposed collagen at the bonding interface and improving bond stability[45,46]. However, the Ca and P ion, isolated, has its release in the short term, and then this release is decreased[47,48]. A recent study showed that the association of monomer dimethylaminododecyl methacrylate (DMADDM), nanosilver particles and nanoparticles of amorphous calcium phosphate (NACP) in commercial adhesives could maintain long-term bonding effectiveness, besides having antimicrobial and remineralizing activity[49].

Conclusion

β-TCP nanoparticles doped with antimicrobial agents $YO_{\beta\text{-TCP@TRI}}$, $YO_{\beta\text{-TCP@CHX}}$, and $YO_{\beta\text{-TCP@BAC}}$ addition could be used to produce orthodontic adhesive with enhanced inhibition of bacterial growth without harming the other properties investigated.

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Table 1. Orthodontic adhesives used in this study.

Orthodontic adhesives	Manufacturer	Compound	Components
YSTICKER ORTHO	Yller Biomateriais Ltda, Pelotas, Brazil	Adhesive	Methacrylate monomers, initiators, stabilizers, inorganic filler
Transbond XT Self Etching Primer	3M/Unitek, Monrovia, CA, USA	Primer & Bond	Fluoride, no filler Methacrylate ester derivative
Transbond XT Light Cure	3M/Unitek, Monrovia, CA, USA	Paste	Quartz silica Bis-GMA, Bisphenol A Bis (2-Hydroxyethyl Ether) Dimethacrylate

Table 2. Means (standard deviation) of demineralization (ΔS) after cariogenic challenge.

Orthodontic adhesives	Local of hardness measurement	
	Under bracket	Around bracket
TB	7,623.5 (4,490.6)^{aA}	11,532.0 (6060.7)^{aA}
YO	9,148.0 (8,199.7)^{aA}	9,656.8 (6,737.9)^{aA}
$YO_{\beta\text{-TCP}}$	6,556.1 (4,296.5)^{aA}	8,771.8 (4,419.6)^{aA}
$YO_{\beta\text{-TC@AgNP}}$	8,685.9 (5,000.8)^{aA}	9,739.0 (4,891.8)^{aA}
$YO_{\beta\text{-TCP@TRI}}$	8,920.8 (5,055.5)^{aA}	7,860.6 (4,111.8)^{aA}
$YO_{\beta\text{-TCP@CHX}}$	5,730.1 (4,127.0)^{aB}	12,475.6 (8,384.6)^{aA}
$YO_{\beta\text{-TCP@BAC}}$	7,795.9 (5,000.8)^{aA}	9,646.0 (6,628.9)^{aA}

Table 3. Results of *in situ* degree of conversion (DC), water sorption (WS), water solubility (SL), bond strength, and failure mode analysis.

Orthodontic adhesives	DC (%)	WS	SL	Bond Strength (Mpa)	Failure mode (n)			
					Type I	Type II	Type III	Type IV
TB	43.5 (8.6)^{b}	0.12 (0.07)^{a}	0.01 (0.06)^{ab}	12.32 (29.82)^{a}	0	5	6	1
YO	55.5 (5.9)^{b}	0.08 (0.07)^{c}	0.01 (0.01)^{a}	9.36 (20.19)^{ab}	0	0	9	3
$YO_{\beta TCP}$	41.7 (2.3)^{b}	0.13 (0.02)^{ab}	0.01 (0.03)^{ab}	12.48 (51.45)^{ab}	0	3	7	2
$YO_{\beta TCP@AgNP}$	38.9 (5.2)^{b}	0.18 (0.08)^{bc}	0.02 (0.03)^{b}	6.99 (20.72)^{bc}	0	0	9	3
$YO_{\beta TCP@TRI}$	89.9 (8.4)^{a}	0.09 (0.14)^{abc}	0.01 (0.03)^{ab}	12.49 (23.37)^{a}	1	3	8	0
$YO_{\beta TCP@CHX}$	57.4 (8.2)^{b}	0.15 (0.07)^{ab}	0.00 (0.03)^{ab}	5.26 (36.08)^{c}	0	10	2	0
$YO_{\beta TCP@BAC}$	40.7 (8.8)^{b}	0.03 (0.04)^{ab}	0.01 (0.01)^{ab}	13.07 (55.29)^{a}	5	1	6	0

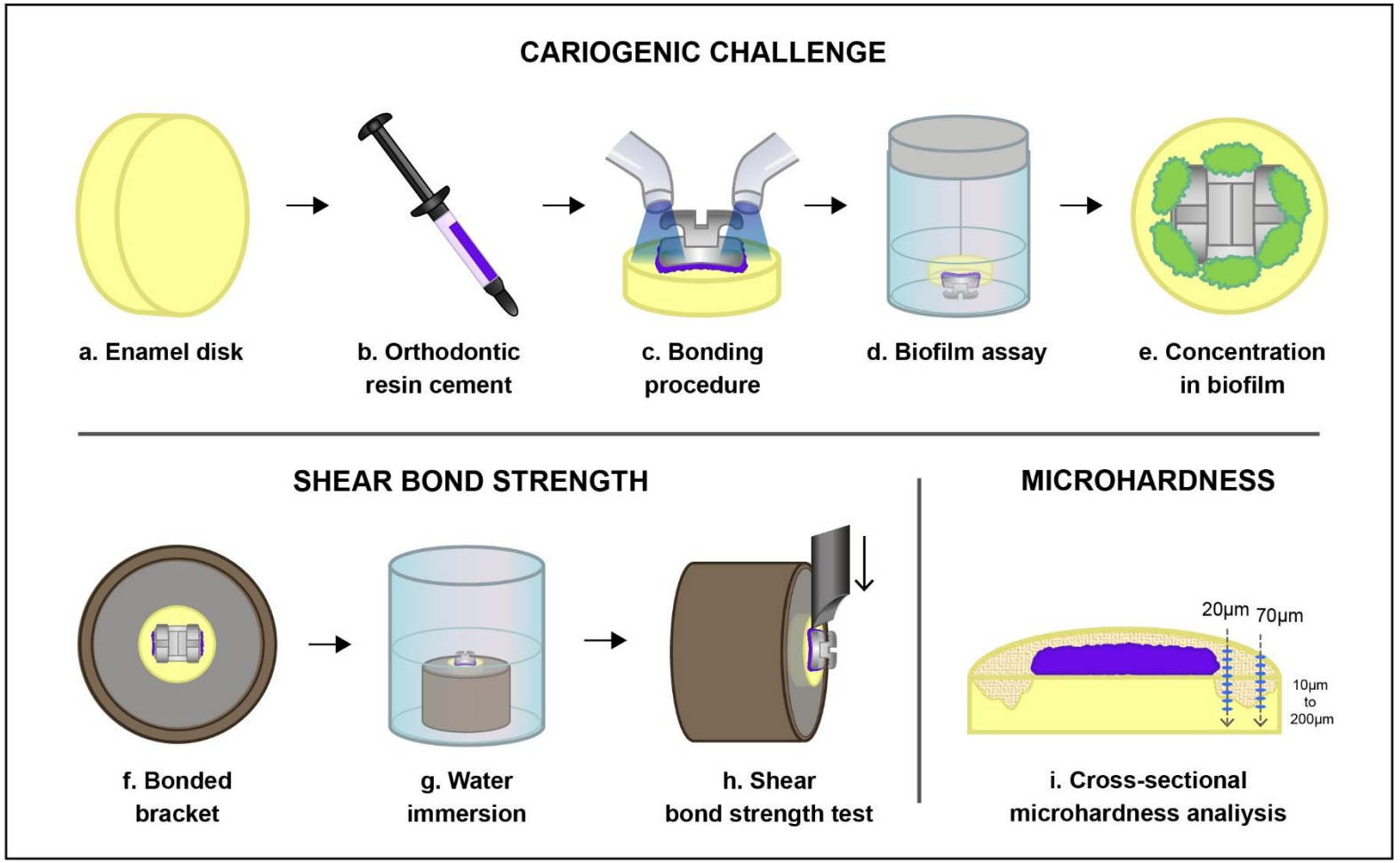

Figura 1. Representative scheme of cariogenic challenge following bond strength test and internal hardness around bracket.

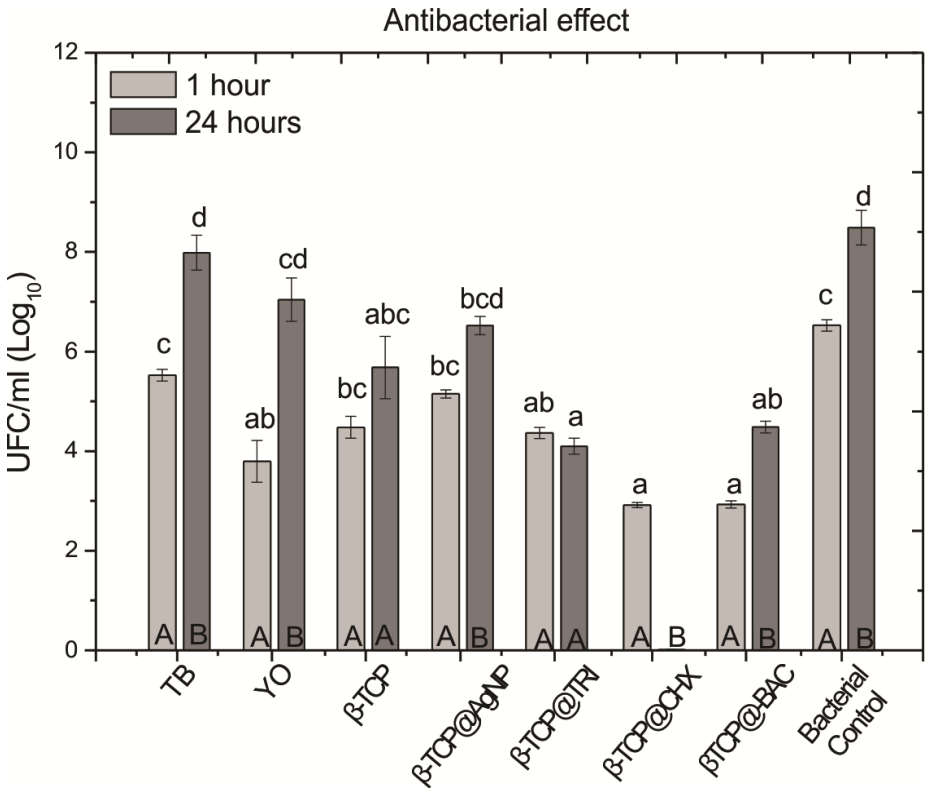

Figure 2. Survival of bacterial after modified direct contact test (CFU/ml). The data were normalized by transforming by log 10. Different capital letters indicate significant statistical difference between the exposure times. Different lowercase letters indicate significant statistical difference between materials (p>0.005).

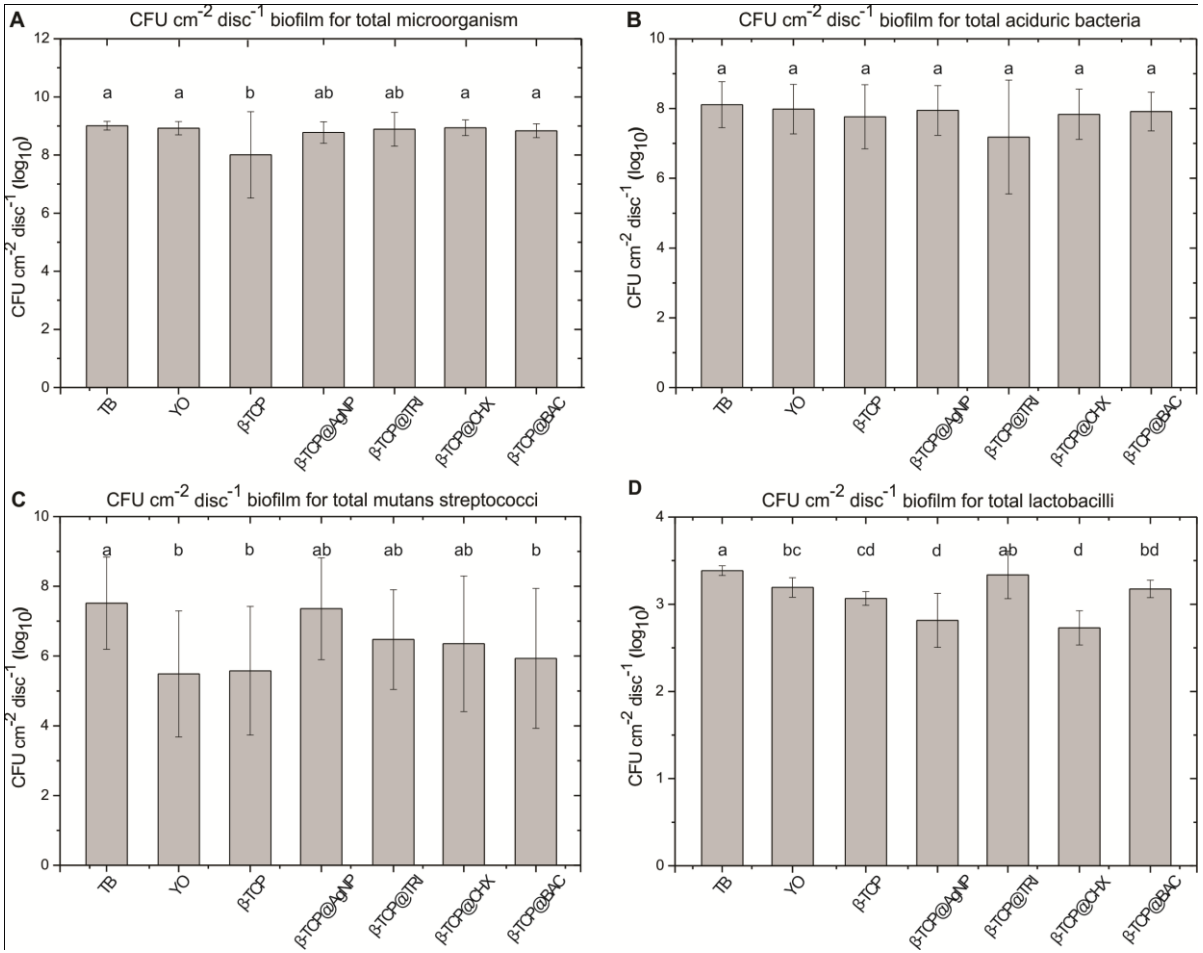

Figure 3. Mean viable bacteria (CFU cm2 dry biofilm weight) in biofilms grown for 72h. The data were normalized by transforming by log10. Within a panel, group values that were identified using similar lower case letters were not significantly different ($p > 0.05$). A= total microorganisms; B= total aciduric bacteria; C = Streptococci mutans; D= total lactobacilli bacteria.

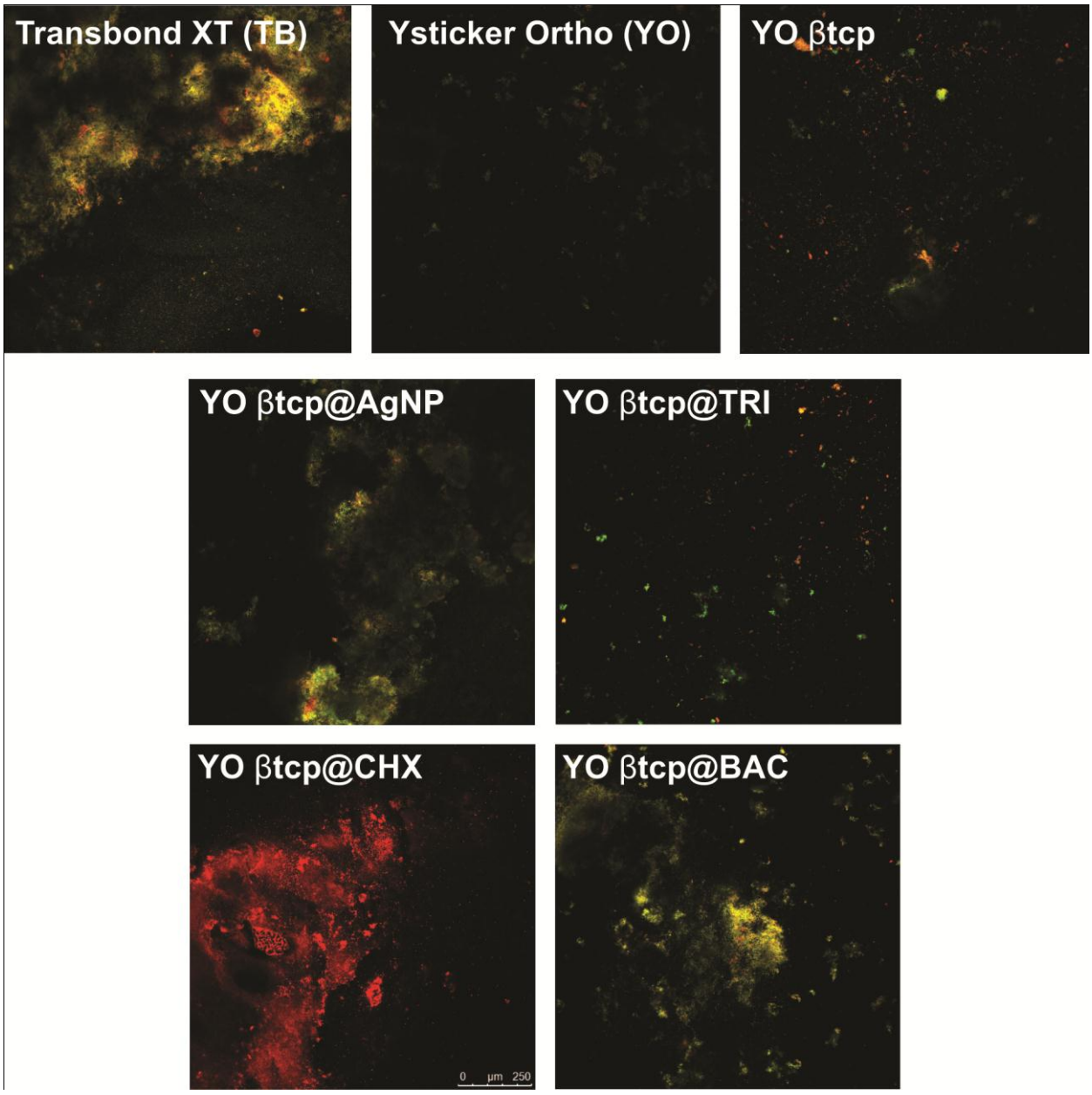

Figure 4. Representative images of cell viability (green pixels: viable cells; red pixels: death cells) from biofilm acquired by confocal microscopy.

Considerações Finais

Dentro daquilo que foi encontrado na revisão sistemática, agentes antimicrobianos estão sendo testados em sistemas de ligação ortodônticos. Além disso, tem demonstrado efetividade na redução do biofilme bacteriano em muitos estudos in vitro. Através do estudo laboratorial, conclui que as nanopartículas de β-TCP dopadas com agentes antimicrobianos podem ser utilizadas para produzir um adesivo ortodôntico com inibição aumentada do crescimento bacteriano sem prejudicar as outras propriedades investigadas. Apesar de serem necessários estudos clínicos para confirmar a efetividade desses materiais no controle antimicrobiano, a revisão sistemática e meta-análise e o estudo laboratorial demonstraram que há evidência de atividade antibacteriana sem prejudicar as outras propriedades investigadas desses adesivos ortodônticos in vitro.

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