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Antibacterial Activity of Various Self-etching Adhesive Systems Against Oral Streptococci

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Clinical Relevance

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 current study evaluated 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 five 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 300 uL of bacterial cell suspension (0.5 MacFarland) were prepared. Holes 6 mm in diameter were made and partially filled with bacteriological agar. Then, 10 uL of each SEA or SEP was dropped and the plates were incubated under microaerophilic atmosphere at 37°C for 48 hours and the diameter of each halo was registered. The results were analyzed by two-way ANOVA and Tukey test. PB exhibited the most effective antibacterial activity against oral streptococci. The performances of SE and FB 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 to all self-etching adhesive systems; on the other hand, *S cricetus*,

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***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.**

INTRODUCTION

The bonding of resin-based composites to dentin can be accomplished by means of etch-and-rinse or self-etch 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, wherein self-etching monomers are applied without further rinsing.[7] Both techniques have been routinely used for promoting the infiltration of resin monomer into the 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 the 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 to avoid the growth of remaining bacteria in the cavity preparation.[11-12] This effect can be achieved by 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 with a low pH and possibly some antibacterial properties. Therefore, not only the antimicrobial agents but also other substances commonly found in the adhesive systems formulas, such as adhesion-promoting monomers that 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 current study evaluated the antibacterial properties of some self-etching/priming solutions against oral streptococci.

METHODS AND MATERIALS

Nine commercially available self-etching adhesives (SEAs) or self-etching primer systems (SEPs) were selected for this experiment (Table 1). A 37% phosphoric acid gel and a 0.12% chlorhexidine solution were used as positive controls. The five microorganisms used in this study were: *Streptococcus oralis* (p12,6.2), *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 cell 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 each bacterial cell suspension was mixed with 65ml of melted BHI agar at 50°C and poured onto a plate.

Eleven sterilized glass cylinders (8 mm x 30 mm) were placed onto each inoculated agar plate before 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 the SEAs (Xeno IV, Clearfil S^3 Bond, Futurabond NR and G-bond), the mixture of the two bottle SEAs (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 a microaerophilic atmosphere at 37°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 they were recorded. The measures considered were the highest diagonal (mm) in the circular inhibitory zones. The mean values were calculated for each experimental group and the data were analyzed by two-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 the tested materials were photographed (Figure 1).

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 ($p<0.00001$).

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*.

Table 1: *Self-etching Adhesives and Self-etching Primers Composition According to the Manufacturers' and Batch Numbers*

Adhesive System (Batch #)	Manufacturer	Type	pH	Components
Clearfil S^3 Bond (00056A)	Kuraray Dental Inc Kurashiki, Japan	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 Kurashiki, Japan	Two bottle Two steps	Primer: 2.0	Primer: MDP, HEMA, hydrophilic dimethacrylate,photo-initiator, water
Clearfil Protect Bond (Primer: B00033B)	Kuraray Dental Inc Kurashiki, Japan	Two bottle Two steps	Primer: 2.0	Primer: MDPB, MDP, HEMA, hydrophilic dimethacrylate, photo-initiator, water
Xeno IV (060926)	Dentsply Caulk Milford, DE, USA	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 St Paul, MN, USA	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 Schaan, Liechtenstein	Two bottle One step	1.7	Primer: dimethacrylate, phosphonic acid acrylate, water, initiators and stabilizers
One-up Bond F Plus (Bottle A: 054 Bottle B: 547)	Tokuyama Dental Corporation Tokyo, Japan	Two bottle One step	A: 0.7 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 Cuxhaven, Germany	Two bottle One step	1.4	dimethacrylates, silicate fillers, initiators, stabilizers, additives
G-bond (0604211)	GC America Inc Alsip, IL, USA	One bottle One step	2.04	4-MET, phosphoric ester-monomer, UDMA, TEGDMA, acetone, water, stabilizer, silica filler, water, photo-initiator

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.

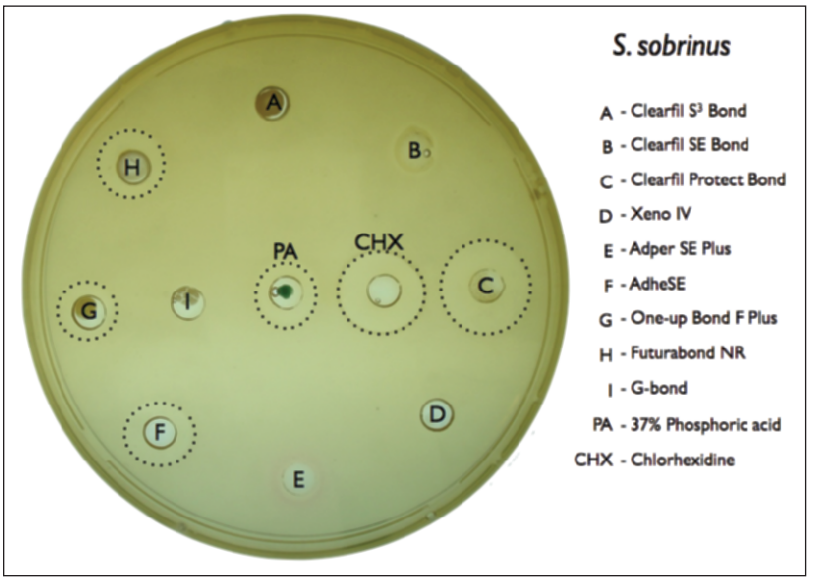

Figure 1: *Representative image of a plate inoculated with* S sobrinus. *Bacterial inhibition growth for the different self-etching adhesive systems can be observed (dashed line).*

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*, the performances of Clearfil SE Bond SEP and Futurabond NR 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 the current study, *S oralis* was the most sensitive for all adhesive systems, and it was the only microorganism 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 the growth of *S mutans* and *S sobrinus, and* Adper SE Plus did not inhibit the growth of *S sanguinis*.

DISCUSSION

Many attempts have been made to produce dental materials that inhibit bacterial growth. The current study evaluated the inhibition of bacterial growth by agar diffusion test of nine SEAs or SEPs against five 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 the 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 progression of caries lesions 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 presented 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 bacterial cell constituents.[18] Also, Clearfil Protect Bond presented antibacterial activity against a wide range of streptococci species with almost the same antibacterial efficiency.[11-12,16,19-23] These results are in agreement with other studies that showed the antibacterial potential of Clearfil Protect Bond 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 an inhibitory effect against bacterial growth and plaque accumulation.[19-20] Thus, this monomer is a better alternative for composing the formulation of adhesive systems or resin composites than the incorporation of other antibacterial substances. Caution is needed, since previous studies have shown that the 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, non-releasing antibacterial 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 current investigation. It is noteworthy that fluoride, present in adhesive systems composition, remains incorporated within the polymer matrix after cure. As for exerting antibacterial activity, fluoride needs to be in the solution, its effect as an antibacterial agent seems to be clinically limited. However, in the current 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 the 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, the performances of Clearfil Protect Bond and Futurabond NR 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 to or lower than chlorhexidine. On the other hand, G-bond and Xeno IV only showed bacterial inhibition against *S oralis*.

The current findings demonstrate different inhibition levels produced by the different SEPs or SEAs against the oral streptococci investigated. As speculated by some authors, the main reasons for the inhibition of bacterial growth are probably due to the cytotoxicity of the monomers or the acidic pH of the self-etching primer.[9] However, Schmalz and others[25] did not find inhibition of bacterial growth to *S mutans* and *S sobrinus* by the monomers HEMA and TEGDMA, suggesting that the main antibacterial effect of the adhesives might result from their low pH.[25] The performance of Futurabond NR was similar to chlorhexidine for all bacteria, probably due to it having the lowest pH system among the 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 the smear layer is partially incorporated into the hybrid layer.[26-28]

No significant differences in bacterial inhibition growth were 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 low pH may inhibit bacterial growth. However, the cleansing effect of acid, followed by water rinsing used in the etch-and-rinse adhesive systems, is limited to a few seconds and its antibacterial activity should not be regarded as reliable.[11]

The authors of the current study 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 on the size of the inhibition zones. As a result, 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 neutralization of the acidic groups due to the buffering action of dentinal fluid and the calcium of hydroxyapatite soon after application.[11,29] Also because, after polymerization the monomers are immobilized into the polymer.[22,30] Then, the antibacterial effect of the self-etching adhesive systems may be clinically restricted to a short time and to the superficial layers of dentin, and they may be considered limited.

Clearfill Protect Bond, which contains the MDPB antimicrobial monomer, may present a better clinical result, since, after curing, part of the molecules with methacryloyl structure are immobilized by co-polymerization with other methacrylate monomers and the other part preserves its antibacterial activity, even after being immobilized. These characteristics make use of materials containing MDPB a potential method for inhibiting bacterial growth before and after cure.[19-20] However, Feuerstein and others[10] examined the immediate and long-term antibacterial effects 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 and others[31] evaluated the cariostatic effects of three adhesive systems by the artificial caries development method using a microbiological model and they found no cariostatic effect, even though there was reduced glucan synthesis provided by the adhesive system containing Clearfil Protect Bond. The choice of an adhesive system for patients with high caries risk should take into consideration the prevention of secondary caries reached by inhibition of residual bacterial growth in the cavity and prevention of invasion through gaps between the composite restoration and cavity walls.[11,23]

CONCLUSIONS

The self-etching adhesives or self-etching primers used in the current 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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