ABSTRACT

Aim: The aim of the present study was to compare the antibacterial activity of a self-etching primer containing antibacterial monomer methacryloyloxydodecylpyridinium bromide (MDPB) (Clearfil protect bond) with a conventional self-etching primer without MDPB (Clearfil SE bond) against *Streptococcus mutans* and the effect of incorporation of MDPB on the tensile bond strength of the experimental self-etching primer (Clearfil protect bond).

Materials and methods: The antibacterial activity of the self-etching primers was assessed using agar disk diffusion method and the diameters of the zones of inhibition were measured and ranked. For tensile bond strength testing, 20 noncarious human molars were selected and randomly divided into two groups comprising 10 teeth in each group. Group I specimens were treated with Clearfil SE bond (without MDPB). Group II specimens were treated with Clearfil protect bond (with MDPB). Composite material was placed incrementally and cured for 40 seconds in all the specimens. Tensile bond strength was estimated using the Instron Universal testing machine at a crosshead speed of 1 mm/min.

Results: The addition of MDPB into a self-etching primer exerts potential antibacterial effect against *S. mutans*. The tensile bond strength of MDPB containing self-etching primer was slightly lower than that of the conventional self-etching Clearfil protect bond primer, but the difference was not statistically significant.

Conclusion: Thus, a self-etching primer containing MDPB will be a boon to adhesive dentistry as it has bactericidal property with adequate tensile bond strength.

Clinical significance: The concept of prevention of extension in adhesive dentistry would result in micro/nanoleakage due to the presence of residual bacteria in the cavity. Self-etching primers with MDPB would improve the longevity of such restorations by providing adequate antibacterial activity without compromising the bond strength.

Keywords: Antibacterial property, Methacryloyloxydodecylpyridinium bromide, Self-etching primers, Tensile bond strength.

INTRODUCTION

Modern dental practice has an increasing demand for esthetic restorations that lead to the extensive use of adhesive dental materials. Many adhesive systems have been developed since Buonocore\(^1\) first described the acid etch technique on enamel. Even some dentin adhesive systems which show high bond strengths in *in vitro* studies have been reported to be incapable of preventing the occurrence of microgaps between the tooth and the restoration\(^2\) and bacteria invaded the cavity floor under these adhesive resin restorations.\(^3\) Polymerization shrinkage and the resultant contraction gaps at the tooth restoration interfaces continue to be a significant problem associated with composite resin restorations.\(^4\) Therefore, dentin bonding systems which possess high bond strength and antibacterial activity even after being placed in the cavity...
would be beneficial for eliminating the harmful effect caused by bacterial microleakage.²

To provide dental adhesive material with antibacterial activity, a new monomer 12-MDPB has been added to the adhesive, which is synthesized by combining the antibacterial agent—quaternary ammonium and a methacryloyl group. The purpose of this in vitro study was to compare the antibacterial activity of a self-etching primer incorporating the antibacterial monomer MDPB (Clearfil protect bond) with a conventional self-etching primer without MDPB (Clearfil SE bond primer) and the effect of MDPB incorporation on the tensile bond strength of the adhesive system.

METHODOLOGY FOR ANTIBACTERIAL ACTIVITY TESTING

- Technique employed: disk diffusion method
- Test organism: S. mutans [American Type Culture Collection (ATCC) 25175].

The agar disk was prepared by inoculating the test organism S. mutans (ATCC 25175) into 2 mL of brain heart infusion (BHI) broth and incubated at 37°C for 3 to 5 hours. The inoculum for the assay was standardized by adjusting the turbidity of the BHI culture to match that of the 0.5 McFarland standards. The standardized inoculum of the test bacteria was seeded on a BHI agar plate by gently swabbing the agar surface with a presterilized cotton swab. For the impregnation of primers, sterile paper disks (Whatman No. 1 filter paper) of 6 mm diameter were selected and placed on the seeded agar plate. The disks were then impregnated with the two primers under study, namely Clearfil SE bond primer and Clearfil protect bond primer and also with known antibacterial agents, such as Zosyn (which is a combination of the beta-lactam antibiotic piperacillin with beta-lactamase inhibitor tazobactam) and cefotaxime which served as positive controls. The agar plate was then incubated at 37°C for 24 to 48 hours.

The antibacterial activity was evidenced by inhibitory zones of clearance around the primer-impregnated disks and their diameters (in mm) measured using sliding calipers and the size of the inhibition zones was calculated and recorded (Fig. 1).

To study the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), the macrobroth dilution method was employed.

Doubling dilution of the two test primers in sterile BHI broth was prepared in sterile test tubes (1/20, 1/40, 1/80, 1/160, and 1/320) and 10 μL of the standardized inoculum was seeded into each of the above dilutions. Growth control was put in parallel. All tubes were incubated at 37°C for 24 hours.

The MIC of the two primers was visually assessed (Fig. 2), and MBC was determined by streaking out a loopful of the broth from the MIC tubes onto a BHI agar plate and incubated for 24 hours. The highest dilution of the primer that showed >90% reduction in growth in comparison to that of the growth control was interpreted as the MBC and recorded (Fig. 3).

METHODOLOGY FOR TENSILE BOND STRENGTH TESTING

A total of 20 recently extracted, intact, human molars were collected. A flat occlusal surface was prepared using water-cooled diamond disk. All prepared teeth were randomly divided into two groups of 10 specimens each. Group I—Clearfil SE bond primer and group II—Clearfil protect bond primer. Apply primer for 20 seconds and air dry for 5 seconds. Apply adhesive on the primed surface and light cure for 20 seconds. Composite resin was placed by incremental technique for standard diameter of 4 mm and height 6 mm in increments of 2 mm thickness. A 26-gauge ligature wire was twisted at one end and loop formed at other end and the twisted end placed inside 2 mm of uncured composite and light cured. Another
Antibacterial Effect and Tensile Bond Strength of Self-etching Adhesive Resins

2 mm of composite was placed on top and light cured for 40 seconds. Tensile bond strength was tested by Instron Universal testing machine at a crosshead speed of 1 mm/min (Figs 4 and 5).

RESULTS

The antibacterial efficacies of the primers with and without MDPB against *S. mutans* (ATCC 25175) are tabulated in Table 1. The results for the macrobroth dilution tests are tabulated in Table 2. The tensile bond strength values of the self-etch adhesive systems with and without MDPB are presented in Table 3. The tensile bond strength values were statistically analyzed using Mann–Whitney U-test and the results are presented in Table 4.

DISCUSSION

The present study compares the antibacterial activity of a mild self-etching primer containing antibacterial monomer MDPB with a conventional self-etching primer and the effect of incorporation of MDPB on the tensile bond strength of the adhesive system. To provide adhesive materials with antibacterial activity, a new monomer, MDPB has been developed, which is a compound of

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**Table 1: Antibacterial efficacies of primers**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Test solution</th>
<th>Zone of inhibition (in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Clearfil SE bond primer</td>
<td>15</td>
</tr>
<tr>
<td>II</td>
<td>Clearfil protect bond primer</td>
<td>16</td>
</tr>
<tr>
<td>III</td>
<td>Cefotaxime</td>
<td>15</td>
</tr>
<tr>
<td>IV</td>
<td>Piperacillin-tazobactam</td>
<td>12</td>
</tr>
</tbody>
</table>

**Table 2: Macrobroth dilution tests**

<table>
<thead>
<tr>
<th>Primer</th>
<th>MIC</th>
<th>MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearfil SE bond (group I)</td>
<td>1/80</td>
<td>1/20</td>
</tr>
<tr>
<td>Clearfil protect bond (group II)</td>
<td>1/320</td>
<td>1/40</td>
</tr>
</tbody>
</table>

**Table 3: Tensile bond strength values**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Group I Clearfil SE bond</th>
<th>Group II Clearfil protect bond</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen 1</td>
<td>15.58</td>
<td>15.77</td>
</tr>
<tr>
<td>Specimen 2</td>
<td>16.61</td>
<td>14.33</td>
</tr>
<tr>
<td>Specimen 3</td>
<td>15.90</td>
<td>15.87</td>
</tr>
<tr>
<td>Specimen 4</td>
<td>17.05</td>
<td>15.96</td>
</tr>
<tr>
<td>Specimen 5</td>
<td>16.46</td>
<td>15.84</td>
</tr>
<tr>
<td>Specimen 6</td>
<td>15.80</td>
<td>16.18</td>
</tr>
<tr>
<td>Specimen 7</td>
<td>16.64</td>
<td>16.21</td>
</tr>
<tr>
<td>Specimen 8</td>
<td>17.35</td>
<td>16.13</td>
</tr>
<tr>
<td>Specimen 9</td>
<td>16.52</td>
<td>16.32</td>
</tr>
<tr>
<td>Specimen 10</td>
<td>15.98</td>
<td>16.26</td>
</tr>
</tbody>
</table>

**Table 4: Mann–Whitney U-test**

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10</td>
<td>16.38</td>
<td>0.5679</td>
<td>0.088</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>15.89</td>
<td>0.5496</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS: Not significant
antibacterial agent quaternary ammonium with a methacryloyl group. The compositions of the primers are as follows:
- Clearfil SE bond primer
- 2-Hydroxyethyl methacrylate
- Hydrophilic dimethacrylate
- 10-Methacryloyloxydecyl dihydrogen phosphate
- N,N-Diethanol-P-toluidine
- D,L-Camphorquinone
- Water
- Clearfil protect bond primer
- 12-MDPB
- 10-Methacryloyloxydecyl dihydrogen phosphate
- Hydroxyethyl methacrylate
- N,N-Diethanol-P-toluidine
- Water.

Incorporation of MDPB is considered to be a potential method of providing dental adhesive system with antibacterial activity before and after curing. Dentin primer is applied to the tooth cavity for promoting bonding of restoration to tooth substrate, and it has been suggested that MDPB-containing primer has a potential benefit to prevent secondary caries caused by residual and invading bacteria in the cavity. At the stage before curing, MDPB-containing primer acts as a bactericidal solution and the cavity prepared for restoration is disinfected by unpolymerized MDPB in the primer.

Mutans streptococci have been associated in particular with the onset of caries and early demineralization. It is also responsible for the development of secondary caries. Thus, S. mutans has been selected as the test microorganism in this study as it has been shown to be highly associated with dental caries. The antibacterial activity of two self-etching primers with and without MDPB was compared in this study by agar diffusion method. The comparison was done with two control groups Zosyn and cefotaxime, which are potent antibiotics against S. mutans.

Agar diffusion method was used in this study, as it is a simple and easy way to determine the antibacterial effects of a liquid substance and this method was previously used in studies. The results of agar disk diffusion method reflect the combination of the amount of antibacterial components included in the materials and their diffusivity within hydrophilic agar. Accordingly, the size of inhibition zones is not an appropriate index for comparison of the intrinsic antibacterial activity of different materials. Therefore, MIC and MBC values of each adhesive were determined in this study.

In MIC/MBC measurements, planktonic microorganisms were used and antibacterial components could come almost freely into contact with bacterial cells. Accordingly, even if the material contains antibacterial components with less diffusivity, the substantive antibacterial activity was available.

The importance of high tensile bond strengths in the adhesion of resin materials to enamel and particularly to dentin for the production of well-sealed and long-lasting restorations cannot be overemphasized. Even if restorative materials have new biological functions, they would not be clinically useful if their original properties are hampered by the addition of new characteristics.

For bond strength testing, freshly extracted caries-free human molars were used in this study. Molars were preferred in this study as flat dentin surface could be prepared which would give a wider area of dentin to be treated and bonded to resin substrate. Flat dentin surface was created using water-cooled disks under careful visual examination.

The composite samples were prepared with a standard dimension of 4 mm diameter and 6 mm height using a polyvinyl mold; 4 mm diameter of polyvinyl mold was used so that it covers the maximum surface of tooth; 6 mm height was kept so that it gives sufficient amount of composite material to perform the tensile bond strength test. Polyvinyl cylinders were used to ensure adequate curing as light can pass through it and facilitate easy removal. Incremental placement of composite and 40 seconds curing of each increment was observed to avoid bonding failure at an undesired site during testing. The test specimens were stored in distilled water for 24 hours at 37°C before testing to simulate the oral conditions.

In this study, to evaluate the tensile bond strength, Instron machine with a crosshead speed of 1 mm/min was used as it is the standard universal testing machine used for various tensile and shear bond tests. In this in vitro study, a tensile type of test was applied to test the bond strength. Normally, in in vivo conditions, dental adhesives used are more likely to be subjected to shear forces, but the ability of the adhesive resin to retain on the tooth surface ultimately depends on the resistance it offers to tensile forces. Hence, tensile bond strength was evaluated in this study.

From the data collected by agar diffusion test, to check the antibacterial potency of Clearfil SE bond (group I) and Clearfil protect bond (with MDPB) (group II) against S. mutans, when compared with two positive controls, cefotaxime (group III) and Zosyn (group IV) which are potent antibiotics against S. mutans, the antibacterial effects of the primers were tabulated by measuring the diameter of the zone of inhibition around the primers (Table 1).

The size of inhibition zone was larger for Clearfil protect bond (with MDPB) than Clearfil SE bond (without MDPB) as well as the positive controls. These results correlate with the results of a previous study done by...
Imazato et al.\textsuperscript{5} where they have showed that the incorporation of MDPB is a potential method for providing dentin primer with antibacterial activity before curing.

Various in vitro studies had been conducted to evaluate the antibacterial effectiveness of MDPB-containing adhesive. Imazato et al.\textsuperscript{12} evaluated the bactericidal effect of a dentin primer incorporating the antibacterial monomer MDPB against bacteria in human carious dentin and also found the MIC and MBC against these bacteria. They concluded that incorporation of MDPB into dentin primer could be beneficial for eliminating the residual bacteria in cavities.

Imazato et al.\textsuperscript{10} revealed that MDPB-containing primer showed an inhibitory effect on the growth of \textit{S. mutans} even after being cured. This may be due to the fact that the antibacterial agent is immobilized by the copolymerization of MDPB and the monomer components in the primer and adhesive after curing, and it shows an inhibitory effect on bacteria, which are in contact with the surface.\textsuperscript{13}

Duyzol et al.\textsuperscript{14} had revealed that incorporation of antibacterial monomer MDPB was effective in providing substantial antibacterial activity and that the bactericidal effects of MDPB-containing primer were greater than those of other total etch and two-step self-etch systems and of chlorhexidine gluconate.

The MIC value for Clearfil SE bond primer (without MDPB) was 1/80 and for Clearfil protect bond primer (with MDPB) was 1/320. No visible bacterial growth was observed in the test tubes containing the experimental primer with MDPB in the macrobroth dilution test. The highest dilution of the primer that showed >90\% reduction in growth in comparison to that of a growth control was interpreted as MBC. The MBC value for Clearfil protect bond primer (with MDPB) was 1/40, whereas for Clearfil SE bond primer (without MDPB) was 1/20. This result shows that Clearfil protect bond primer (with MDPB) showed a greater bactericidal effect than the primer without MDPB and even at 40 times dilution it could kill all detectable bacterial cells.

Imazato et al.\textsuperscript{12} have proposed that a primer is diluted during diffusion into dentin and the bacteria remaining in the deep layer consequently make contact with the diluted primer solution. In a similar study, Imazato et al.\textsuperscript{14} have proposed that even diluted 100 times, the MDPB-containing primer still showed a significant bactericidal effect while the commercially available primers had almost lost their effect at 40 times dilution. However, the antibacterial activity of these commercial products seems to be unreliable as the pH value of the primer increases by the buffering action of the dentinal fluid.\textsuperscript{12} Similar results were obtained in the present study.

Sampath et al.\textsuperscript{15} had evaluated the antibacterial activity of three newer dentin bonding adhesive systems using direct contact test as it relies on direct and close contact between the test microorganism and the testing materials and is virtually independent of the diffusion properties of both the tested materials and the media. They had concluded that Clearfil protect bond showed maximum antibacterial activity.

Therefore, it is considered that the Clearfil protect bond (with MDPB) is able to kill the bacteria, which invaded the deeper layer of the carious lesion and to disinfect the cavity more effectively. Since the bactericidal effect of the MDPB-containing primer is dependent on the killing mechanism of quaternary ammonium, it is more reliable than the commercial self-etching primers for inactivating the residual bacteria in the cavity.\textsuperscript{12}

Taking into consideration the previous findings for experimental primer incorporating MDPB, the results of the present study indicate that the comprehensive antibacterial adhesive system (Clearfil protect bond) employing MDPB-containing primer and adhesive would contribute to prevent the harmful effects caused by oral bacteria \textit{in vivo}.

Recent in vivo animal test by Yoshikawa et al.\textsuperscript{16} proved that the experimental primer containing MDPB might inactivate the residual bacteria in cavities by its antibacterial effects, suggesting its possible clinical benefits. Further studies have proven that MDPB-containing primer inhibits organisms associated with active root caries lesions.\textsuperscript{17} Among the various adhesives tested by Korkmaz et al.\textsuperscript{18} Clearfil protect bond primer based on monomer MDPB was found to be the most potent material against \textit{Lactobacillus acidophilus} and \textit{Lactobacillus casei}.

The results of the present study show that the self-etching primer without MDPB produced better tensile bond strength than the self-etching primer with MDPB, but the difference in their bond strengths was not statistically significant.

Gupta et al.\textsuperscript{19} evaluated the shear bond strength of three different self-etch adhesive systems and concluded that the addition of antimicrobial agent, such as MDPB decreases the bond strength of dentin bonding agent and addition of fluoride further decreases the bond strength more than MDPB.

Modern trend in caries management recommends less surgical intervention, the so-called minimal intervention dentistry. When attention is focused on less removal of tooth structure, it is possible that some active bacteria remain in the cavity.\textsuperscript{2} In addition, the reduction in cariogenic bacteria to eliminate the risk of further demineralization and cavitation is one of the important basic principles in minimum intervention dentistry. In this...
context, adhesive systems with antibacterial effects are considered to be of benefit for achieving a better prognosis. However, the *in vivo* effects of MDPB-containing primer on a variety of bacterial species present in the dentinal lesions including obligate anaerobes remain to be determined. Further, research and clinical trials are necessary to elucidate the benefit of MDPB-containing adhesive systems with other antibacterial materials, such as quaternary ammonium polyethyleneimine nanoparticles and triclosan.

### CONCLUSION

From the results of the present study, it may be concluded that:

- Addition of MDPB into a self-etching primer exerts potential antibacterial effect against *S. mutans*
- The zone of inhibition against *S. mutans* was maximum for MDPB-containing primer when compared with the conventional self-etching primer and the positive controls
- The results of the study further revealed that MDPB-containing self-etching primer retained its antibacterial effect even after 40 times dilution as against the conventional self-etching primer without MDPB which lost its antibacterial effect after 20 times dilution
- The results of the tensile bond strength tests revealed that the tensile bond strength of the MDPB-containing self-etching primer was slightly lower than that of the conventional self-etching primer, but the difference was not statistically significant.

### REFERENCES