Comparative Evaluation of Antibacterial Effects of Three, Seventh Generation Adhesive Systems Against: *Streptococcus mutans*

**ABSTRACT**

**Purpose** To compare the antibacterial efficacy of three bonding agents, ADPER EASY BOND, ONE COAT 7.0, PALFIQUE BOND against *Streptococcus mutans*.

**Materials and Methods** The three seventh generation bonding systems: ADPER EASY BOND, ONE COAT 7.0 and PALFIQUE BOND were taken. A total of 3.8 g/100 mL of Mueller Hinton Agar (MHA) was autoclaved at 110°C for 15 min. Media was then poured in petri plates and solidified at room temperature. After solidification, 3 wells were punched with hollow tube under sterilized conditions. Well 1 as CHX, well 2 as Normal saline (NS) and well 3 A/B/C compounds. About 5 mL of seed culture of *S. mutans* strain MTCC no. 497 was prepared in Mueller Hinton broth. About 6 µL of CHX (+ve control) and NS (−ve control) was added to wells 1 and 2 in each plate. About 100 µL of seed inoculum was spread on each MHA plates. For each bonding agent 10 replicas of experiment was set up at room temperature. A total of 6 µL of bonding agent A, B and C were added to well 3 respectively in each set of experiment. To study zone of inhibition, plates were incubated at 37°C for 12 h.

**Conclusion** Different bonding systems possess different degrees of antibacterial activity. Seventh generation bonding agent of compounds A and C do not have antibacterial action while compound B has.

**KEYWORDS** antibacterial efficacy, inoculums, Mueller Hinton Agar

**INTRODUCTION**

The antibacterial properties of a self-etching system constitute an important issue in operative dentistry and is mandatory as viable bacteria can still be present after cavity preparation and play a vital role in the initiation of tooth disease like dental caries. However, in today’s dentistry, attention has shifted to minimal surgical interventions in which intact tooth structures is preserved to the greatest extent, which sometimes puts pressure on dentists to avoid cavity over-preparation. Moreover, in vivo studies have shown that bacteria may exist on cavity walls, in the smear layer, or at the dentinonamel junction. This unchecked bacterial invasion of dentine leads to inflammatory reaction. There are no instruments or materials available that can verify the presence or absence of bacteria in the prepared floor of a cavity. Hence antibacterial properties in a bonding agent are required.

*Streptococcus mutans* has been strongly implicated as the principal etiological agent in dental caries and is most cariogenic of all the oral streptococci, that breaks down sugar for energy and produces an acidic environment, which demineralizes the superficial structure of the tooth. The result of the conversion disintegrates the coating of the tooth then later dissolves the calcium molecule creating a hole. Transmission of *S. mutans* can be found in people of all ages although it is more common for infants and children. Someone with a healthy oral flora will roughly contain 10,000 CFU/mL of *S. mutans* in their mouth. *Streptococcus mutans* possesses three virulence factors: Water insoluble glycans, acid tolerance, and production...
of lactic acid. There are two possible mechanisms initiating the formation of secondary caries. Firstly, resin polymerization shrinkage causes gaps between the restoration and the cavity walls that can be colonized by oral microorganisms from saliva. Secondly, incomplete caries removal can be another source of microbes. The application of a bonding system with antibacterial activity is a promising solution. Several dentin bonding systems are currently available. The traditional bonding systems demand enamel etching and the application of either a primer and bond (fourth generation) or only a primer/bond (fifth generation). However, the newer generations are self-etch primers/adhesives which no longer demand orthophosphoric acid usage. The sixth generation self-etch adhesives can be either two-step (self-etch primer and bond) or one-step (self-etch adhesive, primer A + B). Procedures all-in-one self-etch adhesives constitute the seventh and eighth generations (solvent-free). Since there are many different bonding systems on the market, and companies are still introducing new ones, it is crucial to know which bonding systems possess the best clinical performance. The success rate of such a restorative procedures relies strictly on the antibacterial activity of the bonding system used. The purpose of this study was to compare the antibacterial efficacy of three bonding agents PALFIQUE BOND (TOCUYAMA), ONE COAT 7.0 (Coltene) and ADPER EASY BOND (3M ESPE) against S. mutans.

MATERIALS AND METHODS

Three commercially available seventh generation bonding systems were investigated.

1. ADPER EASY BOND – 3M ESPE [compound A]
2. ONE COAT 7.0 – Coltene [compound B]
3. PALFIQUE BOND – Tokuyama [compound C]

Agar diffusion assay

The antibacterial properties of these systems against Streptococcus mutans strain MTCC 497 was determined. For this technique 3.8 g/100 mL of MHA was autoclaved at 110°C for 15 min. Autoclaved media was then poured in petri plates and solidified at room temperature. After solidification, 3 wells were punched with hollow tube under sterilized conditions. Well 1 as CHX, well 2 as Normal saline (NS) and well 3 has A/B/C compounds. The 5 mL of seed culture of S. mutans strain MTCC no. 497 was prepared in Mueller Hinton broth. About 6 µL of CHX (taken as +ve control) and NS (as −ve control) were added to wells 1 and 2 in each plate. About 100 µl of seed inoculum was spread plated on each MHA plates. For each bonding agent 10 replicas of experiment was set at room temperature. A total of 6 µL of bonding agent A, B and C were added to well 3 respectively in each set of experiment. To study zone of inhibition, plates were incubated at 37°C for 12 h.

A statistical analysis was conducted to confirm the statistical significance of any differences. As the data was unbalanced and abnormally distributed, the ANOVA test was performed, followed by a multiple-comparison test to test the differences in pairs. A significance level of $P < 0.05$ was assumed. (Tables 1 and 2, Graph 1).

RESULTS

The antibacterial activity of the samples is shown in Figures 1–3.

### Table 1 Antimicrobial analysis.

<table>
<thead>
<tr>
<th>S. no.</th>
<th>A (in cm)</th>
<th>B (in cm)</th>
<th>C (in cm)</th>
<th>CHX (in cm)</th>
<th>NS (in cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.2</td>
<td>1.3</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>1.2</td>
<td>1.4</td>
<td>1.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>1.2</td>
<td>1.3</td>
<td>1.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>1.2</td>
<td>1.0</td>
<td>1.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>1.2</td>
<td>1.4</td>
<td>0.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>1.4</td>
<td>1.0</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>1.3</td>
<td>1.1</td>
<td>1.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>1.3</td>
<td>1.2</td>
<td>1.2</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table 2 Statistical analysis.

<table>
<thead>
<tr>
<th>ANOVA table</th>
<th>Sum of squares (SS)</th>
<th>Mean square (MS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (between columns)</td>
<td>18.96</td>
<td>4.740</td>
</tr>
<tr>
<td>Residual (within columns)</td>
<td>0.6090</td>
<td>0.01353</td>
</tr>
<tr>
<td>Total</td>
<td>19.57</td>
<td></td>
</tr>
<tr>
<td>Tukey’s multiple comparison test</td>
<td>Mean diff.</td>
<td>Significant? $P &lt; 0.05$?</td>
</tr>
<tr>
<td>Compound A vs Compound B</td>
<td>1.310</td>
<td>Yes</td>
</tr>
<tr>
<td>Compound A vs Compound C</td>
<td>0.0000</td>
<td>No</td>
</tr>
<tr>
<td>Compound A vs CHX</td>
<td>1.200</td>
<td>Yes</td>
</tr>
<tr>
<td>Compound A vs NS</td>
<td>0.0000</td>
<td>No</td>
</tr>
<tr>
<td>Compound B vs Compound C</td>
<td>1.310</td>
<td>Yes</td>
</tr>
<tr>
<td>Compound B vs CHX</td>
<td>0.1100</td>
<td>No</td>
</tr>
<tr>
<td>Compound B vs NS</td>
<td>1.310</td>
<td>Yes</td>
</tr>
<tr>
<td>Compound C vs CHX</td>
<td>-1.200</td>
<td>Yes</td>
</tr>
<tr>
<td>Compound C vs NS</td>
<td>0.0000</td>
<td>No</td>
</tr>
<tr>
<td>CHX vs NS</td>
<td>1.200</td>
<td>Yes</td>
</tr>
</tbody>
</table>

→ Zone of inhibition was observed in +ve control well. → No zone of inhibition was observed around compounds A and C. → Bonding agent B act as an inhibitor for bacterial growth forming zone of inhibition with a diameter shown in earlier Table 1.

A statistical analysis was conducted to confirm the statistical significance of any differences. As the data was unbalanced and abnormally distributed, the ANOVA test was performed, followed by a multiple-comparison test to test the differences in pairs. A significance level of $P < 0.05$ was assumed. (Tables 1 and 2, Graph 1).
Comparative evaluation of antibacterial effects of three, seventh generation adhesive systems

DISCUSSION
Operative work in restorative dentistry is now moving away from complete caries removal to an ultraconservative approach, preserving tooth structure and preventing pulpal injury. However, if there is a decreased removal of the tooth structure, it is possible that some active residual bacteria may be left behind in the minimally excavated lesions. The antibacterial properties of self-etching adhesive systems constitute an important issue in restorative dentistry, since viable bacteria can appear even after cavity preparation. It is well known that bacteria, that invade along the tooth-restoration interface, may cause secondary caries and damage to the pulp. Since dentin bonding systems have been developed to minimize both contraction gap formation and the potential for marginal leakage around composite restorations, dentin bonding systems showing antibacterial effect during the placement of the filling would be of significant use to inactivate residual bacteria in the cavity.

The size of the inhibition zone depends on the:

- antibacterial properties of the materials,
- the quantity used,
- the diffusion potential across the culture medium.

The antibacterial activity of dentin bonding agents depends on several factors, including composition and acidity.

The greater the quantity and the higher the diffusion potential, the larger the inhibition zones which can be observed. The quantity of material applied can be easily controlled by an automatic pipette.

The agar disk-diffusion test has been widely accepted as a simple screening method to assess the antibacterial properties of dentin bonding systems.
After being applied to a disc, the investigated material is placed on agar plates and inoculated with oral bacteria. If the material releases any antibacterial components, an inhibition halo is produced. The strength of antibacterial activity is measured by the diameter of the inhibition zone around the material. In this study, a modified agar diffusion method was used. In this, glass cylinders were used instead of absorbent paper disc.

1. Because the application of a two or more component bonding system on an absorbent paper disc may lead to the formation of layers in the disc.
2. To replicate clinical conditions more closely.
3. As LED light source are unable to pass through a paper disc, this does not happen in case for cylinders.

Cylinders also guarantee a strictly limited and replicable area of material application, allowing direct contact between all the components of the adhesive system and the agar, exactly as in the case of adhesive resin application on the dentine surface.

Previous studies concerning the antibacterial effect of commercially marketed dentin bonding systems have generally attributed this effect to their low pH environments. Harper and Loesche in 1984 reported that the pH values of denting bonding systems, which cause 100% killing over a 3 h period, is 3.0 for S. mutans. Thus, ADPER EASY BOND, having a pH of 3.5, does not show appreciable antibacterial activity against it. A recent review on self-etching dentin bonding systems has attributed their antibacterial properties to their low pH or to specific antimicrobial components such as glutaraldehyde and 1,2-methacryloyloxydodecylpyridinium bromide.

Chlorhexidine solution has a broad spectrum of uses against Gram-positive and Gram-negative bacteria. The antimicrobial effect of chlorhexidine is caused by the cationic molecule that binds to the negatively charged bacterial cell walls, thereby altering the cell’s osmotic equilibrium. Chlorhexidine is biocompatible and can be absorbed by dental tissues and mucous membranes. So, chlorhexidine was chosen as the control due to its ability to denature cell wall of bacteria.

Compound B: ONE COAT – (Coltene) consists of:

- Methacrylated phosphoric esters.
- 1,6 hexanediol.
- Polyalkenoic acid.
- Silica fillers.
- Water initiator.
- Stabilizers.

Results show that any of the constituents of compounds A and C, do not possess antibacterial action. Because these bonding agents (A and C) are viscous in nature and not easily spread on the surface. Hence, they have lesser antibacterial action.

The incorporation of antibacterial agents into dentin bonding agents may become an essential factor in inhibiting residual bacteria in the oral cavity following a cavity disinfection procedure. The application of self-etching adhesive materials could contribute toward completely eliminating or at least minimizing the bacteria during tooth preparation.

CONCLUSION

To avoid cariogenic bacterial colonization in the cavity preparation, the adhesive systems should have an antimicrobial effect. Different bonding systems possess different degrees of antibacterial activity. Several generations of bonding agent of compounds A and C do not have antibacterial action while compound B has antibacterial activity against S. mutans and showed statistically significant difference.

REFERENCES

Comparative evaluation of antibacterial effects of three, seventh generation adhesive systems