Antibacterial properties of self-etching dental adhesive systems

Osnat Feuerstein, PhD, DMD; Shlomo Matalon, DMD; Hagay Slutzky, DMD; Ervin I. Weiss, DMD

Self-etching dental adhesive systems have been developed to simplify bonding procedures and to make their application less time-consuming. In two-step systems—the so-called self-priming and self-etching adhesives—the primer and adhesive are combined into one solution. In one-step systems—the so-called all-in-one adhesives—the etchant, primer and adhesive are combined into one solution. Reducing the steps of the adhesive procedure makes it possible to decrease the application time and the risk of error. On the other hand, the bond strengths of the all-in-one adhesives may be lower than those of the two-step systems.[1] A 2004 clinical study showed that simplified systems might restrict the efficacy of the bonding.[2]

Residual bacteria in the oral cavity may remain at the tooth-restoration interface and increase the risk of developing recurrent caries. This is due to the contaminated smear layer, which is partially incorporated into the hybrid layer.[3] In vitro studies have shown that the tooth-restoration interface created when using self-etching adhesive systems do not eliminate microleakage and bacterial penetration, which can lead to secondary caries, the most common reason for dental restoration failure.[4,5]

Therefore, the antibacterial properties of adhesive materials are

ABSTRACT

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Background. Dental adhesives with antibacterial properties may reduce recurrent or secondary caries. The authors conducted a study to examine the immediate and long-lasting antibacterial properties of four self-etching adhesive systems.

Methods. The authors used the agar diffusion test (ADT) and direct contact test (DCT) to measure the antibacterial properties of AdheSe (Ivoclar Vivadent, Schaan, Liechtenstein), Adper Prompt L-Pop (3M ESPE, Seefeld, Germany), Clearfil Protect Bond (Kuraray, Kurashiki, Okayama, Japan) and Xeno III (Dentsply, Konstanz, Germany) on *Streptococcus mutans* after aging samples in phosphate-buffered saline for one, two, seven and 14 days.

Results. Only Clearfil Protect Bond showed an inhibition halo in the ADT. In the DCT, fresh samples of all of the tested materials exhibited potent antibacterial properties, which were maintained by AdheSe for one day and Clearfil Protect Bond for seven days. None of the adhesive systems exhibited any antibacterial properties after 14 days.

Conclusions. All of the tested adhesives had an immediate bactericidal effect on *S. mutans.* None, however, had long-lasting antibacterial properties.

Clinical Implications. The application of self-etching adhesive materials could contribute to the immediate elimination of residual bacteria. The likelihood of developing secondary caries as a consequence of bacterial microleakage may not be affected by the use of the adhesive systems tested in this study.

Key Words. Adhesives; dental bonding; bacteria; microleakage; antibacterial.

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important.[6-11] The antibacterial effects of nonpolymerized or immediately polymerized adhesives are beneficial in the eradication of residual bacteria in the oral cavity.[6] The long-lasting antibacterial activity of polymerized adhesives may be effective in inactivating bacteria that invade the tooth-adhesive interface by microleakage.

Studies have shown the antibacterial effect of adhesive materials is due to their low pH or to specific antibacterial components such as glutaraldehyde[8,11,12] or 12-methacryloyloxydodecylpyridinium bromide (MDPB).[13] Since copolymerization should immobilize MDPB in the adhesive material, adhesives containing MDPB may be effective against bacteria that invade through microleakage.[13]

We conducted a study to examine the immediate and long-lasting antibacterial effect of polymerized self-etching adhesive systems on *Streptoccocus mutans* by using the direct contact test (DCT) and comparing these results with those of the agar diffusion test (ADT).

MATERIALS AND METHODS

Tested materials. We tested the following four commercially available self-etching adhesive systems:

- AdheSe (Ivoclar Vivadent, Schaan, Liechtenstein), a two-step self-etching product;
- Clearfil Protect Bond (Kuraray, Kurashiki, Okayama, Japan), a two-step self-etching product that has adhesives containing MDPB;
- Adper Prompt L-Pop (3M ESPE, Seefeld, Germany), a one-step self-etching product;
- Xeno III (Dentsply, Konstanz, Germany), a one-step self-etching product.

Tested microorganism. We used *S. mutans*, the primary etiologic agent for caries, to test the antimicrobial activity of restorative materials.[14-17] *S. mutans* 27351M (obtained from the Department of Human Microbiology, Sackler School of Medicine, Tel Aviv University, Israel) is naturally resistant to bacitracin. We grew *S. mutans* aerobically from frozen stock cultures in brain-heart infusion (BHI) that contained 8 micrograms of bacitracin per milliliter for 48 hours at 37 C.

Experimental design. We performed an ADT on a mitis salivarius agar plate, in which we punched eight holes that were 4 millimeters in diameter. We inoculated the plate with 200 microliters of freshly grown *S. mutans* (optical density [OD] 650 nanometers = 0.5) spread evenly with a Drigalsky glass stick. After mixing each of the four tested materials, we immediately filled the holes (two holes per material) and light-polymerized them using a light-curing unit (VIP, Bisco, Schaumburg, Ill.) according to the manufacturer's instructions. We incubated the plates for 72 hours at 37 C and then inspected them for the presence of an inhibition zone. We took two perpendicular measurements of the inhibition halo diameter using a digimatic caliper. We repeated the ADT three times.

The DCT described by Weiss and colleagues[18-22] is based on bacterial growth in a 96-well microtiter plate (96 MicroWell Plates, Nunc, Copenhagen, Denmark) (Figure 1). To create samples, we evenly coated the side walls of eight wells with a tested material and polymerized the material using a light-curing unit as follows:

- for AdheSe, we applied the primer and brushed it for 30 seconds and then applied the bonding agent and light cured it for 20 seconds;
- for Adper Prompt L-Pop, we rubbed on the adhesive for 15 seconds, dried it and light cured it for 10 seconds;
- for Clearfil Protect Bond, we applied the primer for 20 seconds and dried it and then applied the bonding agent and light cured it for 10 seconds;
- for Xeno III, we mixed the primer and bonding agent together, applied the mixture for 20 seconds and light cured it for 10 seconds.

We rinsed all of the tested materials with phosphate-buffered saline (PBS) before inoculating them with bacteria.

We placed 10 µL of the bacterial suspension (the *S. mutans* and BHI mixture) (10^6 cells) on the samples. We incubated the plate for one hour at 37 C until the bacterial suspension evaporated to ensure direct contact between the bacteria and the surface of the tested material. We then turned the plate horizontally, added 220 µL of BHI, placed the plate in a vortex at 100 revolutions per minute for two minutes and inserted it into a temperature-controlled spectrophotometer. We recorded the kinetics of the outgrowth in each well (OD at 650 nm) every 30 minutes for at least 16 hours (VersaMax, Molecular Device, Menlo

ABBREVIATION KEY. ADT: Agar diffusion test. **BHI:** Brain-heart infusion. **DCT:** Direct contact test. **DDW:** Double-distilled water. **MDPB:** 12-Methacryloyl oxydodecylpyridinium bromide. **OD:** Optical density. **PBS:** Phosphate-buffered saline.

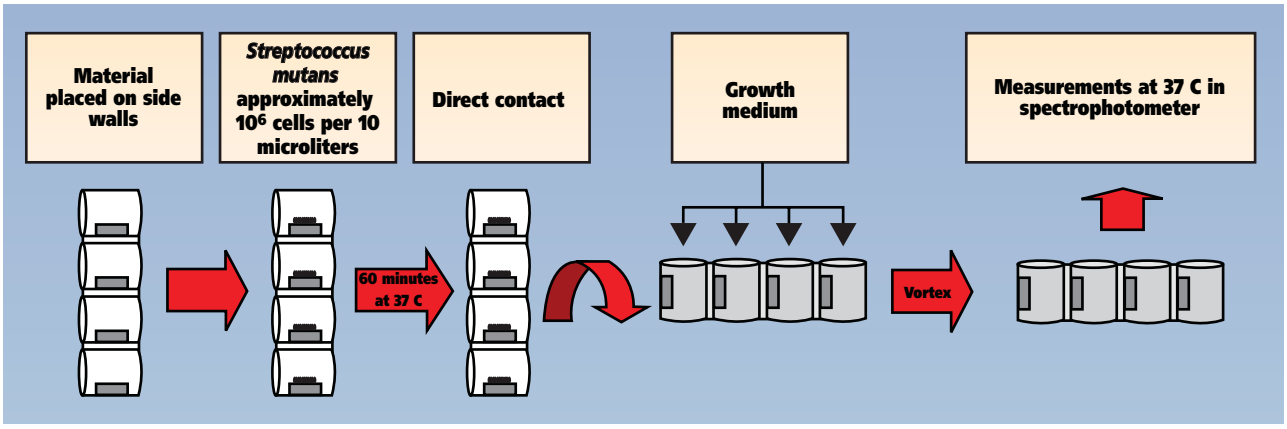

Figure 1. Schematic drawing of direct contact test performed in a 96-well flat-bottomed microtiter plate. Each material was tested eight times. Bacteria were in direct contact with the tested material for one hour. Growth medium was added. The microtiter plate was vortexed for two minutes and inserted into a temperature-controlled spectrophotometer. Bacterial outgrowth was monitored every 30 minutes for at least 16 hours.

Oaks Center, Menlo Park, Calif.) set to 37 C. We performed calibration experiments simultaneously for each plate to obtain a comparative quantitative scale. We placed bacteria (10^6 cells) on each side wall of three wells and added 250 µL of BHI. We performed consecutive fivefold dilution transfers into six sets of three wells. We plotted the average OD measured in three wells simultaneously on a growth curve. In the DCT, each plate was an independent experiment containing positive and negative controls and calibration experiments. The positive control samples were wells with bacterial suspension only, and the negative control samples were wells with tested materials only.

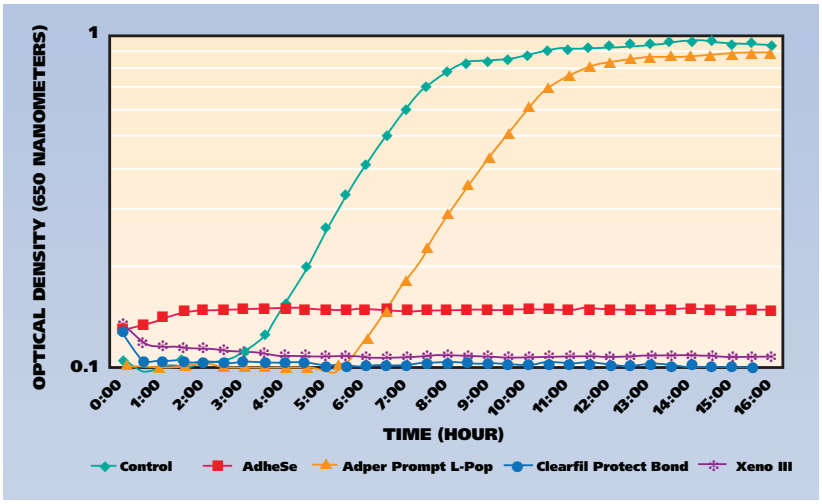

Figure 2. Bacterial outgrowth (measured by changes in optical density [OD]) on the surface of self-etching adhesive materials after aging for one day. Each point is the average OD measured simultaneously in eight wells. AdheSe is manufactured by Ivoclar Vivadent, Schaan, Liechtenstein. Adper Prompt L-Pop is manufactured by 3M ESPE, Seefeld, Germany. Clearfil Protect Bond is manufactured by Kuraray, Kurashiki, Okayama, Japan. Xeno III is manufactured by Dentsply, Konstanz, Germany.

We examined the antibacterial properties of the polymerized tested materials using DCT immediately after polymerization and after aging them in PBS for one, two, seven and 14 days. During the aging process, we renewed the PBS every 24 hours. We performed each experiment at least three times.

pH measurements. We determined the pH values of the self-etching adhesive systems by placing 50 µL of nonpolymerized samples in 1.6 mL cuvettes using a pH meter probe (CyberScan 510, Eutec Instruments, Singapore). Simultaneously with the nonpolymerized samples, we placed two sets of polymerized samples in 15 mL tubes (Corning, Corning, N.Y.) and added 1 mL of double-distilled water (DDW) to each tube. We measured the pH values immediately after and 24 hours after polymerization.

Statistical analysis. We analyzed the growth curves for each well and calculated a regression line on the ascending linear portion of the curve, using the equation $y = ax + b$. This equation provided the value of the slope corresponding to the growth rate. Statistical analysis included two- and one-way analyses of variance (ANOVA) followed by Tukey multiple comparisons.

RESULTS

In the ADT, we found an inhibitory halo (2.0 ± 0.3 mm) only around the Clearfil Protect Bond specimens.

We performed the DCT on eight specimens of

TABLE 1

Bacterial growth rate after direct contact with tested materials.

MATERIAL	BACTERIAL GROWTH RATE*			
	1 Day	2 Days	7 Days	14 Days
Control	80.1 ± 3.8	75.4 ± 2.6	42.9 ± 6.7	76.2 ± 8.2
Adper Prompt L-Pop (3M ESPE, Seefeld, Germany)	64.8 ± 11.7	79.0 ± 6.6	48.4 ± 17.7	60.6 ± 14.0
Xeno III (Dentsply, Konstanz, Germany)	0.3 ± 0.2	90.6 ± 12.2	35.1 ± 12.3	74.8 ± 7.3
AdheSe (Ivoclar Vivadent, Schaan, Liechtenstein)	0.6 ± 0.2	41.6 ± 8.9	45.5 ± 19.1	72.5 ± 15.8
Clearfil Protect Bond (Kuraray, Kurashiki, Okayama, Japan)	0.2 ± 0.2	0.2 ± 0.1	3.6 ± 2.8	66.9 ± 10.7

* The bacterial growth rate is expressed by the slope ($\times 10^3$) of the linear portion of the growth curve (± standard deviation) ($P < .001$, 1-way analysis of variance). The growth rates are the average of three experiments, eight wells in each experiment. The vertical ines connect the values that do not differ significantly (Tukey multiple comparisons).

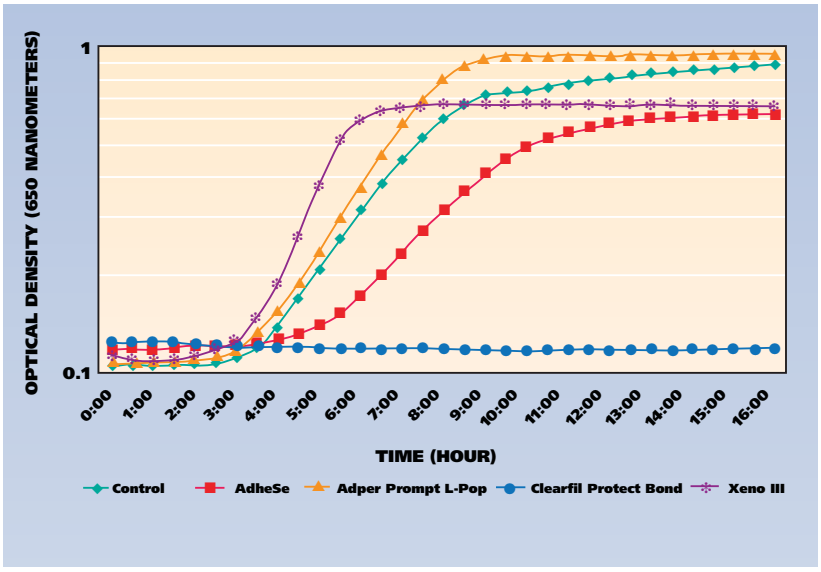

Figure 3. Bacterial outgrowth (measured by changes in optical density [OD]) on the surface of self-etching adhesive materials after aging for two days. Each point is the average OD measured simultaneously in eight wells. AdheSe is manufactured by Ivoclar Vivadent, Schaan, Liechtenstein. Adper Prompt L-Pop is manufactured by 3M ESPE, Seefeld, Germany. Clearfil Protect Bond is manufactured by Kuraray, Kurashiki, Okayama, Japan. Xeno III is manufactured by Dentsply, Konstanz, Germany.

each tested material. We calculated a regression line on the linear portion of the growth curve, which represents the logarithmic growth phase. The multivariate coefficient of determination of all growth curves ranged from $R^2 = 0.91$ to $R^2 = 0.99$. Two-way ANOVA showed a significant difference in bacterial growth rate, both on different adhesive materials ($P < .001$) and at different tested times ($P < .001$). We observed no bacterial growth on the fresh samples of self-etching adhesives ($P < .001$) (data not shown).

After the materials aged for one day, we observed bacterial growth only in the Adper Prompt L-Pop sample wells, which was not significantly different from the control sample ($P < .001$) (Figure 2 and Table 1). After aging for two days, Clearfil Protect Bond sample wells showed no bacterial growth. We observed bacterial growth in the AdheSe, Adper Prompt L-Pop and Xeno III samples, with no significant difference from the control in the Adper Prompt L-Pop and Xeno III samples ($P < .001$) (Figure 3 and Table 1). After aging for seven days, the Clearfil Protect Bond samples still showed antibacterial properties ($P < .001$); the other tested materials did not differ from the control (Table 1). After aging for 14 days, none of the tested self-etching adhesive materials exhibited any antibacterial properties (Table 1).

The pH value measurements of self-etching adhesive materials before, immediately after and 24 hours after polymerization are shown in Table 2.

DISCUSSION

The ADT is used as the standard assay to measure antibacterial properties of materials, despite its limitations[23] (that is, it measures only components that are water-soluble). In our study, we also used the DCT, which measures the effect of direct and close contact between the microorganism and the tested materials. Antibacterial properties are assessed regardless of their solubility and the diffusiveness of components into the surrounding area.[18-22,24]

The results from the ADT showed that only Clearfil Protect Bond exhibited a large inhibition halo, suggesting that soluble antibacterial components were released into the surrounding environment. Under DCT conditions, all of the tested adhesives had an immediate bactericidal effect on *S. mutans*. However, all of the materials lost their

antibacterial properties within 14 days: Adper Prompt L-Pop after 24 hours, AdheSe and Xeno III within 48 hours and Clearfil Protect Bond after 14 days. It is reasonable to assume that their antibacterial components decomposed at varying rates into the surrounding aging liquid. The prolonged antibacterial effect of Clearfil Protect Bond is related to the antibacterial properties of the MDPB molecules,[6,25] which become immobilized after adhesive photopolymerization.[6,25,26] Our findings both in ADT and DCT, however, did not support this assumption. We hypothesize that not all MDPB molecules copolymerize, and those that do not are able to leach and manifest their antimicrobial properties. Once all the MDPB molecules that did not copolymerize are leached out, the antimicrobial effect disappears.

TABLE 2

pH measurements of tested self-etching adhesive materials.

MATERIAL	PH BEFORE POLYMERIZATION	PH OF DDW* IMMEDIATELY AFTER POLYERIZATION	PH OF DDW 24 HOURS AFTER POLYMERIZATION
Control DDW	6.9	6.9	6.9
Adper Prompt L-Pop (3M ESPE, Seefeld, Germany)	1.0	1.72	4.71
Xeno III (Dentsply, Konstanz, Germany)	1.1	2.06	4.74
AdheSe (Ivoclar Vivadent, Schaan, Liechtenstein)	2.0	2.27	4.89
Clearfil Protect Bond (Kuraray, Kurashiki, Okayama, Japan)	1.8	1.72	4.9

* DDW: Double-distilled water.

The advantage of the obtained prolonged antibacterial effect due to possible toxicity of the released antibacterial component on human tissues is questionable.[25] It also is questionable whether this short-term antibacterial period (14 days) has a significant clinical advantage over the 24-hour antibacterial activity exhibited by other bonding systems.

The results we obtained in this study do not support a previously stated hypothesis that the antibacterial effect of self-etching adhesive systems is related to the acidic nature of nonpolymerized materials.[6,27,28] Our results are in agreement with those of Imazato and colleagues[29] who suggested that the benefit of a low pH environment exhibited by dentin bonding systems should be considered as "limited."

CONCLUSION

The application of self-etching adhesive materials could contribute toward completely eliminating bacteria or minimizing it during tooth preparation. Since none of the self-etching adhesive systems tested had long-lasting antibacterial properties, they did not provide a solution to the main cause for secondary caries: bacterial invasion owing to microleakage. ■

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