Antibacterial Properties of Five Bonding Agents

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ABSTRACT

Purpose: This study compared antibacterial properties of five bonding agents with that of a control, Chlorhexidine (2.5%). Products evaluated were the self-etch primers (-P) and adhesives (-A) of Clearfil SE Bond (SE-P; SE-A) [Kuraray Dental], Clearfil Protect Bond (PB-P; PB-A) [Kuraray Dental], Optibond Solo Self-etch (OS-P; OS-A) [Kerr] and the one-bottle products, self-etch Clearfil Tri-S Bond (3S) [Kuraray Dental] and total-etch Adper Scotchbond 1 XT (XT) [3M ESPE].

Methods: Spread plates of three different bacteria (*Streptococcus mutans, Lactobacillus paracasei and Actinomyces naeslundii*) were prepared on Casein-peptone-Soymeal-peptone Agar (CASO-Agar). Controls, Primers, Adhesives, and Primer & Adhesive combinations were placed on standardized, sterilized filtration paper or composite disks and then placed on the inoculated agar and incubated at 37°C for 48 hours. Inhibition zones were measured and data was statistically analyzed using the Student t-test. An additional test was performed by which growth inhibiting of 1/10 and 1/100 dilutions of the test suspensions were measured spectrophotometrically as turbidity at 600nm and expressed as percentage growth (%).

Results: Compared to the controls, the only cured product which produced significant inhibition was Scotchbond 1 XT (XT), and that for *Actinomyces naeslundii* only. The primer of Clearfil Protect Bond (PB-P) showed statistically significant growth inhibition for all three test bacteria, the primer of SE Bond (SE-P) had significant inhibitive properties against *Streptoccocus mutans* and *Actinomyces naeslundii* and the primer of Optibond Solo Self-etch (OS-P) inhibited growth of

Table 1: Bonding agents used in this study - chemical composition

		Composition	
Bonding agent / Manufacturer	**Class**	**Primer**	**Adhesive**
Clearfil SE Bond [Kuraray, Japan]	SEP	MDP, HEMA, Hydrophyllic dimethacrylate, di-camphoroquinoneN, N-diethanol-p-toluidine, water	MDP, Bis-GMA, HEMA, silinated colloidal silica, hydrophilic dimethacrylate, di-camphoroquinoneN, N-diethanol-p-toluidine
Clearfil Protect Bond [Kuraray, Japan]	SEP	MDP, HEMA, Hydrophyllic dimethacrylate, di-camphoroquinoneN, N-diethanol-p-toluidine, water NaF	MDPB, HEMA, silinated colloidal silica, hydrophilic dimethacrylate, di-camphoroquinoneN, N-diethanol-p-toluidine
Optibond Solo Self-etch [Kerr, USA]	SEP	HFGA-GDM, GPDM, Ethanol Water, 4-Methoxyphenol ODMAB, Camphorquinone	Bis-GMA, HEMA, GDM, GPDM, Fumed silicon dioxide Disodium Hexafluorosilicate Barium aluminoborosilicate
Tri-S Bond [Kuraray, Japan]	SB - SE	MDP, Bis-GMA,HEMA, Hydrophobic dimethacrylate, di-Camphorquinone Ethyl alcohol, water, Silinated colloidal silicone	
Adper Scotchbond 1 XT [3M ESPE, USA]	SB	Bis-GMA, HEMA, Dimethacrylates Ethanol, water, photoinitiator, 5nm spherical silica particles Methacrylate copolymer of polyacrylic and polyitaconic acids	

SEP=Self-etching primer/adhesive, SB-SE=Single Bottle Self-etching adhesive, SB=Single Bottle (total-etch) adhesive, MDPB=methacryloyloxydodecyl pyridinium bromide, HEMA=Hydroxyethylmethacrylate, Bis-GMA=bisphenyl glycidylmethacrylate, GDM=Glycerol dimethacrylate, GPDM=Glycerol phosphate dimethacrylate, HFGA-GDM=Hexafluoroglutaric anhydride - Glycerodimethacrylate adduct, ODMAB=2-(Ethylexyl)-4-(dimethylamino)benzoate.

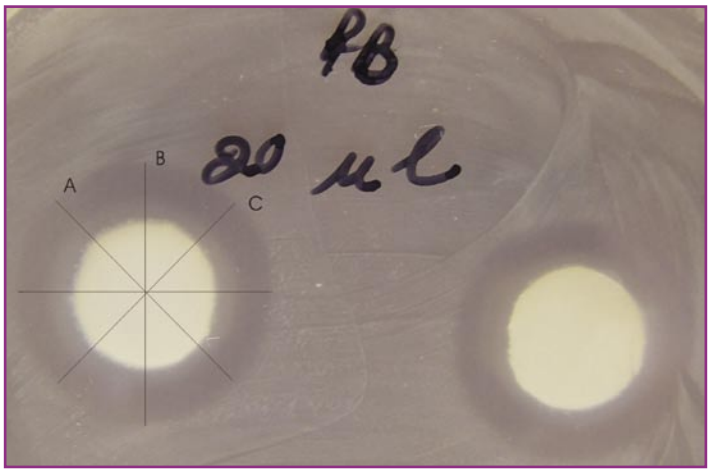

Figure 1: Measuring of inhibition zones [diameter A, B & C -per plate]

Actinomyces naeslundii significantly.

Conclusions: The primers of Clearfil Protect Bond, Clearfil SE Bond, Optibond Solo Self-etch and the product Adper Scotchbond 1 XT may be beneficial in eliminating remaining bacteria after cavity preparation, but further research on a possible long-term antibacterial benefit of these products needs to be undertaken.

INTRODUCTION

Microleakage and associated bacterial penetration and proliferation, leading to pulpal inflammation and secondary caries, remain one of the key problems associated with adhesive dentistry.[1,2] Products with short and/or long-term anti-bacterial properties that could eliminate or inhibit bacterial proliferation of known caries causing bacteria, would be a significant advance in the development of new restorative dental materials. Various studies have therefore examined possible antibacterial properties of commercial restorative products .[3-10] Taking this into account, the ideal restorative product would be a product that kills bacteria upon application and have a long-term, sustainable effect by killing or inhibiting bacteria that subsequently penetrate through microleakage gaps. The aim of the current study is to determine the antibacterial properties of a selection of dentine bonding agents.

MATERIALS AND METHODS

The present study compared antibacterial properties of five bonding agents with controls, Chlorhexidine (2.5%) and sterilized, distilled water. Products evaluated were the primers (-P) and adhesives (-A) of self-etching products Clearfil SE Bond [SE-P; SE-A] (Kuraray, Osaka, Japan), Clearfil Protect Bond [PB-P; PB-A] (Kuraray, Osaka, Japan), Optibond Solo Self-etch [OS-P; OS-A] (Kerr, Orange, CA, USA) and the one-bottle products: self-etching Clearfil Tri-S Bond [3S] (Kuraray, Osaka, Japan) and total-etch Adper Scotchbond 1 XT [XT] (3M ESPE Dental Products Division, St. Paul, MN, USA). Table 1 shows the chemical composition of the bonding agents used.

Forty eight hour cultures of *Streptococcus mutans* ATCC 25175, *Lactobacillus paracasei* A54 and *Actinomyces naeslundii* NCTC 10301 maintained on Casein-peptone-Soymeal-peptone Agar (CASO-Agar) were used. Suspensions of test organisms were prepared in quarter strength Ringer solution until turbidity comparable with MacFarland Standard-1 was achieved. The resulting organism concentrations were 2x108 CFU/ml. 100µl of each suspension was spread onto CASO-Agar by means of the standardized glass spreading technique.[11] The plates were incubated at 37°C for 15 minutes before application of the test materials. 10µl each of the test materials (SE-P, PB-P, OS-P) were placed on sterilized filtration paper disks (5mm diameter). The solvents from the test materials were allowed 5 minutes to evaporate from the disks before the disks were placed on the agar plates. A further 10µl each of the test materials (SE-A, PB-A, OS-A, SE-P&A, PB-P&A, OS-P&A, 3S and XT) were also placed and light-cured on composite disks [Z100, A1, Ø 5mm x 1.5mm (3M ESPE, St. Paul, MN, USA)]. For all product groups five agar plates each were prepared and three paper or composite disks placed on each agar plate (Figure 1).

Table 2: Average zones of inhibition of materials tested [Agar inhibition]

Product	S. mutans	L. paracasei	A. naeslundii
SE Primer (SE-P)	8 ± 1.5 mm	0	5 ± 2 mm
SE Adhesive (SE-A)	0	0	0
SE P & A	0	0	0
PB Primer (PB-P)	8 ± 1 mm	5 ± 2 mm	5 ± 1.5 mm
PB Adhesive (PB-A)	0	0	0
PB P & A	0	0	0
OS Primer (OS-P)	2 ± 1 mm	0	8 ± 2.5 mm
OS Adhesive (OS-A)	0	0	0
OS P & A	0	0	0
Adper Scotchbond 1 XT (XT)	0	0	6 ± 1 mm
Clearfil Tri-S bond (3S)	0	0	0
Chlorhexidine - paper	8 ± 1 mm	9 ± 0.5 mm	9 ± 0.5 mm
Chlorhexidine - composite	8 ± 2 mm	9 ± 1 mm	10 ± 1 mm
Water (paper & composite)	0	0	0

SE - Clearfil SE Bond (Kuraray), PB - Protect Bond (Kuraray), OS - Optibond Solo Self-etch (Kerr),
XT - Adper Scotchbond 1 XT (3M ESPE), 3S - Clearfil Tri-S bond (Kuraray)

Table 3: Percentage bacterial growth measured at 600nm of tested materials: [Adhesives and XT, 3S – not polymerized]

	S. mutans		L. paracasei		A. naeslundii	
Product	1/10	1/100	1/10	1/100	1/10	1/100
SE Primer (SE-P)	0	72%	0	53%	0	40%
SE Adhesive (SE-A)	98%	73%	0	111%	0	90%
PB Primer (PB-P)	0	10%	0	17%	0	14%
PB Adhesive (PB-A)	60%	78%	15%	80%	0	102%
OS Primer (OS-P)	27%	31%	13%	31%	0	0
OS Adhesive (OS-A)	13%	55%	13%	74%	0	30%
Adper Scotchbond 1 XT (XT)	0	64%	0	115%	0	38%
Clearfil Tri-S bond (3S)	0	142%	0	213%	0	233%
Chlorhexidine (CHX)	0	27%	0	34%	0	47%
Water	100%	100%	100%	100%	100%	100%
SE-Clearfil SE Bond (Kuraray), PB-Protect Bond (Kuraray), OS-Optibond Solo Self-etch (Kerr), XT - Adper Scotchbond 1 XT (3M ESPE), 3S - Clearfil Tri-S bond (Kuraray)						

The plates were incubated anaerobically at 37°C for 48hr respectively and the antibacterial activity was evaluated using the conventional agar plate diffusion method.[12-14]

The diameters of the inhibition zones were measured using a micrometre gauge at three different positions for each paper or composite disk (Figure 1). An average (rounded off to the nearest mm) for the nine measurements per plate for the five plates per product was calculated (Table 2) and the data analyzed using the Student t-test to determine significant differences between the individual products and the control Chlorhexidine.

In a secondary study three sets of 1/10 and 1/100 serial dilutions of the test solutions and controls were prepared in Casein-peptone-Soymeal-peptone broth (CASO-broth). Of each dilution, a control, used to zero the spectrophotometer, was prepared. 100µl each of the MacFarland Standard-1 test organism solutions, (the same that were prepared for the first part of the experiment) was inoculated into the prepared serial dilutions tubes and incubated anaerobically at 37°C for 48hr. Turbidity was then determined spectrophotometrically at 600 nm and the inhibiting concentrations of 1/10 and 1/100 dilutions of the test suspensions were expressed as percentage growth (%) (Table 3). The Kruskal-Wallace test was used to determine significant inhibition of growth when compared to Chlorhexidine. Results from the two different tests were not statistically compared to each other.

RESULTS

Agar plate diffusion studies

Results of the agar plate diffusion studies are shown in Table 2. Of all the products cured on the composite disks (SE-A, PB-A, OS-A, XT, 3S,) only Scotchbond 1 XT showed growth inhibition, and then only for *Actinomyces naeslundii*. The chlorhexidine control inhibited all three bacteria significantly when placed on paper and on the composite disks. There was no statistical difference in inhibition amongst the bacteria obtained with the control. Clearfil Protect Bond primer (PB-P) significantly inhibited the growth of all three bacteria tested. However, the Clearfil SE Bond primer (SE-P) inhibited the growth of *S.mutans* and *A. naeslundii*, but not the growth of *L. paracasei*. Optibond Solo Self-etch primer (OS-P) and Scotchbond 1 XT (XT) only inhibited the growth of *A naeslundii*. The growth inhibition by PB-P for *S. mutans* and *A. naeslundii* was not statistically higher than the inhibition obtained from SE-P and OS-P.

Spectrophotometrical studies:

Results of the spectrophotometrical studies are shown in Table 3. At the 1/100 dilution all the products including the chlorhexidine control showed growth to varying degrees for all three bacteria tested. In general, growth was inhibited to some extent, but the product 3S stimulated the growth of the three bacteria while XT and SE-A stimulated the growth of *L. paracasei* only.

At the 1/10 dilution the control showed no growth for all three bacteria. Similarly the products XT, 3S, PB-P and SE-P totally inhibited the growth of all three micro-organisms. All the primers, with the exception of OS-P, were inhibitory towards the bacteria. The other products only inhibited the growth of *S. mutans* and *L. paracasei* to some extent. Interestingly, *A. naeslundii* was totally inhibited by all three products at 1/10 dilution.

DISCUSSION

Microbial communities in dental lesions are known to be diverse. Not only streptococci, but also lactobacilli, gram-positive pleomorphic rods, and obligately anaerobic bacteria have been isolated from dentinal lesions.[15,16] Mutans streptococci, in particular *Streptococcus mutans* and *Streptococcus sobrinus* are associated with the initiation of dental caries whilst lactobacilli are associated with the progression of the carious lesion.[17-19] For the purpose of the present study we chose *Streptococcus mutans*, *Lactobacillus paracasei* and *Actinomyces naeslundii* as representative bacteria important in the development and progression of dental caries.

One of the first studies undertaken on antibacterial effectiveness of dentin bonding systems was performed in 1993.[4]

Since then various studies on the different generations and components of bonding systems to determine possible short and long-term anti-bacterial properties have been reported.[8,9,17,20] Recently an experimental self-etching primer containing the MDPB resin monomer has been evaluated and a variety of studies indicated antibacterial properties against a variety of bacteria.[10, 21--24]

With Clearfil Protect Bond, the monomer MDPB (12-Methacryloyloxy-Dodecyl-Pyridinium Bromide) has been added to the self-etching primer (PB-P) and fluoride (NaF) to the adhesive (PB-A). This is an attempt to include the proven advantages of fluoride as ingredient of restorative materials coupled with an additional antibacterial agent. It is envisaged that the added fluoride will enhance re-mineralization of caries-affected decalcified dentine and assist in stabilizing dentin bonds.[25,26] These features, coupled with additional anti-bacterial properties, are especially important in minimal invasive/intervention dentistry where minimal tooth structure is removed during caries removal, with the possibility that active bacteria may reside in the cavity.[16]

In the present study, compared to the control, the primer of Clearfil Protect Bond (PB-P) showed statistically significant antibacterial activity against *Streptoccus mutans*, *Lactobacillus paracasei* and *Actinomyces naeslundii*, and the primer of Clearfil SE Bond (SE-P) statistically significant antibacterial activity against *Steptococcus mutans* and *Actinomyces naeslundii*. In another study the primer of ABF (Experimental version of Clearfil Protect Bond) was the most bactericidal among the materials tested and the authors concluded that the product MDPB could be beneficial for eliminating the residual bacteria in cavities.[22]

In our study the primer and adhesive and adhesives combinations cured on composite disks had no significant inhibition for most of the organisms tested. This indicates that the cured products released minimal antibacterial substances. One would have expected that those with primers, especially Clearfil Protect Bond to show some inhibitive properties. It was found that curing resulted in some products having a limited antibacterial efficacy.[22,27] A study found that the adhesive of Clearfil Protect Bond (PB-A [ABF]) did not produce any inhibition in a similar agar well technique study.[28] Imazato *et al* (2003) did report antibacterial properties of cured resin containing the monomer MDPD. This possible 'inactivation' of inhibitive properties needs to be further investigated, especially to see if any long-term leaching of antibacterial components might take place.[24]

Some products seem to stimulate bacterial growth (values above 100%) as presented in Table 2 (1/100 of SE-A, XT & 3S). Although Clearfil Tri-S Bond (3S) seems to stimulate bacterial growth, it is unknown at this stage if this is a true stimulation of growth or increase in turbidity as result of polymerization of the product due to light exposure. Further research involving incubation without microorganisms may shed some light on this phenomenon.

CONCLUSION

Since this research product only compared possible antibacterial properties for a selection of bonding agents, and no product had a significant advantage in the cured state, the authors did not discuss the possible antibacterial properties of each ingredient found in the different products evaluated.

Judging from the results obtained the primers of Clearfil Protect bond (PB-P), Clearfil SE Bond (SE-P) and Optibond Solo Self-etch (OS-P) may have an immediate, upon application inhibitive effect on residual bacteria found in the cavity after cavity preparation. However, since the cured test specimens did not show a significant inhibition of all bacteria tested, it is doubtful whether, other than the possible immediate anti-cariogenic effect by some product groups, a long-term, beneficial, anti-cariogenic advantage will be applicable.

Since Lactobasilli is involved in caries progression and the 'anti-bacterial' Protect Bond primer (PB-P) was the only product that significantly inhibited *Lactobacillus paracasei* in the Agar test, this product may delay or limit caries progression. [17-19]

The 1/10 test dilutions produced what appeared to be more sensitive results whereby all product groups significantly inhibited growth for all the evaluated bacteria, with the exception of the adhesives of SE Bond (SE-A) and Protect Bond (PB-A) for *Steptococcus mutans* only. Since this test evaluated only product in the uncured state, the results obtained can also be expected to be different when applied and cured in the cavity.

Further research on the long-term efficacy, the limited antibacterial properties of the cured product, the phenomenon where some product seems to 'stimulate growth, as well as the antibacterial properties of the individual chemical ingredients may be of use.

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Additional references (9-28) are available on www.sada.co.za