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CAMILA MACHADO ESTEVES

AÇÃO ANTIBACTERIANA E POTENCIAL CARIOSTÁTICO DE SISTEMAS ADESIVOS AUTOCONDICIONANTES

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Dissertação apresentada à Universidade Guarulhos para obtenção do título de Mestre em Odontologia.

Área de Concentração em Dentística.
Orientador: Prof. Dr. José Augusto Rodrigues
Co-orientador: Prof. Dr. André Figueiredo Reis

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A Comissão Julgadora dos trabalhos de Defesa de Dissertação de MESTRADO, intitulada "AÇÃO ANTIBACTERIANA DE SISTEMAS ADESIVOS AUTOCONDICIONANTES" em sessão pública realizada em 18 de Fevereiro de 2010, considerou a candidata Camila Machado Esteves aprovada.

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1. Prof. Dr. José Augusto Rodrigues (UnG)_____

2. Profa. Dra. Cecilia Pedroso Turssi (UNIUBE)_____

3. Profa. Dra. Alessandra Cassoni Ferreira (UnG)_____

Guarulhos, 18 de Fevereiro de 2010.

Dedico esta dissertação,

À minha família,

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RESUMO

Este trabalho avaliou *in vitro* a ação antibacteriana e *in situ* o efeito cariostático de sistema adesivos auto-condicionante. Em uma seqüência lógica, o assunto foi abordado por intermédio do desenvolvimento de três artigos científicos. No primeiro foi realizada uma revisão bibliográfica sobre o potencial antibacteriano e ação cariostática de sistemas adesivos auto-condicionantes. No segundo foi avaliado o halo de inibição proporcionado por 9 adesivos autocondicionantes (Clearfil S3, One-Up Bond, Futurabond, GBond, Xeno IV, Clearfil SE Bond, Clearfil Protect Bond, Adper SE Plus e AdheSE) contra as bactérias orais *Streptococcus oralis*, *Streptococcus sanguinis*, *Streptococcus cricetus*, *Streptocccus* mutans e *Streptococcus sobrinus* e no terceiro trabalho, blocos de esmalte dental humano de 4X4X3 mm receberam preparos cavitários (1,6 mm ∅) e foram restaurados utilizando-se 4 sistemas adesivos auto-condicionantes (Clearfil SE Bond, Clearfil Protect Bond, Adper SE Plus e One-Up Bond), uma resina composta (Z350) e um cimento de ionômero de vidro convencional (Ketac-Fil) que foram submetidos a desafios cariogênicos *in situ*. Os estudos encontrados na literatura apontam atividade antibacteriana *in vitro* do sistema autocondicionante que possui o monômero com efeito antibacteriano, que foi confirmado no estudo *in vitro* sendo similar ou superior à clorexidina, porém os outros sistemas adesivos também apresentaram halos de inibição possivelmente provocados pelo baixo pH dos monômeros. Entretanto, os sistemas adesivos avaliados *in situ* não demonstraram efeito cariostático, mesmo possuindo flúor ou o monômero que possui ação antibacteriana *in vitro*. Conclui-se que a ação do monômero antimicrobiano, do flúor ou do baixo pH que proporcionam efeito antibacteriano *in vitro* clinicamente não são capazes de prevenir o desenvolvimento de lesões cariosas frente a um alto desafio cariogênico.

Palavras-Chave: Bactéria, cárie dental, sistemas adesivos auto-condicionante, compósitos resinosos, cimento de ionômero de vidro, fluoretos, esmalte dental, microdureza.

ABSTRACT

This study evaluated *in vitro* the antibacterial activity and *in situ* the cariostatic effect of self-etching adhesives system. In a logical sequence, three scientific articles were developed. The first was a literature review about the use of self-etching adhesives systems in the prevention of dental caries. The second evaluated the inhibition zone 9 self-etching adhesives (Clearfil S3, One-Up Bond Futurabond, GBondi, Xeno IV, Clearfil SE Bond, Clearfil Protect Bond, Adper SE Plus and AdheSE) against the oral bacteria *Streptococcus oralis*, *Streptococcus sanguinis*, *Streptococcus cricetus*, *Streptocccus mutans* and *Streptococcus sobrinus* and in the third article, human enamel blocks of 4X4X3 mm were prepared with cavities (1.6 mm □) and were restored using 4 self-etching adhesives systems (Clearfil SE Bond, Clearfil Protect Bond, Adper SE Plus and One-Up Bond) and a conventional glass ionomer (Ketac-Fil). They were submitted to cariogenic challenge *in situ*. The studies in the literature describe the *in vitro* effectiveness of self-etching adhesive systems with antibacterial monomers, which was confirmed in the second study, which the antibacterial monomer was similar or superior to chlorhexidine against the Streptococci, but the other adhesive systems also showed bacterial inhibition possibly caused by the low pH of the monomers. However, the adhesive systems evaluated *in situ* showed no cariostatic effect, even containing fluoride or the antibacterial monomer. It can be concluded that the action of antimicrobial monomer, fluoride or low pH that provide antibacterial action *in vitro* clinically are not able to prevent the development of caries lesions.

Keywords: Bacteria, dental caries, adhesives systems, self-etching, resin composite, glass-ionomer cement, fluoride, dental enamel, microhardness.

SUMÁRIO

1. Introdução

A Odontologia adesiva teve início com o estudo de Buonocore em 1955, no qual foi descrito o condicionamento ácido do esmalte como uma simples técnica para aumentar a adesão das resinas acrílicas. Deste ponto em diante, a união de sistemas resinosos ao esmalte e à dentina tornou-se um método que está presente em grande parte dos procedimentos restauradores diretos e indiretos realizados na Odontologia (Baratieri et al. 2001, De Munck et al. 2005) .

Com o aperfeiçoamento da técnica do condicionamento ácido foram desenvolvidos sistemas adesivos que possibilitaram tanto o tratamento com ácido fosfórico do esmalte como o da dentina objetivando a formação da camada híbrida utilizando uma combinação de monômeros hidrófilos e hidrófobos (Nakabayashi, 1992). Estes procedimentos garantiram maior previsibilidade e sucesso a longo prazo aos procedimentos restauradores (Baratieri et al. 2001).

Entretanto, os sistemas adesivos ainda não alcançaram a excelência clínica desejada em função da sensibilidade da técnica. Algumas limitações, tais como o rompimento da linha de união e a subsequente formação de fendas devido à força gerada durante a contração de polimerização e/ou o preenchimento incompleto pelo adesivo da dentina desmineralizada acarretam frequentemente em sensibilidade pós-operatória, aumento do risco relativo de cárie dental e possivelmente em recorrência da lesão devido a presença de nichos estagnação de placa (Gwinnett and Yu, 1995; Sano et al., 1999; Sarrett, 2005).

Para sanar essas limitações e diminuir o risco de erro clínico foram desenvolvidos os sistemas adesivos autocondicionantes. Estes sistemas são compostos por monômeros ácidos, que promovem a desmineralização dentinária e simultaneamente se infiltram nos espaços desmineralizados, preenchendo-os e diminuindo o risco de sensibilidade pós-operatória (Sundfeld et al., 2005).

Assim, com esses sistemas não é necessária a utilização do tratamento ácido, e subseqüente lavagem e secagem do mesmo, o que elimina a fase

crítica de controle da umidade na superfície dentinária necessária para a aplicação do adesivo hidrofílico (De Munck, 2005; Sundfeld et al., 2005; Feuerstein et al., 2007).

Contudo, os sistemas autocondicionantes não apresentam capacidade de desmineralização similar aos sistemas adesivos que empregam o condicionamento ácido e a resistência de união ao esmalte dental ainda é controversa sendo ainda recomendado o condicionamento ácido do mesmo (Van Landuyt et al., 2009). Desta forma, caso o sistema adesivo não suporte as forças geradas pela contração de polimerização, ou mesmo os esforços mastigatórios, pode haver um rompimento da linha de união esmalte/adesivo resultando na formação de uma fenda. É importante salientar que a formação de uma fenda não determina o desenvolvimento de lesões cariosas, entretanto, pode se tornar um nicho de estagnação de biofilme bacteriano e subsequentemente favorecer o seu desenvolvimento (Bergenholtz et al., 1982; Karanika-Kouma et al., 2001; Sarrett, 2005; Feuerstein et al., 2007).

Outro fator importante na formação de cáries secundárias é detectar clinicamente o limite entre a dentina afetada e a infectada. Após o preparo cavitário, a resina adesiva deve infiltrar nessa dentina e irá envolver as bactérias na camada híbrida, o que pode ser uma das razões para o fracasso das restaurações caso haja rompimento na camada adesiva (Kidd, 2001; Brunthaler, 2003). Assim sendo, como ainda não foi desenvolvido um sistema adesivo que impeça a formação de fendas e subsequente microinfiltração, o uso de formulações com monômeros que possuem propriedades antibacterianas pode ser uma importante ferramenta na prevenção de cárie secundária (Imazato et al. 2001; Imazato et al. 2006).

Os sistemas adesivos convencionais são compostos por materiais resinosos que apresentam pouca ou nenhuma atividade antimicrobiana e tais propriedades são obtidas pela incorporação de outras moléculas, tais como o glutaraldeido, fluoretos e monômeros como o 12-metacriloiloxidodecilpiridínio de Brometo (MDPB) (Imazato et al., 1998a). Estudos demonstraram claramente por meio de técnicas de cultura microbiológica o potencial efeito inibidor dos sistemas adesivos auto-condicionante contendo esses componentes contra diferentes espécies de estreptococos. (Tziafas et al., 2007), *Actinomyces viscosus* e *Lactobacilus casei.* (Imazato et al., 1998b) porém, esta metodologia

não prediz a duração deste efeito.

Todavia, não se encontra na literatura nenhum relato de caso ou estudo clínico que demonstre este efeito, sendo assim a proposta do presente trabalho foi revisar a literatura e avaliar o potencial de sistemas adesivos autocondicionantes com componentes antibacterianos por meio do estudo *in vitro* da inibição de crescimento de estreptococos, do estudo do seu potencial de prevenção do desenvolvimento *in situ* de cárie dental.

2. Proposição

O objetivo deste estudo foi analisar o potencial antibacteriano e ação cariostática de sistemas adesivos autocondicionantes por meio do desenvolvimento de três artigos científicos.

3. Desenvolvimento

Capítulo 1- Atividade antibacteriana de Sistemas adesivos autocondicionantes - Camila Esteves, André Figueiredo Reis, José Augusto Rodrigues

Artigo aceito para publicação na Revista Saúde UnG-on line

Capítulo 2- Antibacterial activity of various self-etching adhesive systems against oral streprococci - Camila Esteves, André Figueiredo Reis, Claudia Ota-Tsuzuki, José Augusto Rodrigues

Artigo aceito para publicação no periódico Operative Dentistry.

Capítulo 3- *In situ* caries development around composite resin restored with self-etching adhesive - Camila Esteves, André Figueiredo Reis, José Augusto Rodrigues

Artigo em preparação para submissão no periódico Dental Materials.

Capítulo 1- Atividade antibacteriana de Sistemas adesivos autocondicionantes - Camila Esteves, André Figueiredo Reis, José Augusto Rodrigues

Artigo aceito para publicação na Revista Saúde UnG-on line (Anexo 2).

ANTIBACTERIAL ACTIVITY OF SELF-ETCHING ADHESIVE SYSTEMS

Resumo: Este trabalho de revisão de literatura foi realizado com o objetivo de discutir as propriedades antibacterianas dos sistemas adesivos autocondicionantes. A evolução das propriedades bioativas dos materiais são uma tendência atual e a atividade antibacteriana é uma propriedade importante na redução das bactérias nas superfícies restauradas, prevenindo a formação de cáries secundárias. Esta propriedade pode ser obtida com a incorporação de substâncias ou pela substituição dos monômeros convencionais por outros com potencial antibacteriano. Apesar da literatura mostrar que a adição de substâncias como o glutaraldeído, e a clorexidina promoverem efeito antibacteriano, sua presença na matriz resinosa pode prejudicar as propriedades físicas dos materiais. Já a incorporação de flúor tem efeito restrito, pois os fluoretos permanecem imobilizados na matriz resinosa. A incorporação de monômeros como o 12-metacriloiloxidodecilpiridínio de Brometo (MDPB) e o dimetil metacrilato de cloreto de amônia (DMAE-CB) demonstram grande efeito antibacteriano sem a alteração das propriedades mecânicas, porém sua efetividade clínica ainda não está estabelecida. Além disso, o baixo pH dos sistemas adesivos autocondicionantes também pode atuar contra algumas bactérias. Pode-se concluir que o efeito antibacteriano dos sistemas adesivos autocondicionantes ocorre somente no momento da aplicação na cavidade, visto que o pH ácido é neutralizado e os componentes antibacterianos permanecem no interior da matriz resinosa após a polimerização. Essa inclusão de componentes antibacterianos nos sistemas adesivos, foi indicado pelos estudos *in vitro* por ser uma propriedade bioativa promissora, porém ainda existe a necessidade de estudos *in situ* e *in vivo* que comprovem a ação antibacteriana clinica dos sistemas adesivos autocondicionantes.

PALAVRAS-CHAVE: Atividade antibacteriana, sistemas adesivos autocondicionantes, cáries secundária.

ABSTRACT: This review of literature was conducted to discuss the antibacterial properties of the self-etching adhesives systems. The development of bioactive properties of the materials is a current trend and the antibacterial property is important in reducing bacteria on surfaces restored, preventing the formation of caries around the restorations. The antibacterial property can be obtained by incorporating substances or by the substitution of conventional monomers for others with antibacterial potential. Although literature shows that addition of antibacterial substances such as glutaraldehyde and chlorhexidine promote antibacterial effect, its presence in the resin matrix can impair its physical properties. Since the incorporation of fluoride have limited effect because the fluoride will remain trapped into the resin matrix. The incorporation of monomers such as 12-methacryloyloxydodecylpyridinium bromide (MDPB) and methacryloxytethocetyl dimethyl ammonium chloride (DMAE-CB) have demonstrated a strong antibacterial effect without impairing its mechanical properties, but its clinical effectiveness is not yet established. Furthermore, the low pH of the self-etching adhesives systems can also work against some bacteria. It can be concluded that the antibacterial effect of self-etching adhesive systems occurs only at the application moment in the cavity, whereas the acidic pH is neutralized and the antibacterial components may not be released from the resin matrix after polymerization. The inclusion of antibacterial compounds in the adhesive systems was indicated by *in vitro* studies to be a promising bioactive property, but there is still a need for *in situ* and *in vivo* studies confirm the clinical effectiveness of antibacterial potential of the self-etching adhesives systems.

KEYWORDS: Antibacterial activity. Adhesive systems. Secondary caries.

Introdução

A união duradoura de sistemas resinosos ao esmalte e a dentina tem sido a grande busca da Odontologia contemporânea. Esta busca teve início com o estudo de Buonocore (1955)[1], que descreveu o condicionamento ácido do esmalte como uma simples técnica para aumentar a adesão de resinas acrílicas. Com o aperfeiçoamento da técnica do condicionamento ácido foram desenvolvidos sistemas adesivos que possibilitaram tanto o tratamento com ácido fosfórico do esmalte como o da dentina, objetivando a formação da camada híbrida[2]. Além disso, a utilização de uma combinação de monômeros hidrófilos e hidrófobos tem garantido maior previsibilidade e sucesso a longo prazo dos procedimentos adesivos restauradores[3,4].

Entretanto, em função da sensibilidade da técnica os sistemas adesivos ainda não alcançaram a excelência clínica desejada[5]. Um dos principais princípios que devem ser respeitados é o de que a dentina deve permanecer úmida para a formação adequada da camada híbrida[6]. Caso esta regra não seja respeitada pode ocorrer o rompimento da linha de união e consequentemente a formação de fendas devido à força gerada durante a contração de polimerização das resinas restauradoras ou durante os esforços mastigatórios[7-9]. Além disso, o preenchimento incompleto da dentina desmineralizada pela resina adesiva após o condicionamento ácido faz com que este espaço seja ocupado por água levando a uma degradação hidrolítica da camada híbrida e clinicamente pode acarretar em sensibilidade pós-operatória, formação de um nicho de estagnação de biofilme e possivelmente em recorrência da lesão cariosa[7,8].

Frente a essas limitações foram desenvolvidos os sistemas adesivos autocondicionantes, que por serem compostos por monômeros ácidos promovem a desmineralização dentinária enquanto se infiltram na estrutura dental para formação da camada híbrida[10,11]. Outra vantagem apresentada por essa técnica é a eliminação da fase de lavagem e secagem do substrato após o condicionamento ácido, o que elimina a fase crítica de controle da umidade na superfície dentinária necessária para a aplicação do adesivo acarretando em menos erros técnicos cometidos pelo cirurgião dentista[11-12].

É importante salientar que o rompimento da camada híbrida com a formação de uma fenda entre esmalte e resina não determina o desenvolvimento de lesões cariosas secundárias, mas este pode servir como um nicho retentor de biofilme bacteriano e desta forma pode ser um fator que favoreça sua instalação e progressão, principalmente em pacientes de alto risco[12-14]. Apesar da Dentística restauradora moderna estar baseada nos princípios da cariologia, e o foco do tratamento restaurador é a redução do risco do indivíduo, torna-se desejável que o material restaurador e sistema adesivo apresentem uma ação antibacteriana ou cariostática para ajudar no controle do desenvolvimento de lesões secundárias[15-17]. Desta forma, o presente estudo tem como objetivo caracterizar por meio de uma revisão de literatura o potencial antibacteriano de sistemas adesivos autocondicionantes.

Potencial antibacteriano de sistemas adesivos autocondicionantes

Os sistemas adesivos autocondicionantes são compostos por materiais resinosos convencionais com pH ácido que apresentam pouca ou nenhuma atividade antimicrobiana[18,19]. Entretanto, tais propriedades podem ser alteradas com a incorporação de outras moléculas com potencial antibacteriano[20]. As principais moléculas já descritas na literatura científica e que foram adicionadas aos sistemas adesivos foram o glutaraldeído[21,22], flúor[23]; 12-metacriloiloxidodecilpiridínio (MDPB; Figura 1)[18-20,24], e recentemente o dimetil metacrilato de cloreto de amônia (DMAE-CB, Figura 2)[25]. Além destas moléculas, o baixo pH dos agentes autocondicionantes também tem sido sugerido como responsável por uma ação antibacteriana[18,21,22].

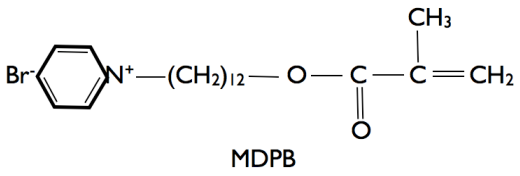

Figura 1- Molécula do monômero MDPB, adaptado de Li et al. 2009[61].

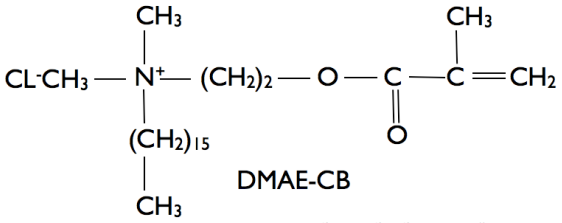

Figura 2- Molécula do monômero DMAE-CB, adaptado de Li et al. 2009[61].

Estudos *in vitro* analisam a atividade antibacteriana dos compósitos e seus constituintes e normalmente empregam testes em meio Agar. De uma forma geral, os materiais são inoculados em uma placa de Agar contra bactérias orais semeadas no meio de cultura; normalmente espécies relacionadas com o desenvolvimento da doença cárie, tais como espécies de estreptococos e lactobacilos[20,26]. Nesse método, o efeito dos componentes antimicrobianos formam uma zona de inibição de crescimento das bactérias ao redor do mesmo.

Por meio desta técnica de cultura microbiológica contra diferentes espécies de estreptococcus[12,27] e lactobacilus[18], vários estudos observaram que após a incorporação de determinados monômeros nos agentes adesivos os mesmos inibiram o crescimento destas espécies[18,20,26].

A ação antibacteriana nos sistemas adesivos pode ser obtida pela adição de agentes antibacterianos na matriz da resina como no caso da adição de clorexidina, glutaraldeido e flúor, ou pela imobilização do componente antimicrobiano na composição dos monômeros presentes na matriz resinosa como no caso da adição dos monômeros MDPB e DMAE-CB[18-25].

Pela sua comprovada capacidade antimicrobiana e de inibição de formação de biofilme nas superfícies dentais, alguns estudos incorporaram experimentalmente agentes antibacterianos como o digluconato de clorexidina em alguns sistemas adesivos e em resinas compostas e obtiveram efeito antibacteriano[28,29]. Observa-se que a liberação desses agentes pelos sistemas resinosos pode exercer efeito tóxico ou induzir a mudança de população de microrganismos. Entretanto, este efeito pode ter sua eficiência esgotada com tempo, além de deteriorar as propriedades mecânicas dos materiais restauradores[28-30]. Os primeiros estudos que tentaram incorporar esse agente

antimicrobiano apresentaram deficiência na resistência mecânica e adesiva, e o potencial preventivo desses sistemas adesivos ainda são questionados, principalmente em relação a sua atividade após foto-ativação[12,31,32] e ainda não existe no mercado um sistema adesivo auto-condicionante com clorexidina.

O glutaraldeído é um forte desinfetante frequentemente utilizado nos consultórios dentários para esterilização dos instrumentais, dessa forma este componente foi adicionado nos sistemas adesivos com uso de condicionamento ácido prévio como GLUMA (Heraeus Kulzer; Figura 3), Syntac (Vivadent), ProBOND (LD Caulk/Dentsplay) desejando-se obter esse efeito[21,22]. Feltron *et al*. (1989)[33], confeccionaram cavidades em dentes de primatas e aplicaram compósitos contendo o adesivo GLUMA. Os autores deixaram estas cavidades abertas durante 48h e observaram que após este tempo o sistema adesivo aplicado foi eficaz na eliminação de bactérias que invadem os túbulos dentinários. Corroborando com estes resultados, todos os estudos de sistemas adesivos contendo glutaraldeído no método cultura produziram halo de inibição contra *Streptococus*, *Lactobacilos* e *Actinomyces*[21,22,34]. Desta forma, os estudos sugerem que este resultado mostrado pelo glutaraldeído é devido ao seu efeito antibacteriano, uma vez que os adesivos que não possuem glutaraldeído ou qualquer outro agente antimicrobiano na sua composição não demonstraram inibição[21].

Figura 3 – Sistema adesivo Gluma que contém glutaraldeído (Heraeus Kulzer).

Entretanto, o efeito do glutaraldeído pode ser limitado a poucos dias após a aplicação. Walter em (2007)[35] observaram, por meio da aplicação e foto-ativação de sistemas adesivos em uma membrana permeável e, subsequente inserção em meio de cultura, que todos os adesivos que continham glutaraldeído exibiram

zonas de inibição contra *Streptococcus mutans*, *Streptococcus sobrinus*, *Lactobacilus acidophilus* e *Actinomyces viscosus*. Porém, este efeito teve a duração inferior a uma semana. Entretanto, não se encontra disponível atualmente no mercado Odontológico sistemas adesivos autocondicionantes contendo glutaraldeído.

Outra alternativa para prover ação antibacteriana ou cariostática aos sistemas adesivos é a incorporação de íons flúor cuja posterior liberação pode inibir a formação de lesões cariosas[36,37]. Existe uma grande variedade de mecanismos envolvendo os efeitos cariostáticos do flúor, incluindo a redução nos fenômenos de desmineralização e na formação de placa bacteriana. A liberação de flúor nos materiais restauradores é bem conhecida nos cimentos de ionômero de vidro convencionais e também pode ser observada nos híbridos de ionômero de vidro e resina composta[14,38]. Kerber & Donly (1993)[39] demonstraram que sistemas adesivos dentinários acrescidos com fluoreto podem promover uma zona de inibição de cárie de até 0,25 mm da margem da cavidade.

Entretanto, o efeito cariostático do flúor adicionado nos adesivos parece ser controverso. Rodrigues et. al. (2005)[38], compararam *in vitro* a ação cariostática de ionômeros de vidro convencionais e híbridos e de uma resina composta contendo fluoretos e cálcio. Os autores observaram que a resina composta não apresentou efeito cariostático e os mesmos justificaram este resultado devido ao efeito cariostático do fluoreto e do cálcio só ocorrer quando os mesmos entram em solução na cavidade bucal. Corroborando com estes resultados Hara et al. (2005)[40] também questionaram os efeitos cariostáticos de agentes adesivos contendo flúor em um estudo *in vitro* que comparou quatro agentes adesivos contendo flúor (Optibond Solo, One-up Bond F, Prime & Bond NT e Tenure Quick) com um cimento de ionômero de vidro. Corpos-de-prova de dentina bovina restaurados com estes agentes adesivos e com o cimento de ionômero de vidro foram submetidos à indução de cárie secundária. Os autores relataram que, apesar dos sistemas adesivos liberarem flúor, os mesmos não foram capazes de inibir a formação de lesões de cárie secundária como ocorrido com o cimento de ionômero de vidro. Posteriormente, Peris et al. (2007)[41] observaram que o flúor liberado de sistemas adesivos autocondicionantes (Optibond Solo

Plus e Clearfil Protect Bond) não foi capaz de promover um efeito cariostático frente ao desafio cariogênico *in vitro* e resultou em cárie secundária ao redor de restaurações realizadas em dentes bovinos e consequentemente na redução da força de união.

A imobilização do componente antimicrobiano na matriz resinosa pela incorporação de monômeros com ação antibacteriana parece ser a opção mais adequada para alcançar um sistema adesivo que possua um efetiva ação antibacteriana sem prejuízos no processo de adesão ao esmalte e dentina. Partindo deste principio, Imazato (1994)[42] preconizou a incorporação do monômero MDPB. Este monômero foi inicialmente sintetizado e incorporado nos compósitos resinosos e possui propriedades antibacterianas.

Os estudos iniciais apresentaram resultados promissores em relação à inibição de estreptococos sem alterar as propriedades mecânicas de força de compressão, tensão diametral, resistência, capacidade de polimerização e microdureza do mesmo[42,43]. Após os estudos que introduziram o MDPB como antibacteriano em resinas compostas, ele foi inserido experimentalmente nos sistemas adesivos[24]. A adição de MDPB nas resinas e nos componentes dos sistemas adesivos resultou em produtos com propriedades físicas e mecânicas compatíveis com outros materiais restauradores livres de MDPB. Além disso, os mesmos resultados físico-mecânicos foram observados após a foto-polimerização do mesmo.

Ainda em fase experimental, Imazato et al. (2001)[15] demonstraram que o monômero MDPB possui alta capacidade bactericida *in vitro* promovendo um grande halo de inibição para 11 diferentes espécies de microrganismos anaeróbicos, e quando aplicado em amostras de dentina cariada, mesmo diluído 40 vezes, ainda pode inibir o crescimento de microrganismos totais comparado a outros sistemas adesivos que não apresentaram este efeito. Em 2006, Imazato et al.[16] demonstraram por meio da medição do halo de inibição de crescimento de microrganismos em placas de Agar e pela contagem de microrganismos viáveis, que no substrato dentinário preparado *in vitro* um primer com uma concentração de 5% de MDPB (Clearfil Protect Bond) possuía uma grande atividade antimicrobiana contra *S. mutans*, *L. casei* e *A. naeslundii*.

Porém, Gondim et al. (2008)[44] afirmam que a foto-ativação reduz significativamente a ação antibacteriana dos sistemas adesivos autocondicionantes, incluindo o que possui o monômero MDPB, mas este ainda apresentava um leve efeito contra as bactérias mesmo após a polimerização. Thomé et al. (2009)[45] afirmam que uma vantagem importante desses materiais é o fato de que após a polimerização, o componente antibacteriano permanece imobilizado na matriz da resina. Deste modo o efeito é imobilizado, não havendo a necessidade de degradação do agente. Teoricamente a ação antibacteriana não ocorre pela decomposição do material, e sim pela inibição da adesão bacteriana na superfície do material restaurador. Quando ocorre o contato da membrana citoplasmática com o monômero esta se rompe e há um extravasamento do conteúdo celular e a destruição da mesma[16,46].

Por outro lado, alguns estudos observaram efeito antibacteriano *in vitro* em sistemas adesivos autocondicionantes, mesmo quando não apresentavam componentes antibacterianos em suas formulações. Tal efeito foi atribuído ao seu baixo pH[19,21,47]. Ohmori et al. (1999)[48] também demonstraram formação de halo de inibição contra *S. mutans* e efeito antibacteriano em cavidades confeccionadas *in vitro* em dentes bovinos para uma série de adesivos autocondicionantes que não possuíam monômeros antibacterianos e tal efeito foi atribuído ao pH ácido. Karanika-Kouma et al. (2001)[14] observaram a formação de halos de inibição contra *L. salivarius*, *S. sorbinus*, *A. viscosus* e *S. mutans* por um material ionomérico e adesivos convencionais e tal ação foi atribuída ao baixo pH dos mesmos. Outros estudos também associam efeitos antibacterianos de sistemas adesivos ao baixo pH[19,22,47].

Discussão

Devido a alta prevalência de cáries secundárias após o tratamento restaurador[49], existe recentemente um aumento de interesse na capacidade terapêutica dos materiais restauradores.

Desta forma, o uso de materiais restauradores inteligentes, ou seja

materiais bioativos, que tem a capacidade de inibir o desenvolvimento de biofilme, desmineralização ao redor das restaurações, proporcionar sua reminerização e que tem ação direta contra microrganismos cariogênicos, além das propriedades básicas dos biomateriais tem crescido dentro do mercado odontológico.

Assim sendo, o desenvolvimento de compósitos e sistemas adesivos com propriedades bioativas é uma tendência futura dentro da Odontologia. Os sistemas adesivos autocondicionantes que possuem atividades antimicrobianas podem ser classificados como material com propriedades bioativas. Desta forma, este trabalho teve como objetivo realizar uma revisão de literatura em relação ao uso destes sistemas adesivos e a sua ação antimicrobiana em estudos *in vitro*, visto que até o presente momento não existem estudos *in situ* ou *in vivo* em humanos.

Desde o século passado vários estudos tem examinado as propriedades antibacterianas dos compósitos odontológicos e seus constituintes[31,38, 45,50-53].

O conhecimento atual sobre o efeito antimicrobiano dos sistemas adesivos autocondicionantes foi na maioria proveniente de estudos *in vitro*. De forma geral, o método de cultura vem sendo o mais utilizado por ser um teste simples e fácil para determinação do efeito antimicrobiano destes materiais. Estudos utilizando glutaraldeído[21,22] e MDPB[20,54] demonstraram efeito inibidor quando aplicado em placas de Agar contra diferentes espécies de estreptococos e lactobacilos.

Dessa forma, baseando-se apenas nos resultados dos estudos *in vitro*, o uso destes agentes parece ser promissor. Entretanto, cabe ressaltar que o efeito *in vitro* pode não ser o mesmo apresentado *in vivo*. Estudos já demonstraram que as bactérias são mais susceptíveis a agentes antibacterianos quando estão em meios de cultura do que quando estão crescendo na forma de biofilme sobre a superfície dental[55]. Assim é importante analisar os efeitos em estudos *in situ* e *in vivo*. Como o desenvolvimento de lesões cariosas é demorado e a indução de lesão de cárie nos dentes de pacientes é inviável, estudos *in situ* são mais adequados e necessários para comprovar e eficácia dos sistemas adesivos autocondicionantes.

Existe somente um sistema adesivo no mercado odontológico que possui o MDPB (Clearfil Protect Bond - Kuraray; Figura 4), e não existem estudos

longitudinais comprovando seu efeito antibacteriano na prevenção de lesões secundárias.

Figura 4- Sistema adesivo autocondicionante com o monômero MDPB- Clearfil Protect Bond.

O resultado mais próximo da situação clínica é o de Lobo et al. (2005)[56], que avaliou o efeito cariostático de três sistemas adesivos por meio do desenvolvimento de lesões cariosas artificiais, com um modelo microbiológico, ao redor de cavidades preparadas em dentes bovinos. Nenhum dos adesivos apresentou efeito cariostático, porém, foi observado que os adesivos com flúor ou com o MDPB diminuíram a síntese de polissacarídeos extracelulares necessários para adesão nas superfícies dentais e utilizados como fonte de energia (glucanos) pelas bactérias, demonstrando que elas foram afetadas.

Feuerstein et al. (2007)[12] relatam que os sistemas adesivos autocondicionantes podem ter um efeito antibacteriano imediato pela presença dos monômeros antibacterianos, porém, após a foto-ativação e a longo prazo é possivelmente limitado pois se restringem a matriz resinosa. Além disso, se restringem a interface das restaurações, com uma espessura de 2-3 micrômetros,[32,57] que possivelmente terá pouco efeito antibacteriano proporcionalmente à área adjacente na qual as bactérias podem se aderir.

Além disso, o efeito antibacteriano atribuído ao pH ácido dos sistemas autocondicionantes também deve ser considerado limitado pois com sua reação com a dentina ocorre uma neutralização gradual[19,48,58,59] e possivelmente perda do efeito. Assim, somente a aplicação do sistema com característica ácida não garante uma ação cariostática, além disso o efeito desse adesivo em bactérias acidogênicas como as espécies de Lactobacilos a longo prazo é questionável.

Schmalz et al. (2004)[26] analisaram a sua ação antimicrobiana em meio de cultura de materiais com pH igual ou inferior a 1,5 e apesar de efetivos para algumas espécies bacterianas, para os Lactobacilos, que são ácidos tolerantes, este efeito é mínimo[60].

Desta forma, o benefício antibacteriano dos sistemas autocondicionantes deve ser focado na modificação dos monômeros, visto que a adição de substâncias antibacterianas pode comprometer suas propriedades físico-mecânicas, e como ficam incorporadas na matriz[24], necessitam serem liberadas para o meio, o que somente ocorre pela sua degradação. Mesmo assim, a efetividade clínica dos monômeros antibacterianos ainda continua questionável embora seja promissora.

Conclusão

O efeito antibacteriano dos sistemas adesivos autocondicionantes ocorre somente no momento da aplicação na cavidade, visto que o pH ácido é neutralizado e os componentes antimicrobianos permanecem no interior da matriz resinosa após a polimerização. Existe a necessidade de estudos *in situ* e *in vivo* que comprovem a ação antimicrobiana dos sistemas adesivos clinicamente.

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Capítulo 2- Antibacterial activity of various self-etching adhesive systems against oral streprococci - Camila Esteves, André Figueiredo Reis, Claudia Ota-Tsuzuki, José Augusto Rodrigues

Artigo aceito para publicação no periódico Operative Dentistry (Anexo 3).

Running title: self-etching adhesives antibacterial activity

Clinical relevance statement: An antimicrobial effect is desired for adhesive systems in order to avoid cariogenic bacterial colonization and also the growth of remaining bacteria in the cavity preparation.

Summary

The antibacterial properties of self-etching adhesive systems constitute an important issue in Operative Dentistry since viable bacteria can still be present after cavity preparation. The aim of this study was to evaluate the antibacterial activity of five one-step self-etching adhesives (SEAs) and four self-etching primers (SEPs) against oral streptococci. Clearfil S3 (S3), One-Up Bond F Plus (OU), Futurabond NR (FB), GBond (GB) Xeno IV (X4), Clearfil SE Bond (SE), Clearfil Protect Bond (PB), Adper SE Plus (AS) and AdheSE (AD) were tested for antibacterial activity against 5 streptococci species: *S. oralis*, *S. sanguinis*, *S. cricetus*, *S. mutans* and *S. sobrinus*. Chlorhexidine (0.12%) and phosphoric acid (37%) gel were used as control. The agar diffusion test method was used. Plates containing BHI agar and 300uL of bacterial cell suspension (0.5 MacFarland) were prepared. Holes of 6mm diameter were made and partially filled with bacteriological agar. Then, 10uL of each SEA or SEP was dropped and the plates were incubated under microaerofiliae atmosphere at 37^{o} C for 48h and the diameter of each halo was registered. Results were analyzed by 2-way ANOVA and Tukey test. PB exhibited the most effective antibacterial activity against oral streptococci. SE and FB performances were similar or better than Chlorhexidine for all bacteria. S3, X4, AS, AD, OU and GB showed significantly lower inhibition values. Among the species tested, *S. oralis* was the most sensitive for all self-

etching adhesive systems; on the other hand *S. cricetus*, *S. mutans* and *S. sobrinus* were more resistant. Among the self-etching adhesive systems evaluated, Clearfil Protect Bond exhibited the most effective antibacterial activity against oral streptococci.

Keywords: Self-etching adhesives, Bacteria, Microbiology, Self-etching primer adhesive.

INTRODUCTION

Bonding of resin-based composites to dentin can be accomplished by means of etch-and-rinse or self-etching adhesive systems. The etch-and-rinse technique has been considered sensitive.[1-4] Incomplete resin infiltration and evidence of phase separation within resin-dentin interfaces and its detrimental effects have been demonstrated.[5,6] With the attempt to reduce technique sensitivity, a second approach was developed, in which self-etching monomers are applied without further rinsing.[7] Both techniques have been routinely used for promoting the infiltration of resin monomer into dentin surface and forming a hybrid layer which is the key for resin/dentin bonding.

This step should promote an effective, long-lasting seal of tooth structures, in order to avoid gap formation during cavity restoration. However, gap formation between composite and cavity walls, as a result of polymerization shrinkage stress, is still an important concern, since these sites could be colonized by oral bacteria from saliva leading to development of secondary caries.[8-10]

An antimicrobial effect is desired for restorative composites and adhesive systems in order to avoid cariogenic bacterial colonization and also the growth of remaining bacteria in the cavity preparation.[11,12] This effect can be achieved by the incorporation of antimicrobial agents such as glutaraldehydes, fluorides or antibacterial monomers in the adhesive systems formulation.[9,11-15] Generally, adhesive monomers of self-etching adhesive systems present a hydrophilic group at one end of the molecule, which is usually an acid such as hydrogen phosphate or carboxylate. These traits provide these materials a low pH and possibly some antibacterial properties. Therefore, not only the antimicrobial agents but also other substances commonly found in adhesive systems formula such as adhesion-promoting monomers, which are acidic in different degrees, might be able to exert some activity against bacterial growth.[16-17]

Although several studies describe the antibacterial effects of the MDPB (12-methacryloyloxydodecylpyridinium bromide) monomer, little is known about the antimicrobial effects of other self-etching adhesive systems. As the antibacterial effect occurs during application due to the low pH of these materials, other commercially available self-etching adhesive systems could also exhibit this antibacterial effect. Thus, the aim of this study was to evaluate the antibacterial property of some self-etching/priming solutions against oral streptococci.

MATERIALS AND METHODS

Nine commercially available self-etching adhesives (SEAs) or self-etching primer systems (SEPs) were selected for this experiment (Table 1).

Table 1- Self-etching adhesives and self-etching primers composition according manufactures and batch numbers

Adhesive system (Batch number) Manufacturer	*Type*	*pH*	*Components*
Clearfil S^3 Bond (00056A) Kuraray Dental Inc.	One bottle One step	2.7	MDP, Bis-GMA, HEMA, photo-initiator, ethanol, water, silanated colloidal silica
Clearfil SE Bond (Primer: 704) Kuraray Dental Inc.	Two bottle Two steps	Primer: 2.0	Primer: MDP, HEMA, hydrophilic dimethacrylate, photo-initiator, water
Clearfil Protect Bond (Primer: B00033B) Kuraray Dental Inc.	Two bottle Two steps	Primer: 2.0	Primer: MDPB, MDP, HEMA, hydrophilic dimethacrylate, photo-initiator, water
Xeno IV (060926) Dentsply Caulk	One bottle One step	2.1	PENTA, Mono-, Di- and Trimethacrylate resins, cetylamine hydrofluoride, acetone–water
Adper SE Plus (Liquid A: 8AP Liquid B: 8AP) 3M ESPE	Two bottle One step	Not available	Liquid A: Water, HEMA Liquid B: Surface Treated Zirconia, Triethylene Glycoldimethacrylate, Di-Hema Phosphates, Mono Hema Phosphate, Methacrylated Pyrophosphates, Hema Phosphate Phosphoric Acids-6-Methacryloxy-Hexylesters Mixture, 1,6-Hexanediol Dimethacrylate, Diurethane Dimethacrylate, Trimethylolpropane Trimethacrylate, Ethyl 4-Dimethyl Aminobenzoate, Dl-Camphorquinone
AdheSE (Primer: K29856 Bond: K29858) Ivoclar Vivadent	Two bottle One step	1.7	Primer: dimethacrylat, phosphonic acid acrylate, water, initiators and stabilizers
One-up Bond F Plus (Bottle A: 054 Bottle B: 547) Tokuyama Dental Corporation	Two bottle One step	Bonding agent A: 0.7 Bonding agent B: 7.7	Bonding Agent A: MAC-10, photo-initiator, methacryloylalkyl acid phosphate, multi-functional methacrylic monomers Bonding Agent B: MMA, HEMA, water, F-deliverable micro-filler (fluoro-alumino-silicate glass), photo-initiator
Futurabond NR (Liquid 1: 0818019 Liquid 2: 0818169) Voco	Two bottle One step	1.4	dimethacrylates, silicate fillers, initiators, stabilizers, additives
G-bond (0604211) GC America Inc.	One bottle One step	2.0	4-MET, phosphoric ester-monomer, UDMA, TEGDMA, acetone, water, stabilizer, silica filler, water, photo-initiator

A 37% phosphoric acid gel and a 0.12% chlorhexidine solution were used as positive controls. The 5 microorganisms used in this study were: *Streptococcus oralis* (p.12,6.2), *Streptococcus cricetus* (ATCC 19642),

Streptococcus sanguinis (ATCC 10556), *Streptococcus mutans* (ATCC 25175), and *Streptococcus sobrinus* (ATCC 33478).

Fresh cultures (24 hours) of the strains were obtained by seeding them on Brain Heart Infusion (BHI) agar. From these cultures, bacterial cells suspensions in a sterile phosphate buffer solution (PBS, pH 7.0) were obtained adjusting the turbidity to the 0.5 Mac Farland scale. Then, 975uL of bacterial cell suspension was mixed with 65ml of melted BHI agar at 50^{o}C and poured onto a plate.

Eleven sterilized glass cylinders (8mmX30mm) were placed onto each inoculated agar plate before the agar solidification. As soon as the agar had been solidified, the cylinders were removed and the resultant holes were filled with 150uL of melted BHI agar, in this way shallow holes were obtained on the surface agar. Then, volumes of 10uL of each SEAs (Xeno IV, Clearfil S^{3} Bond, Futurabond NR and G-bond), the mixture of the two bottle SEA (One-up Bond F), only the SEP of the two bottle self-etching primer systems (AdheSE, Adper SE Plus, Clearfil SE Bond and Clearfil Protect Bond) or control materials were applied at the shallow holes. The plates were incubated under microaerofilie atmosphere at 37^{o} C for 48 hours (n=3).

The inhibition of microbial growth was evaluated by measuring the bacterial growth inhibition halos formed around the holes containing the tested materials after the incubation period and recorded. The measures considered were the highest diagonal (mm) in the circular inhibitory zones. The mean values were calculated for each experimental group and data were analyzed by 2-way (“Material” x “Bacteria”) analysis of variance (ANOVA) and Tukey test at the 5% confidence level. Representative inoculated Agar plates showing the inhibition zones around the hole containing tested materials were photographed (figure 1).

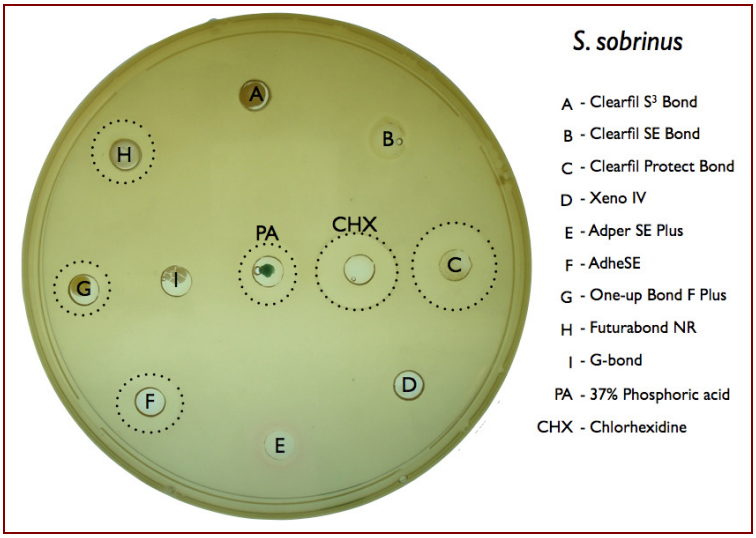

Figure 1- Representative photograph of a plate inoculated with *S. sobrinus.* Bacterial inhibition growth for the different self-etching adhesive systems can be observed (dashed line).

RESULTS

Mean diameters and standard deviation values of antibacterial inhibition zones for the SEAs, 0.12% Chlorhexidine solution and 37% phosphoric acid are shown in table 2. Two-way ANOVA indicated significant differences for the factor "Materials" ($p<0.00001$), "Bacteria" ($p<0.00001$), and for the interaction between factors ($p<0.00001$).

Table 2- Mean values (mm) and standard deviation of bacterial inhibition for the different self-etching adhesive systems tested.

	S. oralis	*S. cricetus*	*S. sanguinis*	*S. mutans*	*S. sobrinus*
Clearfil S^3 Bond	15.0 ±3.6 BCa	10.6 ±1.1 Da	10.6 ±2.1 BCa	0.0 ±0.0 Db	0.0 ±0.0 Db
Clearfil SE Bond	25,0 ±2.0 Aa	18.6 ±1.1 BCab	15.6 ±6.1 ABb	13.3 ±1.1 CDb	13.3 ±0.6 BCb
Clearfil Protect Bond	23.3 ±4.2 Ab	30.3 ±0.6 Aa	19.3 0.6 Ab	23.3 ±2.1 Ab	21.3 ±0.6 AB
Xeno IV	27.6 ±0.6 Aa	0.0 ±0.0 Eb	0.0 ±0.0 Db	0.0 ±0.0 Db	0.0 ±0.0 DB
Adper SE Plus	13.3 ±0.6 Ca	16.3 ±1.5 BCDa	3.0 ±5.2 CDc	6.6 ±5.7 BCDc	10.3 ±0.6 Cab
AdheSE	13.6 ±0.6 BCa	11.0 ±0.0 CDa	11.6 ±1.5 ABa	10.6 ±1.1 Ca	13.3 ±2.5 BCa
One-up Bond F Plus	15.0 ±1.0 BCa	16.0 ±1.0 BCDa	12.3 ±1.5 ABab	8.0 ±7.0 Cb	13.0 ±1.0 BCab
Futurabond NR	21.3 ±1.5 ABa	19.0 ±2.0 BA	19.0 ±1.0 Aa	20.0 ±1.0 ABa	15.0 ±1.0 ABCa
G bond	12.6 ±0.6 Ca	0.0 ±0.0 Eb	3.0 ±5.2 CDb	0.0 ±0.0 Db	0.0 ±0.0 Db
37% phosphoric acid	25.0 ±3.0 Aa	20.3 ±4.5 Bab	15.3 ±13.4 ABb	18.6 ±2.1 ABab	17.6 ±3.0 ABCb
Chlorhexidine	15.0 ±0.0 BCa	21.0 ±1.0 Ba	18.6 ±1.1 Aa	20.6 ±1.1 ABa	20.0 ±0.0 ABa

Means followed by different letters (upper case - column, lower case - row) are significantly different by Tukey test.

Clearfil Protect Bond SEP exhibited the most effective antibacterial activity against oral streptococci, promoting inhibition zones significantly higher than 0.12% Chlorhexidine for *S. cricetus* and *S. oralis*. Moreover, the inhibition zones of 0.12% Chlorhexidine did not differ from 37% phosphoric acid inhibition zones except for *S. oralis*. Except for *S. oralis*, Clearfil SE Bond SEP and Futurabond NR performances were similar to 0.12% Chlorhexidine solution. One Up Bond formed a lower inhibition zone than 0.12% Chlorhexidine for *S. mutans*.

Among the species used in this study, *S. oralis* was the most sensitive for all adhesive systems, and it was the only microorganisms inhibited by G-Bond and Xeno IV. There were no differences in the inhibition zones formed by 0.12% Chlorhexidine, Futurabond NR and AdheSE for *S. oralis*, *S. sanguinis* and *S. sobrinus*. Clearfil S3 did not inhibit *S. mutans* and *S. Sobrinus* growth. Adper SE Plus did not inhibit *S. sanguinis* growth.

DISCUSSION

Many attempts have been made to produce dental materials that inhibit bacterial growth. This study evaluated the inhibition of bacterial growth by agar diffusion test of 9 SEAs or SEPs against 5 microorganisms. The bacterial species used in this study are related to dental caries disease. Among them, *S. oralis* and *S. sanguinis* are commonly found colonizing healthy oral cavity and are related to the initial colonization of dental surfaces or to fissures and smaller caries lesions. *S. mutans, S. sobrinus* and *S. cricetus* are species related to the caries lesions progression or to established caries lesions.

All SEAs or SEPs presented some antibacterial effect, however, they resulted in different mean values of inhibition zones. The only tested adhesive system that presents an antibacterial agent was Clearfil Protect Bond, which was as effective as Chlorhexidine with regard to the inhibition of bacterial growth. Chlorhexidine solution is a well-known wide range antimicrobial agent capable of reducing plaque formation due to its ability to denature the bacterial cell constituents.[18] Also Clearfil Protect Bond presented antibacterial activity against a wide range of streptococci species and with almost the same antibacterial

efficiency.[11,12,16,19-23] These results are in agreement with other studies, which showed the antibacterial potencial of Clearfil Protect Bont to reduce *in vitro S. mutans, A. viscosus, L. casei, L. salivarius, L. acidophillus* or in dentin samples without detrimental effects on bond strength or degree of conversion compared with other self-etching adhesives.[9,21]

Clearfil Protect Bond SEP presents the MDPB monomer, which is synthesized by combining a methacryloyl group with a quaternary ammonium that presents antibacterial properties with inhibitory effect against bacterial growth and plaque accumulation.[19-20] Thus, this monomer is a better alternative to compose the formulation of adhesive systems or resin composites than incorporation of other antibacterial substances. Caution is needed since previous studies have shown that incorporation of antibacterial agents could impair mechanical properties and the release of the agent from the material could result in further changes in physical properties.[19-20,24] For these reasons, the non-releasing antibacterial agent from adhesive systems able to provide adhesion and inhibit secondary caries are desired. Although the bonding agent of Clearfil Protect Bond presents NaF crystals in its composition, it was not evaluated in the present investigation. It is noteworthy that fluoride present in adhesive systems composition remains incorporated within the polymer matrix after cure. As for exerting an antibacterial activity fluoride needs to be in solution, its effect as an antibacterial agent seems to be limited clinically. However, in the present study fluoride might have played a role in the antibacterial effect of One-up Bond F Plus since it had not been photo-activated during the assay and antibacterial effect was similar to Chlorhexidine except for *S. mutans*.[13,14,25]

Except for the presence of the MDPB monomer, the composition of Clearfil SE Bond and Clearfil Protect Bond are very similar. No significant differences were observed with regard to the inhibition of bacterial growth for *S. oralis* and *S. cricetus* species. Also, Clearfil Protect Bond and Futurabond NR performances were similar except for *S. cricetus*. Then, the antibacterial effect could not be attributed to the presence of the MDPB monomer per se. In addition, the growth inhibition of cariogenic bacteria by Clearfil Protect Bond and Futurabond NR did not differ from Chlorhexidine to *S. cricetus*, *S. sanguinis*, *S. mutans* and *S. sobrinus* and was higher than Chlorhexidine to *S. oralis*. The

other SEPs and SEAs presented bacterial inhibition growth similar or lower than Chlorhexidine. On the other hand, G-bond and Xeno IV showed bacterial inhibition only against *S. oralis.*

As speculated by some authors, the main reasons for the inhibition of bacterial growth are probably because of the cytotoxicity of the monomers or the acidic pH of the self-etching primer.[9] However, Schmalz et al (2004)[25] did not find inhibition of bacterial growth to *S. mutans* and *S. sobrinus* by the monomers HEMA and TEGDMA suggesting that main antibacterial effect of the adhesives might be result of their low pH.[25] Futurabond NR performance was similar to Chlorhexidine for all bacteria probably due to be the lowest pH system among SEPs and SEAs evaluated. Because of the low pH SEPs and SEAs are expected to exert some antibacterial effect in dentin substrate before the restoration step, especially because dentin is not etched with phosphoric acid and rinsed with water, and smear layer is partially incorporated into the hybrid layer.[26-28]

No significant differences in the bacterial inhibition growth was observed when Chlorhexidine was compared to the 37% phosphoric acid gel group, which represented the etch-and-rinse adhesive systems. These results reinforce the hypothesis that the low pH may inhibit bacterial growth. However, the cleansing effect by acid followed by water rinsing used in the etch-and-rinse adhesive systems is limited to few seconds and its antibacterial activity should not be regarded as reliable.[11]

The authors are aware that this study does not resemble clinical conditions, the agar diffusion method is generally used to investigate the antibacterial activity of materials from which an antibacterial component leaches out and the activity is determined based upon the size of the inhibition zones. So this methodology cannot predict whether the antibacterial activity observed would last for extended periods or if this property is restricted to the moment of the adhesive application and photo-activation. Little antibacterial effect may be expected from these adhesive systems during clinical application, since it has been suggested that the acid process is stopped by acidic groups neutralization due to buffering action of dentinal fluid and calcium of hydroxyapatite soon after application[11,29] and because after polymerization the monomers are immobilized

into the polymer.[22,30] Then, the antibacterial effect of the self-etching adhesive systems clinically may be restricted to a short time and to the superficial layers of dentin, and may be considered limited.

Clearfill Protect Bond which contains the MDPB antimicrobial monomer may present a better clinical result, since, after curing, part of molecules with the methacryloyl structure is immobilized by co-polimerization with other methacrylate monomers and the other part preserve its antibacterial activity even after being immobilized. These characteristics make the use of materials containing MDPB a potential method to inhibit bacterial growth before and after cure.[19-20]

However, Feuerstein et al. (2007)[10] examined the immediate and long-term antibacterial effect of polymerized self-etching adhesive systems *in vitro* and found bacterial inhibition within a 14-day period for Clearfil Protect Bond. The other self-etching adhesive systems presented bacterial inhibition for 24 or 48 hours. Lobo et al. (2005),[31] evaluated the cariostatic effects of 3 adhesive systems by artificial caries development method using a microbiological model and found no cariostatic effect, even though there was reduced glucan synthesis provided by the adhesive system containing the Clearfil Protect Bond. The choice for an adhesive system for patients with high caries risk should consider the prevention of secondary caries reached by inhibition of residual bacterial growth in the cavity and should prevent invasion through gaps between composite restoration and cavity walls.[11,23]

CONCLUSIONS

The self-etching adhesives or self-etching primers used in this study demonstrated different levels of inhibition for the oral streptococci tested. Clearfil Protect Bond self-etching primer exhibited the most effective antibacterial activity against oral streptococci.

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Capítulo 3- In situ caries development around cavities restored with self-etching adhesive systems - Camila Esteves, André Figueiredo Reis, José Augusto Rodrigues

Running title: self-etching adhesives antibacterial activity

Clinical relevance statement: An antibacterial effect is desired for adhesive systems in order to avoid cariogenic bacterial colonization and secondary caries development.

Abstract

The aim of this study was to evaluate *in situ* the development of caries around cavities restored with self-etching adhesive systems. Methods: Enamel blocks were obtained from human third molars and received a 1.6mm-diameter cylindrical standardized cavity preparation. Prepared cavities were randomly distributed into six groups: G1- One-up Bond F Plus (Tokuyama); G2- Adper SE Plus (3M ESPE); G3- Clearfil Protect Bond (Kuraray); G4- Clearfil SE Bond (Kuraray); G5- Ketac-Fil (3M ESPE); and G6- composite resin with no adhesive system. Superficial mircrohardness was analyzed right after restorations were performed (KHN). The restored enamel blocks were fixed in palatal intra-oral appliances worn *in situ* for 23 human volunteers, which dropped a 20% sucrose solution 8 times a day. After 21 days they were removed and the final microhardness was analyzed. Microhadness data was evaluated by repeated measures ANOVA and Tukey test. Percentage of mineral loss was evaluated by 1-way ANOVA and Tukey test ($p<0.05$). Results showed that all groups developed dental caries around restorations. Reduction in enamel microhardness and mineral loss were observed. However, the glass ionomer cement was the only material that could reduce caries development. In spite of of the presence of fluoride or antibacterial monomers, the self-etching adhesive systems were not able to prevent or reduce dental caries development *in situ*.

Keywords: Self-etching adhesives, Bacteria, *In situ*, Microhardness, Enamel, Dental Caries, Composite Resin, Glass Ionomer Cement.

INTRODUCTION

Adhesive techniques have been routinely used to promote the infiltration of resin monomers into enamel/dentin surfaces and form a hybrid layer, which is the key for resin/dentin bonding[1-7]. This step should promote an effective, long-lasting seal of tooth structures, in order to avoid gap formation during cavity restoration. However, gap formation between composite and cavity walls, as a result of polymerization shrinkage stress, is still an important concern, since these sites could be colonized by oral bacteria from saliva leading to development of secondary caries.[8-10]

Self-etching adhesive systems (SEAs) were developed to reduce the technique sensitivity of etch-and-rinse adhesive systems. Incomplete resin infiltration, and evidence of phase separation within resin-dentin interfaces and its detrimental effects have been demonstrated.[5,6] The self-etching monomers are applied without further rinsing as they demineralize and infiltrate the substrate simultaneously.[7] However, the bond strength of SEAs to enamel are lower than etch-and-rinse adhesive systems and resin-enamel bond durability is still a controversy.[7] For this reason, an antimicrobial effect is desired for SEAs in order to avoid cariogenic bacterial colonization and also the growth of remaining bacteria in the cavity preparation.[11,12]

This effect is supposedly achieved by the incorporation of antimicrobial agents such as glutaraldehydes, fluorides or antibacterial monomers in the adhesive systems formulation.[9,11-15] Also, adhesive monomers of self-etching adhesive systems present a hydrophilic group at one end of the molecule, which is usually an acid such as hydrogen phosphate or carboxylate. These traits provide these materials a low pH and possibly some antibacterial properties.[10] Therefore, not only the antimicrobial agents but also other substances commonly found in adhesive systems formula such as adhesion-promoting monomers, which are acidic in different degrees, might be able to exert some activity against bacterial growth.[16-17]

Although several studies also describe the antibacterial effects of the MDPB (12-methacryloyloxydodecylpyridinium bromide) monomer, little is known about its antimicrobial effects and about the other self-etching adhesive systems when used in high caries risk clinical situations. Thus, the aim of this study was to evaluate *in situ* caries development around cavities restored with SEAs. The

hypothesis of the present study is that SEAs with antibacterial action can inhibit secondary caries lesion development.

MATERIALS AND METHODS

Experimental design

The Ethics Research Committee at the Guarulhos University approved the present research protocol (#51/2008). Each human volunteer wore an intra-oral appliance containing 6 enamel blocks, each one from one of the 6 different groups (Factor “restorative technique”): G1- One-up Bond F Plus (Tokuyama); G2- Adper SE Plus (3M ESPE); G3- Clearfil Protect Bond (Kuraray); G4- Clearfil SE Bond (Kuraray); G5- Ketac-Fil (3M ESPE); and G6- composite resin with no adhesive system (Table 1). Each volunteer worn the appliance for 21 days and was considered as one block. The quantitative variable response “development of artificial caries-like lesion” was evaluated twice blindly by Knoop Microhardness test, prior to caries challenge and after caries development (Factor “Time”). The microhardness data was used to analyze the percentage of mineral loss.

Table 1- Dental restorative materials composition according to manufacturers and batch numbers.

Group	Material (Batch number) Manufacturer	*Type*	*Components*
G1	One-up Bond F Plus (Bottle A: 054 Bottle B: 547) Tokuyama Dental Corporation + Z 350	Two bottle One step	Bonding Agent A: MAC-10, photo-initiator, methacryloylalkyl acid phosphate, multi-functional methacrylic monomers Bonding Agent B: MMA, HEMA, water, F-deliverable micro-filler (fluoro-alumino-silicate glass), photo-initiator
G2	Adper SE Plus (Liquid A: 8AP Liquid B: 8AP) 3M ESPE + Z 350	Two bottle One step	Liquid A: Water, HEMA Liquid B: Surface Treated Zirconia, TEGDMA, Di-Hema Phosphates, Mono Hema Phosphate, Methacrylated Pyrophosphates, Hema Phosphate Phosphoric Acids-6-Methacryloxy-Hexylesters Mixture, 1,6-Hexanediol Dimethacrylate, Diurethane Dimethacrylate, Trimethylolpropane Trimethacrylate, Ethyl 4-Dimethyl Aminobenzoate, Dl-Camphorquinone
G3	Clearfil Protect Bond (Primer: B00047B Bond: 00074A) Kuraray Dental Inc. + Z 350	Two bottle Two steps	Primer: MDPB, MDP, HEMA, hydrophilic dimethacrylate, photo-initiator, water Bond: MDP, Bis-GMA, HEMA, hydrophilic dimethacrylate, di-camphorquinone, N,N-diethanol-p-toluidine, silanated colloidal silica, surface treated sodium fluoride
G4	Clearfil SE Bond (Primer: 00896A Bond: 01320A) Kuraray Dental Inc. + Z350	Two bottle Two steps	Primer: MDP, HEMA, hydrophilic dimethacrylate, photo-initiator, water Bond: MDP, Bis-GMA, HEMA, camphoroquinone, hydrophobic dimethacrylate, N,N-diethanol p-toluidine bond, silanated colloidal silica
G5	Ketac-Fil (liquid: 276077 Powder: 248437) 3M/ ESPE, Seefeld, Germany	Glass ionomer cement	Powder: glass powder 100% Liquid: water 60-65%, polyethylene, polycarbonic acid 30-40%, tartaric acid 5-10%
G6	Z350 (7EC) 3M ESPE No adhesive system	Composite resin	Silane Treated Ceramic, Silane Treated Silica, BISEMA, UDMA, BISGMA, TEGDMA.

MDP: Methacryloyloxydecyl dihydrogen phosphate; MDPB: Methacryloyloxydodecylpyridinium bromide; HEMA: Hydroxy ethyl methacrylate; BISEMA: Bisphenol A Polyethylene Glycol Diether Dimethacrylate ; UDMA: Diurethane Dimethacrylate ; Bis-GMA: Bisphenol A Diglycidyl Ether Methacrylate; TEGDMA: Triethylene Glycol Dimethacrylate

Human enamel slabs preparation

Forty-five unerupted human third molars were selected and stored in a 0.1% thymol solution for no more than 30 days. The teeth were soft-tissue debrided and cleaned with water/pumice slurry and rubber cups in a low-speed handpiece (Kavo do Brasil, Joinville, SC, Brazil). The crowns were sectioned to obtain 138 enamel slabs (4x4x3mm^3) from the middle of the crowns, using double-faced diamond discs #7020 (KG Sorensen, Barueri, SP, Brazil, 06454-920).[14,18]

Enamel slabs were immersed in containers with distilled and deionized water. They were steam sterilized (Vitale 12L, Cristófoli Equipamentos de Biossegurança Ltda., Campo Mourão- Brazil) for 20 minutes at 121^o C before cavity preparation.[18-19]

Cavity preparation and restoration

Standardized circular cavities were prepared in the center of the enamel slabs. Cavities of approximately 1.6 mm in diameter and 1.6 mm depth were prepared at high speed with diamond burs #2292 (KG Sorensen, Barueri, SP, Brazil, 06454-920) under a constant water spray coolant.[14,18]

After the cavity preparations, the slabs were randomized among the restorative material groups (Table 1), and the cavities in 23 blocks were restored, with one sample from each group, in one increment, according to the manufacturers' instructions.

Cavities in groups G1 to G4 were restored with one of the four self-etching adhesive systems and Z350 composite resin. For G1 (One-up Bond F Plus), liquids A and B were mixed and applied for 10s in the cavity and light-cured for 10s by an Optilux 501 light unit (Demetron/Kerr, Danbury, CT, USA). The power density was measured by placing the light tip at the radiometer of the light unit. The light-cure unit had a light tip diameter of 11 mm with an irradiance of 700 mW/cm^2. For G2 (Adper SE Plus) liquid A was applied followed by liquid B for 20s, after that it was gently air flowed for 10s and liquid B was re-applied, air flowed and light-cured for 10s. For G3 (Clearfil Protect Bond) and G4 (Clearfil SE Bond) the primer solution was applied for 20s, gently air-blowed for 10s, the bonding agent was applied, gently air-thinned and light cured for 10s. For G5, Ketac-Fil was hand-mixed, inserted into the cavity with a Centrix injector, protected with a mylar strip (Dentart, Polidental, São Paulo, Brazil; dimension $10x120x0.05mm^3$) for 5 min, coated with Vitremer Finish Gloss and light-activated for 20s. For G6, no adhesive system was applied, the cavities were filled with Z350 composite resin, which was inserted and light-activated for 20s.

All restored slabs were immediately polished using the Sof-lex (3M ESPE) disks system for 15s with each disk and the surface microhardness was evaluated.[18]

Microhardness test

Microhardness measurements were performed in each enamel slab soon after restorative procedures prior to caries challenge (initial) and after *in situ* caries development (final). Knoop microhardness was measured keeping the long axis of the diamond parallel to the outer enamel surface using a

microhardness tester (Microdurômetro Digital 10A 1000 HVS-1000A, Panambra Ind. e Tec. S.A., São Paulo, SP, Brazil). In each one of the tests time, 4 indentations were made on each specimen applying 25g load for 5s in each 100 µm around the restoration margins in the upper, left, right, and bottom sides. For the final measurements the initial microhardness indentations were localized, and the final indentations were performed in the right side as described by Perito et al. (2009)[18].

Volunteer selection and in situ caries challenge

Twenty-four undergraduate students were selected as volunters to wear intra-oral appliances with the restored enamel slabs in the *in situ* phase. Before participating in the study, subjects were informed of the objectives, benefits, and possible risks involved in this study, and accepted to participate only after signing an informed consent form. The volunteers were evaluated clinically and were required to present all maxillary teeth free of caries. The exclusion criteria included presence of a fixed or removable denture, presence of orthodontic appliances, pregnant or nursing women and smokers. The subjects included 24 adults (14 female and 10 male) ranging from 20 to 34 years with a mean age of 27.

Each subject received instructions on how to perform the Bass tooth brushing technique using a toothbrush (Oral-B® Indicator® Plus #35, Oral-B Gilette do Brasil, Manaus, Brazil). A non-fluoride toothpaste (Sanikids, SANiFill® Facilit Odontológica e perfumaria LTDA, Curitiba- Pr; Brazil) was used by each subject during the study period. Each patient was given a complete prophylaxis one week before beginning the *in situ* treatment (wash out period). Stone casts were obtained and palatal intra-oral appliances with 6 individual windows were produced.

Soon after initial microhardness analysis, the slabs were fixed with wax in the palatal intra-oral appliances with nylon mesh, leaving a space of 1 mm between the slab surface and the mesh, to allow biofilm accumulation, as described by Benelli et al.[20]

The volunteers wore the appliances for 21 consecutive days, brushed their teeth exclusively with the non-fluoride-containing dentifrice, toothbrush, and

dental floss supplied by the researchers. The volunteers were advised to remove the appliances only to eat, drink, and perform oral hygiene. Eight times a day, a drop of 20% sucrose solution, was applied to each window to induce a high cariogenic challenge, followed by a 5-min wait before replacing the appliance in the mouth.

After *in situ* caries development, the palatal intra-oral appliances were removed and a final microhardness analysis was performed.[18] One representative specimen of each group was evaluated by scanning electron microscopy (SEM - FEI; Quanta 600F, Nederland, NE).

Statistical analysis

The statistical analysis considered the average of the four Knoop microhardness initial and final indentations from each specimen (Table 2). Data was submitted to repeated measures Analysis of Variance, which showed statistical significant differences, followed by Tukey test ($p< 0.05$). Then, the mineral loss was determined by the following calculation: M(KHN)= (KHNi - KHNf)/KHNi, where KHNi is the initial Knoop hardness number, and KHNf represents the final Knoop hardness number, as described by Watanabe et al. 2005[21] (Table 2), for statistical analysis One-way ANOVA was done followed by Tukey test ($p< 0.05$).

RESULTS

There were statistically significant differences in the microhardness values for the factors “Restorative Technique” and “Time” and for the interaction between factors ($p< 0.0001$). There were no significant differences among groups in the initial evaluation time, but in the final evaluation, G5 (glass ionomer cement) presented significantly higher microhardness values than the others groups. All groups presented a significant reduction in microhardness after the cariogenic chalenge (Table 2). Significantly lower mineral loss percentage was observed for G5, while no significant difference was observed among G1, G2, G3, G4 and G6.

Table 2- Mean Knoop microhardness values in KHN (SD) for the different restorative techniques before and after the *in situ* cariogenic challenge; and the percentage of mineral loss per group (SD).

Group	Initial Microhardness	*Final microhardness*	*Percentage of mineral loss*
G1- One-up Bond F Plus	402.3 (81.7) Aa	85.2 (78.3) Bb	78.7% (19.6) B
G2- Adper SE Plus	389.6 (75.0) Aa	106.2 (96.9) Bb	70.4% (28.0) B
G3- Clearfil Protect Bond	414.5 (75.5) Aa	112.3 (90.4) Bb	72.4% (21.9) B
G4- Clearfil SE Bond	393.4 (64.6) Aa	105.8 (111.7) Bb	72.6% (28.2) B
G5- Ketac-Fil	398.3 (51.2) Aa	265.7 (107.2) Ab	33.1% (26.3) A
G6- Z-350	406.5 (73.8) Aa	63.9 (66.1) Bb	83.3% (18.0) B
Means followed by different letters (upper case - column, lower case - row) are significantly different by Tukey test. ($p < 0.05$)			

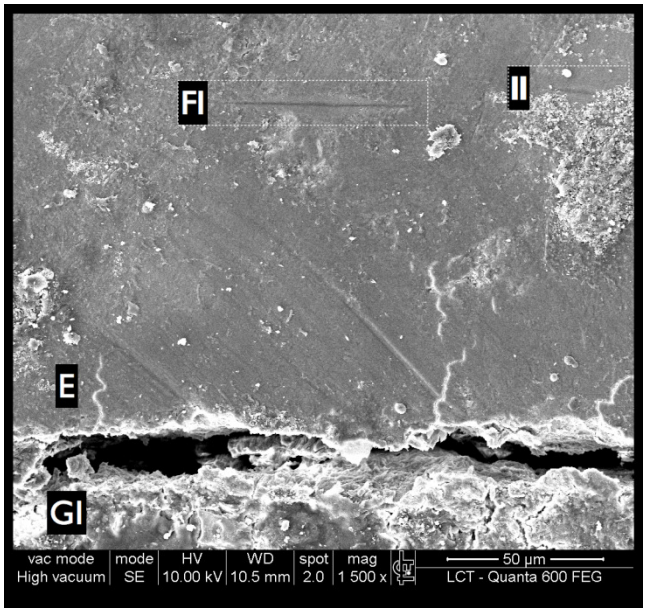

Figure 1. Scanning electron microscopic (SEM) photograph of enamel restored surface. II- initial microhardness indentation FI- final microhardness indentation; E- enamel; GI- glass iomoner cement.

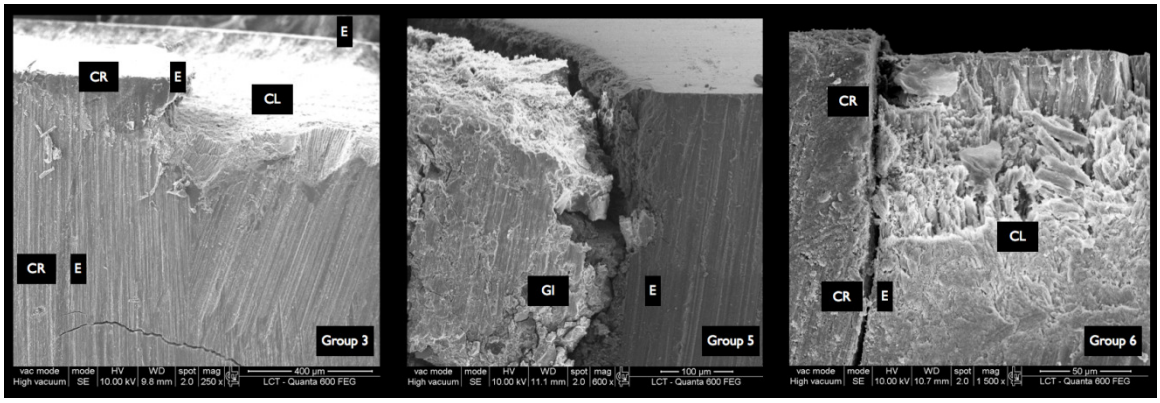

Figure 2. Scanning electron microscopic (SEM) photographs of transversal section of Group 3, 5 and 6. CR- composite resin; E- enamel; GI- glass iomoner cement; CL- Caries lesion.

DISCUSSION

Many efforts have been made to produce dental materials with cariostatic properties that can inhibit dental caries development such as glass ionomer cements[14]. This study analyzed *in situ* the development of caries lesions around standardized composite resin restorations performed with self-etching adhesive systems with antibacterial properties due to the presence of fluoride or MDPB monomer.

Two monomers with antibacterial properties have been recently developed.[22-26] The MDPB monomer is synthesized by combining methacryloyl groups with quaternary ammoniums that presents antibacterial properties with inhibitory effect against bacterial growth and plaque accumulation (MDPB and DMAE-CB).[22-26] Before polymerization, quaternary ammonium monomers are assumed to exert biocide activity by reacting with negatively charged bacterial surface, causing disruption of cell membranes, and subsequently leading to the increase of cell permeability and the loss of cytoplasmatic content.[26] After polymerization, these monomers can exert contact antibacterial activity by inhibition on the bacterial growth and adherence.[26] It is well know that Clearfil Protect Bond presents *in vitro* antibacterial activity against a wide range of streptococci species and with almost the same antibacterial efficiency.[11,12,16,22-29] This self-etching primer system also shows antibacterial potencial to reduce *A. viscosus, L. casei, L. salivarius, L. acidophillus* in *in vitro* cultures or in dentin sample cultures without detrimental effects to bond strength or degree of conversion compared with other self-etching adhesives.[9,25,27] Thus, an

antibacterial monomer seemed to be a better alternative to compose the formulation of adhesive systems or resin composites than incorporation of other antibacterial substances, since previous studies have shown that incorporation of antibacterial agents, such as chlorhexidine, could impair mechanical properties and release of the agent from the material could result in further changes in physical properties.[24,30]

However, in the present study, Clearfil Protect Bond (G3) was not able to prevent or reduce dental caries development in spite of the presence of the MDPB monomer in the primer solution and NaF crystals in the bonding resin, and showed a 72.4% mineral loss with no significant differences from the other SEAs or from the group restored with composite resin only (G6).

The group restored with conventional glass ionomer cement (G5) also developed dental caries, but its microhardness values were significantly higher than the other SEAs or the control group, indicating its ability to reduce dental caries development. In addition, this group presented significantly lower mineral loss. This result is due to the ability of glass ionomer cements to release fluoride over an extended period without affecting physical properties. In addition, it presents the advantage of, in a clinical situation, uptake fluoride during topical fluoride treatment, acting as a fluoride reservoir and release fluoride ions over a relatively long period.[31] But it probably did not occur in this study because a non-fluoride toothpaste was used by the patients.

The benefit of fluoride incorporation to composite resins has been reported.[24,32] It is noteworthy that fluoride in adhesive systems remain incorporated within the polymer matrix after cure. However, for exerting an antibacterial activity, fluoride needs to be in solution, as occured in the group restored with glass ionomer cement (G5), which was able to reduce caries lesion progress. Then, the fluoride present in SEAs as an antibacterial agent seems to be limited clinically, since fluoride is enveloped by adhesive resin. As in Clearfil Protect Bond, the antibacterial effects of fluoride in One-up Bond F Plus, which shows *in vitro* antibacterial effect, was also not able to prevent or reduce dental caries development *in situ*.[13,14,33]

Except for the presence of the MDPB monomer, the composition of Clearfil SE Bond (G4) and Clearfil Protect Bond is very similar. No significant differences were observed with regard to caries development. Also Adper SE Plus (G2) showed the same pattern of caries development of the other SEAs experimental groups and did not differ from control group restored with composite resin only.

However, Feuerstein et al. (2007)[10] examined the immediate and long-term antibacterial effect of polymerized self-etching adhesive systems *in vitro* and found bacterial inhibition within a 14-day period for Clearfil Protect Bond. The other self-etching adhesive systems presented bacterial inhibition for 24 or 48 hours.[10] Lobo et al. (2005),[34] evaluated the cariostatic effects of 3 adhesive systems by artificial caries development method using a microbiological model and found no cariostatic effect, even though there was reduced extracellular glucan synthesis provided by the adhesive system containing the Clearfil Protect Bond. Since glucans are synthesized by bacteria as an essential mechanism for cellular adhesion and biofilm formation, few antibacterial effect may be expected by quaternary ammonium monomers. Thus, the choice of an adhesive system for patients with high caries risk should also consider the prevention of secondary caries reached by inhibition of residual bacterial growth in the cavity and should prevent invasion through gaps between composite resin and cavity walls.[11,29]

Schmalz et al (2004)[33] found no *in vitro* antibacterial effect for the the monomers HEMA and TEGDMA against *S. mutans* and *S. sobrinus*.[33] Then, the main antibacterial effect of the SEAs might be result of their low pH. However, the application time of SEAs is limited to no more than a minute and the pH is easily buffered in contact with mineralized dentin. Then, their antibacterial activity should not be regarded as reliable after polymerization.[11]

The authors are aware that this study model resembles clinical conditions of high caries risk and in a less intense caries challenge the antibacterial effect could be perceived. Pinto et al. (2009)[35] after an *in situ* challenge of 14 days with 20% sucrose solution applied 8 times a day found that the Clearfil Protect Bond adhesive promoted less demineralization around restorations on bovine enamel.[35] Even though no inhibition in caries development was observed for the

self-etching adhesive systems tested in this study, the antibacterial effect of fluoride and MDPB monomer are well documented *in vitro* by the agar diffusion method and must be considered. This method is generally used to investigate the antibacterial activity of materials from which an antibacterial component leaches out and the activity is determined based upon the size of the inhibition zones and must be one of the first steps in the evaluation of a material prior to clinical evaluations. However, this methodology cannot predict exactly whether the antibacterial activity observed would last or also if it is restricted to the moment of the adhesive application.

Thus, the association of previous *in vitro* studies with this *in situ* investigation shows that little antibacterial effect may be expected from these adhesive systems during clinical application. Because, the buffering action of dentinal fluid and calcium of hydroxyapatite buffer the acidic action of self-etching monomers soon after application,[11,36-37] and because after polymerization fluoride and antibacterial monomers are immobilized into the polymer matrix.[27,32] Then, the antibacterial effect of the self-etching adhesive systems can be considered limited, because it can be restricted clinically to a short time and to the superficial layers of the adhesive surface.

CONCLUSIONS

Regardless of the presence of fluoride or the MDPB antibacterial monomer, the self-etching adhesives systems were not able to prevent or reduce dental caries development *in situ*.

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