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Comparison of the Antibacterial Activity of Different Self-etching Primers and Adhesives

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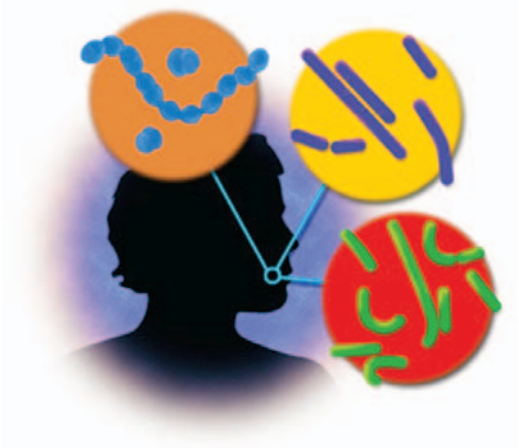

Aim: The aim of this study was to evaluate the antibacterial effects of different one-step and two-step self-etching primer/adhesives on *Streptococcus mutans (S. mutans)*, *Lactobacillus casei (L. casei)*, and *Lactobacillus acidophilus (L. acidophilus)*.

Methods and Materials: The antibacterial effects of Clearfil Protect Bond Primer and Bonding agent; AdheSE Primer and Bonding agent; Adper Prompt L-Pop; Futurabond NR; Clearfil Tri S Bond; and Cervitec (positive control, 1% chlorhexidine varnish) were tested against standard strains of *S. mutans*, *L. Casei*, and *L. acidophilus* using the disk diffusion method. Standard filter paper disks (n=5) impregnated with 20 microL of each material were prepared. After incubation at 37ºC for 48 hours in a 5-10% CO_2 atmosphere, the diameter of inhibition zones were measured in millimeters. Data were analyzed using one way analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA). Duncan's Multiple Range Test was used for pairwise comparison.

Results: The size of inhibition zones produced by primer/adhesives varied among the brands. AdheSE Primer: *S. mutans* (20.6±1.51); *L. casei* (14.8±1.78); *L. acidophilus* (11.4±0.54). Adper Prompt L-Pop: *S. mutans* (19.6±1.51); *L. casei* (13.8±1.64); *L. acidophilus* (13.8±1.09). Cervitec: *S. mutans* (23±0.00); *L. casei* (27±0.70); *L. acidophilus* (22.4±0.54). Clearfil Protect Bond Primer: *S. mutans* (17±0.00); *L. casei* (17.6±0.54); *L. acidophilus* (22.4±0.54). Futurabond NR was found effective only against *S. mutans* (14.6±1.67). Of all the materials tested, AdheSE Bonding agent, Clearfil Protect Bond Bonding agent, and Clearfil Tri S Bond exhibited no inhibition zone (-) for all bacteria tested.

Conclusion: Among the adhesives tested Clearafil Protect Bond Primer based upon monomer methacryloyloxydodecylpyridiniium bromide (MDPB) was found to be the most potent material against *L. acidophilus* and *L. casei*. AdheSE Primer and Adper Prompt L-Pop are highly effective against *S. mutans*.

Clinical Significance: Compared with other adhesive systems, Clearfil Protect Bond Primer (containing MDPB) showed a high antibacterial effect against all microorganizms tested. Two-step, self-etching primer/adhesive system Clearfil Protect Bond might be a suitable choice under minimally invasive restorations. The recently developed one-step, self-etching system Clearfil Tri S Bond showed no antibacterial effect against microorgazims tested.

Keywords: Antibacterial activity, self-etching primer, adhesive system

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Introduction

Even though current composite resin/adhesive systems bond to human dentin with bond strength of 20 MPa *in vitro*, new adhesive systems are not capable of totally prohibiting gap formation between the cavity preparation and restorative material because of the composite resin polymerization shrinkage.[1] Gaps between restorations and cavity walls could be colonized by oral microorganizms from saliva. It is well known bacteria invade along the tooth restoration interface and may cause secondary caries and damage to the pulp.[2,3]

Another source of bacteria may be incompletely removed caries. The presence or absence of bacteria after caries removal cannot be verifyed objectively. Caries disclosing dyes have been proposed as an objective method to determine whether caries is removed, but these dyes do not accurately reflect the actual bacterial status of dentin.[4]

Several different self-etching primer/adhesive systems are currently available. Self-etching systems not requiring a separate acid conditioning step are less likely to result in a discrepancy between the depth of demineralization and the depth of resin infiltration since both processes occur simultaneously.[5,6] Two-step self-etching primers eliminate the conditioning steps,[7] however, a separate bonding step is essential to couple the primed tooth substrate to the resin composite. Recently, one-step self-etching adhesives or the so-called “all in one adhesives” have been introduced which combines the conditioner, primer, and bonding resin to allow a single-step application.[8] Using self-etching primer systems in which the smear layer is not washed away, residual bacteria can be anticipated. Therefore adhesive systems with antibacterial activity could be useful in the destruction of bacteria capable of causing harmful effects. This would lead to a better prognoses associated with minimal restorative treatments of dental caries.

Clearfil Protect Bond with antibacterial properties which also releases fluoride was developed. The self-etching primer in this system contains the antibacterial monomer methacryloyloxydodecylpyridiniium bromide (MDPB).[9] Imazato el al.[10-11] have reported that unpolymerized MDPB shows strong bactericidal activity, and residual bacteria in the cavity can

be inactivated when a MDPB-containing adhesive system is applied.

Recently developed self-etching primer systems provide better clinical results by reducing the risk of conditioned surfaces being contaminated.[12] However, little is known about their antibacterial effects. The purpose of this *in vitro* study was to evaluate the antibacterial effects of different one and two-step self-etching primer/adhesives on *Streptococcus mutans (S. Mutans)*, *Lactobacillus casei (L. casei)*, and *Lactobacillus acidophilus (L. acidophilus)*.

Methods and Materials

Test Materials

Adhesives tested in the study included three "one-step" and two "two-step" self etching adhesives along with a 1% chlorhexidine varnish (Cervitec, Vivadent-Vivacare, Schaan, Liechtenstein). Characteristics, compositions, and pH levels of products tested are listed in Table 1.

The materials (Clearfil Protect Bond Bonding agent, AdheSE Bonding agent, Adper Prompt L-Pop, Futurabond NR, and Clearfil Tri S Bond) were applied and cured using a Halogen light curing unit (Hilux Expert, Benlioglu Dental, Bülbülderesi Cad.8'A, Ankara, Turkey) according to the manufacturers' instructions.

Clearfil Protect Bond Primer (20 seconds), AdheSE Primer (30 seconds), and Cervitec (30 seconds) were applied on sterilized paper disks and gently air-dried (nonpolymerized).

Preparation of Standard Disks

Standard paper 6.35 mm Ø, thickness 0.88 mm disks (Whatman, Schleicher & Schvell, Maidstone, England) were sterilized. For each test material and bacteria, five disks were prepared by dropping 20 µl of material with a micropipet onto sterilized paper disks. All the disks were allowed to dry in an incubator and stored in the refrigerator until they were used.

Table1. Self-etching adhesive systems used.

Adhesive	Manufacturer	Composition	pH
AdheSE (two-step self-etching)	Ivoclar Vivadent, Amherst, NY, USA	**Primer:** Phosphoric acid acrylate Bis-acrylamide, water, initiators and stabilizers **Adhesive:** Dimethcrylate, HEMA, highly dispersed silicon oxide, initiators, stabilizers	1.7
Adper Prompt L-Pop (one-step self-etching)	3M ESPE, St. Paul, MN, USA	**Adhesive (Liquid 1):** Methacrylated phosphoric esters, Bis-GMA, initiators based on camphorquinone, stabilizers **Adhesive (Liquid 2):** Water, HEMA, Polykenoic acid, co-polymer, stabilizers	1.5
Clearfil Protect Bond (two-step self-etching)	Kuraray Co, Osaka, Japan	**Primer:** MDPB, MDP,hydrophobic dimethacrylate, HEMA, water, photoinitiators, etc. **Bond:** MDP, HEMA, silanated colloidal silica, Bis-GMA, NAF, photoinitiators, etc.	1.9
Clearfil Tri S Bond (one-step self-etching)	Kuraray Co, Osaka, Japan	MDP, Bis-GMA, HEMA, Hydrophobic dimethacrylate dl-Camphorquinone, Ethyl alcohol, water, silanated colloidal silica	2.7
Futurabond NR, Single Dose (one-step self-etching)	Voco, Cuxhaven, Germany	**Liquid A:** Polyfunctional adhesive monomers (methacryl phosphorus acid ester and corbonic acid modified methacrylic ester) **Liquid B:** Water	1.4

Bacteria Used

The antibacterial activity of each material was tested against the following bacteria: *S. mutans* (ATCC 25175, American Type Culture Collections), *L. casei* (RSKK 731, Refik Saydam Central National Institute of Health, Ankara, Turkey), and *L. acidophilus* (RSKK 03037, Refik Saydam Central National Institute of Health, Ankara, Turkey). All the strains were inoculated onto the Trypticase soy agar (TSA, Merck Darmstadt, Germany) plates which were incubated at at 37ºC for 48 hours under 5-10% CO_2. Before the test, bacterial colonies taken from the pure cultures of each strain were suspended in sterile saline to give a concentration of 0.5 McFarland turbidity standard (app.1.5 x 10^8 cfu/ml). After 15 minutes, these suspensions were used in a disk diffusion test.

Disk Diffusion Test

The test was performed according to CLSI (Clinical and Laboratory Standards Institute, formerly NCCLS) requirements.[13] Briefly, bacterial suspensions were inoculated onto the entire surface of the TSA plates by a steril cotton swab. Before the disks were placed, the plates were allowed to dry. Using an applicator the disks were arranged at required distances from the edge of the plate and between each other. The plates were inverted and incubated at 37ºC for 48 hours under 5-10% CO_2. The growth inhibition zone diameters around the disks including the disk diameter were measured as milimetres. The results were expressed as mean diameters ± standard deviations.

Statistical Analysis

Data were analyzed using one way analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) tests. Duncan's Multiple Range Test was used for pairwise comparison.

Results

The inhibitory effects of the test and control materials on the test microorganisms are shown in Figure 1.

The mean values of the growth inhibition zones for each test and control material are shown in Table 2. The size of inhibition zones produced by adhesives varied among the brands.

AdheSE Bonding agent, Clearfil Protect Bond Bonding agent, and Clearfil Tri S Bond exhibited no inhibition zone for all bacteria tested. Futurabond NR showed no inhibition for *L. casei* and *L. acidophilus*. Cervitec (positive control,1% chlorhexidine varnish) exhibited the largest zone of inhibition against the *S. mutans* and *L. casei* ($p<0.05$). Cervitec and Clearfil Protect Bond Primer produced similar inhibition zone against *L. acidophilus*.

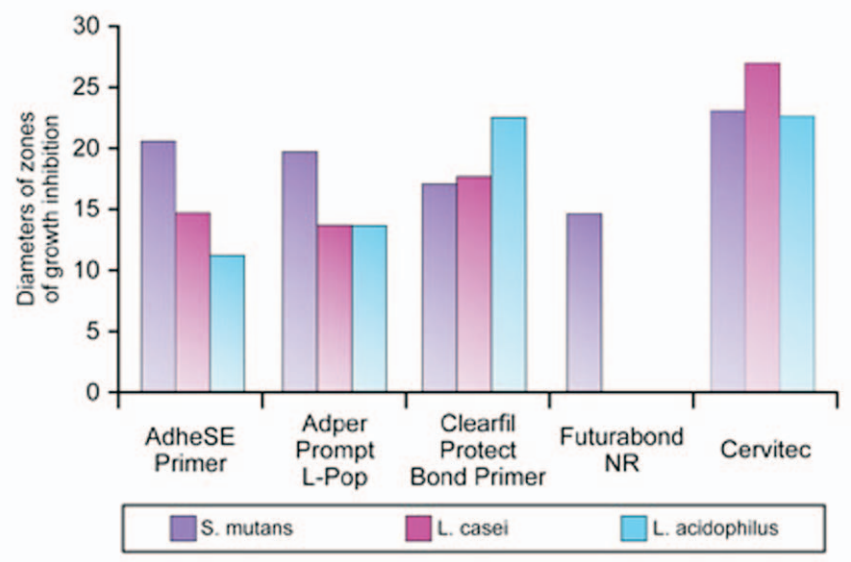

Figure 1. Comparison of test materials against tested bacteria according to growth inhibition zone diameters.

Table 2. Growth inhibition zone diameters (mm) (mean ± SD).

Test Materials (n=5)	*S. mutans* (ATCC 25175)	*L. casei* (RSKK 731)	*L. acidophilus* (RSKK 03037)
AdheSE Primer	20.6±1.51	14.8±1.78	11.4±0.54
AdheSE Bond Bonding agent	-	-	-
Adper Prompt L-Pop	19.6±1.51	13.8±1.64	13.8±1.09
Clearfil Protect Bond Bonding agent	-	-	-
Clearfil Protect Bond Primer	17±0.00	17.6±0.54	22.4±0.54
Clearfil Tri S Bond	-	-	-
Futurabond NR	14.6±1.67	-	-
Cervitec	23±0.00	27±0.70	22.4±0.54
(-):No inhibition zone			

Among the adhesives tested Clearfil Protect Bond Primer was found to be the most potent material against *L. acidophilus* and *L. casei* ($p<0.05$). AdheSE Primer and Adper Prompt L-Pop are highly effective against *S. mutans* ($p<0.05$).

Discussion

Current adhesive research is focused on the simplification of the application procedure.[14] Reduction of application steps should reduce manipulation time and abate technique sensitivity, thus, improving bonding effectiveness.[15]

Secondary caries is the most frequent reason for the replacement of restorative materials including resin composites.[16] Many clinicians prefer using a cavity disinfectant such as chlorhexidine or peroxide in the treatment of dentinal caries because they are unsure if the lesion has been completely removed. Clearfil Protect Bond has been developed as a self-etching/priming system with cavity disinfecting effects by incorporating the antibacterial monomer MDPB into the primer solution.[17]

Several species of bacteria have been isolated from dental plaque associated with caries lesions, there is evidence that *Streptococcus* and *Lactobacilli* are the major human dental pathogens. *S. mutans* strains have been shown to be associated with dental caries. Together with *Lactobacilli*, they are regarded as significant odontopathogens.[18] *S. mutans*, found in plaque in

the early stages of caries, is the primary bacterial agent for dental caries. *L. acidophilus* isolated from carious lesions and *L. casei* are present in large numbers in dental plaque.[19] Therefore, *S. mutans*, *L. acidophilus*, and *L. casei* were chosen as the test microorganisms in this study.

Antibacterial activity of dentin bonding agents depend upon several factors including the composition and acidity.[10] In previous studies concerning the antibacterial activities of dentin bonding systems it has been reported this effect might be a result of their low pH environment.[20] In the present study self-etchants (Futurabond NR, Adper Prompt L-Pop) had an acidic pH ≤1.5. However, some bacteria such as *Lactobacilli*

species are acid tolerant.[21] Finally, the antibacterial effect of Clearfil Protect Bond primer has been demonstrated to be related to its antibacterial monomer content.[10] Therefore, for the self-etchants tested, low pH may play only a minor role, if any, as the source of antibacterial activitiy.[22]

In the present study no inhibition zone for all microorganisms was noted for AdheSE bond and Clearfil Protect Bond. Because only the AdheSE Primer and Clearfil Protect Bond Primer have antibacterial effects it is not suprising their corresponding bond products showed no antibacterial effects. One step self-etching Clearfil Tri S Bond showed no inhibition zone for all bacteria tested; Futurabond NR showed no inhibition zone for *L. casei* and *L. acidophilus*.

The antibacterial activitiy of dentin primers is usually evaluated using the agar well or disk diffusion method.[10,22] In order to save the material the disk diffusion method was preferred for the present study. Chlorhexidine varnish (1%) was used as the positive control because its antibacterial activity is well-established and its widespread clinical use.[23] As was expected, chlorhexidine varnish produced consistently large growth inhibititation zones for all tested materials. The cured MDPB containing Clearfil Protect Bond Primer was not included in the test groups. According to results of Imazato et al.[10] the cured MDPB containing primer produced no inhibition zones. When the adhesive systems are applied to dentin *in vivo*, the acidic primers are buffered by the dentin substrate, however, the extent to which this influences the antibacterial effect of self-etching primers is still unknown.[24] There might be some properties of dentin or the overlying restorative material that change the effectiveness of some of the materials tested.[25] Further investigations simulating clinical situations should be developed in order to confirm the results.

Conclusion

The antibacterial activity of simplified adhesives varies greatly amoung commercial brands. Among the adhesives tested Clearfil Protect Bond Primer based upon MDPB was found to be the most potent material against *L. acidophilus* and *L. casei*. AdheSE Primer and Adper Prompt L-Pop are highly effective against *S. mutans*.

Clinical Significance

Compared with other adhesive systems, Clearfil Protect Bond Primer (containing MDPB) showed a high antibacterial effect against all microorganizms tested. Two-step, self-etching primer/adhesive system Clearfil Protect Bond might be a suitable choice under minimally invasive restorations. The recently developed one-step, self-etching system Clearfil Tri S Bond showed no antibacterial effect against microorgazims tested.

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