Antibacterial-containing dental adhesives' effects on oral pathogens and on *Streptococcus mutans* biofilm: Current perspectives

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Abstract: Purpose: To describe the literature findings regarding commercially available antibacterial-containing dental adhesives and the futures perspectives of this field. **Results:** High-risk caries patients could yield benefits from restorative materials containing antibacterial properties in order to reduce the recurrent caries formation. Dental adhesives with antibacterial agents may reduce restoration replacement, as recurrent caries is still one of the major reasons for replacing a resin restoration. Literature results of three commercially available adhesives: Gluma 2Bond, Clearfil SE Protect and Peak Universal Bond, containing glutaraldehyde, MDPB and chlorhexidine, respectively indicates that Clearfil SE Protect seems to have better results against oral pathogens and on *Streptococcus mutans* biofilm. Besides the promising findings, clinical studies are still necessary in order to validate the clinical efficacy when exposed to a more complex environment and the long-term effect of either commercially available materials, experimental antibacterial monomers or antibacterial incorporations. As a suggestion of this article and according to the current scientific trends in this specific field, future directions should focus on restorative materials with therapeutic components targeting the virulence factors of cariogenic biofilm with minimal toxicity and side effects, and long-term action. (*Am J Dent* 2018;31:(Sp Is B):37B-41B).

Clinical significance: Antibacterial-containing dental adhesives may have therapeutic effects, working as an additional source to reduce recurrent caries development in patients with high-risk of caries, and consequently the reduction in restoration replacements.

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Introduction

Practitioners have spent a lot of time replacing or performing resin restorations due to recurrent caries formation, tooth fractures, restoration fractures, loss of marginal integrity or lack of marginal sealing and non-carious cervical lesions, such as erosion, abrasion and abfraction.[1-6] To restore small and middle-size cavities, resin-based composites have been used due to their outstanding esthetic appeal[7] and excellent adhesive strength to dentin and enamel in combination with bonding agents. Several dental adhesive systems are commercially available for clinical use and are classified according to their application mode.[8]

Etch-and-rinse adhesives can be applied in two or three steps and their main characteristic is the application of an adhesive after phosphoric acid etching in wet demineralized dentin. Three-step etch-and-rinse adhesives use a primer, which is generally an aqueous solution containing HEMA (2-hydroxyethyl methacrylate), while two-step etch-and-rinse adhesives present a combination of primer and bonding resin in a single bottle, which contains organic solvents, such as alcohol or acetone.[9]

Self-etch adhesives are applied in one or two steps and the main compositional characteristic is the presence of functional monomers, which are responsible to etch and infiltrate into mineralized tooth structures. Two-step self-etch adhesives use an acidic primer followed by a bonding or hydrophobic resin. Single-step or all-in-one self-etching systems are user-friendly bonding agents; however, many studies have criticized this category of adhesives regarding clinical durability.[10]

Besides resin monomers, chemical initiators and organic solvents, dental adhesives may contain filler, fluoride, desensitizing or antimicrobial agents.[8] Many compounds and substances, such as triclosan, dimethylaminododecyl methacrylate (DMADDM), silver nanoparticles, doxycycline-encapsulated halloysite nanotube, zinc methacrylate, methacryloxylethyl cetyl dimethyl ammonium chloride (DMAE-CB) have been incorporated into dental bonding agents in order to promote antibacterial activity.[11-15]

Antibacterial properties in adhesive systems or composites are considered a viable option to reduce the bacterial colonization around dental restorations, prevent recurrent caries by suppressing biofilm formation and acid production, and thereby reduce restoration replacement.[16-18] Although extensive research on antibacterial agents incorporated into dental adhesives or antibacterial monomer syntheses is available, just a few commercial adhesives contains antimicrobial agents, such as Clearfil SE Protect[a] (methacryloyloxydodecylpyridinium bromide, MDPB), Gluma 2Bond[b] (glutaraldehyde) and Peak Universal Bond[c] (chlorhexidine).[19,20]

The most well-known adhesive with antimicrobial activity is Clearfil SE Protect, a two-step self-etch system that contains MDPB in the primer solution. MDPB is a polymerizable quaternary ammonium methacrylate that copolymerizes with other adhesive monomers and disrupts the bacterial cell membrane when bacterium is in direct contact with the adhesive layer (by contact of the negatively charged bacteria with positively charged quaternary ammonium).[21,22] Antibacterial monomers that copolymerize with other adhesive monomers may provide long-term antibacterial activity.[23] After the development of MDPB, several other monomers with quaternary ammonium have been synthesized and incorporated into dental materials as antibacterial agents.[24-26] Despite the in-

creased development and evaluation of experimental antibacterial monomers, containing or based on substances with broad antimicrobial action, as antibacterial agents, the focus of this article is to discuss commercially available dental adhesives and their future perspectives.

Regarding the commercially available dental adhesives containing antibacterial agents, using a direct contact method the Clearfil SE Protect was tested against four facultative bacteria and four strict anaerobic microorganisms and had a bactericidal effect against *Fusobacterium nucleatum* after 10 minutes, against *Streptococcus mutans*, *Porphyromonas gingivalis*, *Prevotella intermedia* and *Prevotella nigrescens* after 30 minutes and against *Staphylococcus aureus* and *Lactobacillus casei* after 24 hours.[19] Another study[20] showed antimicrobial effects against oral pathogens by inhibition halo method and the decrease of viability of *S. mutans* biofilm grown on top of the adhesive layer, compared to Clearfil SE Bond. The same adhesive was tested in simulated Class I restorations and a significant reduction in formation of biofilm of *S. mutans* was also achieved, when compared to an adhesive without antibacterial agent.[27] In situ studies[28,29] indicate that Clearfil SE Protect is capable of controlling the caries progression in enamel at the restoration interface under conditions of high cariogenic challenge, compared to an adhesive with fluoride in its composition. Likewise, an in vivo study[30] showed a reduction in caries formation around brackets after 30 days compared to conventional methods. In addition, it was reported[31] that *E. faecalis* and *S. mutans* were not able to adapt to MDPB, which may suggest a lower risk of producing drug resistance.

A two-step etch-and-rinse adhesive, Gluma 2Bond, contains 5% glutaraldehyde, which is a desensitizing and strong antibacterial agent.[32-34] This adhesive showed bactericidal contact activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Lactobacillus casei*, *Streptococcus mutans*, *Prevotella nigrescens* and *Fusobacterium nucleatum* after 24 hours and against *Porphyromonas gingivalis* and *Prevotella intermedia* after 1 hour.[19] The qualitative analysis of *S. mutans* biofilm using scanning electron microscopy showed a decrease of colonies when using Gluma 2Bond compared to a similar adhesive without glutaraldehyde; a result that was confirmed by colony counting.[20] Another study[35] also investigated dental adhesives containing glutaraldehyde (Gluma Primer[b] and Syntac Classic System[c]) and glutaraldehyde present in Gluma Primer[b] and Syntac Adhesive[c] appears to be effective against infected dentin. An in vivo study[36] also showed the dentin disinfecting capacity of a glutaraldehyde-containing adhesive compared to an adhesive without antibacterial agent. Glutaraldehyde-containing bonding agents have been criticized due to toxicity and mutagenic potential of this type of aldehyde. These effects were already described.[37,38]

Peak Universal Bond contains 0.2% chlorhexidine di(acetate), which is a cationic polybiguanide, bisphenol component containing chlorine that reacts with the negatively charged microbial cell surface, destroying its membrane. Chlorhexidine has a wide spectrum of action against gram-positive and gram-negative organisms, facultative, anaerobes, aerobes and fungi.[39-41] This two-step etch-and-rinse adhesive demonstrated bactericidal contact activity only for strict anaerobic microorganisms (*Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens* and *Fusobacterium nucleatum* after 24 hours).[19] No effect against *Streptococcus mutans* biofilm was observed for this adhesive, compared to the same adhesive without chlorhexidine. However, these adhesives (with and without chlorhexidine) presented a reduction in biofilm of *S. mutans* similar to Clearfil SE Protect, which implies that other components, such as adhesive monomers and solvents may have antibacterial activity.[20] These results suggest that chlorhexidine may stay trapped in the polymer chain, without the release properties.[19,20] In another study,[42] Peak Universal Bond presented a lower *S. mutans* biofilm formation compared to the same adhesive version without chlorhexidine; however the specimen preparation was different and the incubation time was lower. In addition, this non-light-cured adhesive presented an inhibition halo against some bacteria, suggesting that it may work as a cavity disinfectant.[20] Also, for Peak Universal Bond, an inhibition halo for *S. mutans* was identified when it was not light-cured.[43]

The complex interactions between the specific oral bacteria, salivary constituents, dietary carbohydrate, and tooth surface modulates the transition from a condition of health to a diseased state by the establishment of cariogenic biofilms and consequently surface cavitation by acid dissolution, resulting in dental caries.[44] Regarding the role of the aforementioned bacteria at the pathogenesis of caries disease, *S. mutans* is considered the main pathogen involved in caries formation.[45] *S. mutans* is not always the most predominant at the initial colonizing community, however the primary role of *S. mutans* resides with its ability to assemble an insoluble polymeric matrix, forming the core of the matrix-scaffold in cariogenic biofilms.[46] Besides the extracellular polysaccharides production, the virulence of *S. mutans* is also associated to the production of weak acids from sugars, to adapt to large fluctuations in pH, oxygen tension and nutrient availability.[47,48]

Other microorganisms present in the complex oral microbiota also play an important role in caries disease development and progression.[20] *Lactobacillus casei* is an acidogenic and acid tolerant bacteria that can grow and survive in an acidic environment;[49,50] *Staphylococcus aureus* is found in individuals with aggressive periodontitis[51] and *Enterococcus faecalis* is associated with chronic periodontitis and frequently is the only species that persists in endodontically treated teeth.[20,52,53]

Strict anaerobic bacteria are more related to periodontal disease and can be found in cariogenic biofilm around the gingival margin.[54] Due to further accumulation of biofilm, the number of obligatory anaerobic bacteria increase, changing the antimicrobial biofilm composition from streptococcus-dominated to *Actinomyces* spp. that is involved in root caries, and *P. gingivalis* involved and periodontal disease.[19,54,55] *P. intermedia* is also a periodontal pathogen found in patients with early periodontitis, advanced periodontitis, and acute necrotizing ulcerative gingivitis.[56,57] *P. nigrescens* also plays a role in the pathogenesis of periodontal disease, gingivitis and some odontogenic infections.[58,59] *F. nucleatum* is frequently associated with periodontal diseases and is commonly found in human dental plaque with a crucial role in plaque development.[19,60-62]

Clearfil SE Protect, Gluma 2Bond and Peak Universal Bond

present dentin bond strengths around 40 MPa and did not differ among them when the specimens were analyzed after artificial saliva storage for 1 year. These adhesives form a hybrid layer and resin tags, which represent the bonding mechanism of contemporary bonding agents. Thus, the presence of antimicrobial components in the composition of adhesives seems to not interfere in the bond strength and bonding mechanism.[19,20]

Bonding agents containing antibacterial compounds are indicated for patients with very poor oral health, due to the high probability of recurrent caries development. Elderly patients with greater incidence of root caries and patients who have limitations to promote their own oral hygiene may also benefit when restorations are performed with materials containing antibacterial agents. Although some advantages have been extensively reported in the dental literature, there are concerns regarding the side effects produced by antibacterial agents and little clinical evidence that supports the in vitro findings has been reported.[24,33] Also, the antibacterial activity in multispecies biofilm may be lower compared to results with planktonic bacteria,[63] considering that the bacteria are protected by a diffusion barrier, the extracellular matrix.[64] Another concern regarding in vitro tests remains on the interaction between the adhesive layer and the saliva pellicle. Some publications suggested that the saliva pellicle could attenuate antibacterial properties of underlying surfaces.[65,66] However, the antibacterial effect of Gluma 2Bond and Clearfil Protect Bond was expressed in the biofilm of *S. mutans,* even covered with clarified saliva.[20]

One of the major side effects related to substances with broad antimicrobial spectra into restorative materials is the oral health resident bacterial interference and the promotion of bacterial resistance, producing undesirable outcomes on oral health.[67] In order to reduce these side effects, the incorporation of natural products are been proposed, as a result of the lower probability of producing bacterial resistance. Natural products are considered a potential alternative approach to the current chemotherapeutic strategies, owing to the fact that natural products are a safer technology, biologically and environmentally, when compared to compounds synthetized by chemical or physical methods.[68,69]

Propolis is a natural product composed of a resinous substance collected by *Apis mellifera* bees from various plant sources. It is considered a nontoxic natural product with a complex chemical composition and exhibits a wide range of biological activities, including antimicrobial, anti-inflammatory, anesthetic, and cytostatic properties.[70,71] Two components were isolated from a Brazilian propolis, apigenin and tt-farnesol, and may represent an important alternative to current antibacterial agents, seeing that they can reduce the expression of virulence of *S. mutans* without necessarily suppressing the resident oral microbiota.[68]

Apigenin (4′,5,7-Trihydroxyflavone) is a potent inhibitor of water-insoluble glucan synthesis (inhibitor of glucosyltransferases B and C), while tt-farnesol (trans,trans-3,7,11-trimethyl-2,6,10-dodecatrien-1-ol) changes the permeability and fluidity of the cell membrane by its lipophilic properties, affecting its glycolytic activity, production-secretion of glucosyltransferases and acidurance.[72,73] They can be used separately or together, and seem to be more effective in the presence of fluoride.[68]

One study[67] incorporated these components into comercial bonding agents that contain fluoride (Patent: BR 10 2014 024497 5): Clearfil S3 Bond Plus, a single-step self-etch adhesive and Optibond S,[e] a two-step etch-and-rinse adhesive. The results were promising and may represent a novel alternative to decrease the cariogenicity of the biofilm around dental restorations, without suppressing the target microorganism. The addition of apigenin or appigenin and tt-farnesol to Clearfil S3 Bond Plus were more efficient regarding the reduction of virulence of *S. mutans* compared to Optibond S and they did not interfere on the adhesion mechanism of both adhesives.[67] Clearfil S3 Bond Plus containing apigenin reduced the amount of insoluble and intracellular polysaccharides of *S. mutans* biofilm grown for 5 days on top of the adhesive layer covered with clarified saliva.[67]

The new approach of incorporating anti-caries agents that are less likely to induce bacterial resistance into restorative materials could yield benefits in terms of enhanced durability of composite restorations, mainly in areas where biofilms accumulate, such as the interproximal and cervical regions of the teeth, by targeting the main virulence factors of *S. mutans* biofilm, namely the insoluble polysaccharides and intracellular polysaccharides.[67] The reduction of both polysaccharides could affect the *S. mutans* ability to colonize the tooth surface and become the dominant bacteria and expressing it's virulence.[74] Although this approach is considered promising, further studies are necessary to clarify the effect on multispecies biofilm, on long-term action, and in vivo conditions (animal studies or long-term clinical trials).

Following the same trend of incorporating natural products that have antibacterial properties into dental materials, chitosan and Epigallocatechin-3-gallate (EGCG) were also investigated when added to dental adhesives. The antibacterial activity of chitosan remains on the interaction between the positively charged chitosan and the negatively charged bacteria cell surface, causing the cell wall rupture.[75,76] When added to dental adhesives the antibacterial effect has been reported.[76,77] Conversely one study[78] showed the absence of antibacterial activity of chitosan into a dental adhesive. EGCG, a flavonoid produced by *Camellia sinensis* plant (green tea), may be capable of suppressing gtf B, C, and D gene expression, disrupting *S. mutans* biofilm formation. This compound was able to express antibacterial activity when incorporated into dental adhesives in some concentrations.[79] In addition, the increased research in natural products brings new alternative formulations to oral health care, including antibacterial, antifungal, and anti-caries properties, still poorly explored in the dental biomaterials field.

Conclusions and future perspectives

Dental adhesive systems containing antibacterial or anti-caries agents show remarkable results against oral pathogens in in vitro studies. MDPB containing adhesives had greater results and is extensively explored in the dental literature. These antibacterial findings suggest a favorable indication of antibacterial dental adhesives for patients with high caries risk. Incorporation of natural products into restorative materials that can act on the *S. mutans* virulence factors can be considered a new approach in order to reduce recurrent caries formation,

without killing the target organism. Besides the promising findings, clinical studies are still necessary in order to validate the clinical efficacy when exposed to a more complex environment and the long-term effect of either commercially available materials, experimental antibacterial monomers or antibacterial incorporations. Future directions in research should focus on restorative materials with therapeutic components targeting the virulence factors of cariogenic biofilm with minimal toxicity, side effects, and with long-term action.

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