

# To Evaluate the Antibacterial Activity of Different Self Etching Adhesive Systems against Streptococcus mutans using Agar Diffusion Test

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## ABSTRACT

**Aim:** To evaluate the antibacterial activity of different self-etching adhesive systems against streptococcus mutans using agar diffusion test. **Material method:** The antibacterial activity of Clearfill protect bond, Adper prompt and Xeno111 on streptococcus mutans were evaluated using Agar diffusion test. 15 brain heart infusion plates were swabbed with streptococcus mutans and four 6mm wells were punched on each plate. The plates were incubated at 37-degree centigrade for 24 hrs. Antibacterial actions of these adhesive materials were tested by measuring the zones of inhibition as per the groups. **Results:** Among the tested materials Clearfill protect bond exhibited maximum antibacterial activity against S.mutans. Adper prompt and Xeno111 showed limited antibacterial activity. **Conclusion:** The incorporation of MDPB into dentin bonding agents may become an essential factor in inhibiting residual bacteria in the cavity.

**KEYWORDS:** Dentin bonding. Agar test, antibacterial, Adhesives.

## INTRODUCTION

The modern dental practice has an increasing demand for esthetic restorations that leads to an extensive use of adhesive dental materials. Many adhesive systems have been developed since BUONOCORE (1955) first described acids etch technique on enamel.<sup>1</sup> Hembree and Andrews (1978) have demonstrated that polymerization shrinkage of composite resins can cause significant micro leakage on margins that do not involve the enamel, which results in gaps in tooth/ restoration interface.<sup>2</sup> To minimize contraction gap formation and potential for bacterial leakage, dentin adhesives have been developed. However, so far no adhesive system has been proven to be completely effective.<sup>3</sup> Thus, polymerization shrinkage and the resultant contraction gaps associated with composite restorations continue to be a problem at the tooth restoration interface.

Newer adhesive systems namely selfetching primer/adhesive systems are currently available. Selfetching dental adhesive systems have been developed to simplify bonding procedures and to make their application less time consuming.<sup>4</sup> In -vitro studies have shown that the tooth restoration interface created when using self-etching adhesive systems do not eliminate micro leakage and bacterial penetration which can lead

to secondary caries the most common reason for dental restoration failure.<sup>5</sup> Therefore, the adhesive materials with antibacterial properties are important. MDPB is a compound of antibacterial quaternary ammonium with a methacryloyl group which shows antibacterial activity against oral streptococci.<sup>6</sup> Since copolymerization should immobilize MDPB in adhesive material, adhesives containing MDPB may be effective against bacteria that invade through micro leakage. Clearfill protect bond with antibacterial properties which also releases fluoride was developed.<sup>7</sup> The self-etching primer in this system contains the anti-bacterial monomer Methacryloyloxy Dodecyl Pyridinium Bromide (MDPB).<sup>8</sup>

## MATERIALS & METHODS

**Tested materials:** Three Self etching adhesive systems were; Clear fill protect bond (Kuraray), Adper Prompt (3M ESPE), and Xeno 111 (Dentsply). All these Materials were handled and polymerized according to the manufacturer's instructions.

**Tested Micro organism:** We used Streptococcus mutans (MTCC 890), the primary etiological agent for caries to test the antibacterial activity of self etching adhesive system. Streptococcus mutans (obtained from Microbiology Institute Chandigarh) is naturally resistant

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to bacitracin. We grew *S. mutans* aerobically from frozen stock cultures in brain heart infusion.

### EXPERIMENTAL DESIGN

A single isolated colony of *Streptococcus mutans* from blood agar plate is inoculated in brain heart infusion broth for 4-6 hrs. The broth is adjusted to 0.5 Mc Farland standards ( $1.5 \times 10^8$  CFU/ML). 15 brain heart infusion plates were swabbed with streptococcus mutans and four 6mm wells were punched in each plate. The plates were divided into 3 groups.

**Group A.** The wells were filled with Clearfill protect bond. Liquid A,( primer) was applied to the well with the help of a disposable brush and left in place for 20 secs. After this liquid B (bond) was applied to an entire surface of well with a disposable brush. To create a uniform bond film a gentle oil-free air flow was used, and light cured for 10 secs as per manufacturer instructions.

**Group B.** The wells were filled with Adper Prompt. 1 drop of liquid 1 was mixed with 1 drop of liquid 2 in mixing well. The mixture was then applied to the well made in a plate with the help of disposable brush for 15secs. A gentle stream of air was used to thoroughly dry the adhesive and light cured for 10 secs as per manufacturer instructions.

**Group C.** The wells were filled with Xeno 111. 1 drop of Liquid A was mixed with 1 drop of liquid B in a mixing well. The mixture was then applied to the well made in the plate with the help of disposable brush for 20 sec. A gentle stream of air was used until there is no more flow at least for 2 sec. Cure for 10 sec. as per manufacturer instructions.

The plates were then incubated at 37-degree centigrade for 24 hrs. Antibacterial actions of these adhesive materials were tested by measuring the zones of inhibition as per the groups. Reliability of the results is confirmed by repeating each test twenty times.

### STATISTICAL ANALYSIS

All the values were expressed in the form of mean SEM. The antibacterial activity of each dentin bonding agent was analyzed statistically using the Kruskal wallis test with a level of significance set up  $P < 0.01$ .

The significance between groups was done using unpaired student 't' test.

## RESULTS

The mean inhibition zone diameter (mm) according to agar diffusion test for the study groups are shown in table 1. Clearfill protect bond was found to be the most antibacterial material, followed by Adper and Xeno 111 against streptococcus mutans( Table 1).

On comparing Mean and SD **Group A** (Figure 1)clearfill protect bond ( $19 \pm 1.1239$ ) showed maximum zone of inhibition followed by **Group B** (Figure 2) Adper protect ( $10.7 \pm 0.7327$ ) and **Group C** (Figure 3)Xeno111 ( $10.2 \pm 0.8335$ ) showed fewer inhibition zones .

Groups	Mean $\pm$ SD	SEM
Group A Clearfill protect bond	$19 \pm 1.1239$	0.2513
Group B Adper prompt	$10.7 \pm 0.7327$	0.1638
Group C Xeno 111	$10.2 \pm 0.8335$	0.1864

Table 1: Mean  $\pm$  standard deviation and standard error of mean between the groups

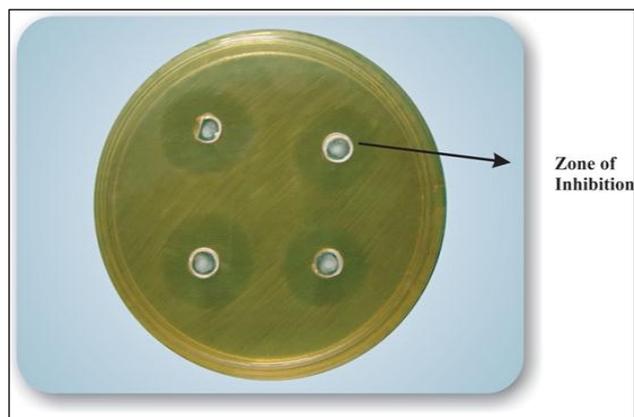


Figure 1 : Showing zone of inhibition in Group A (Clearfil Protect bond )

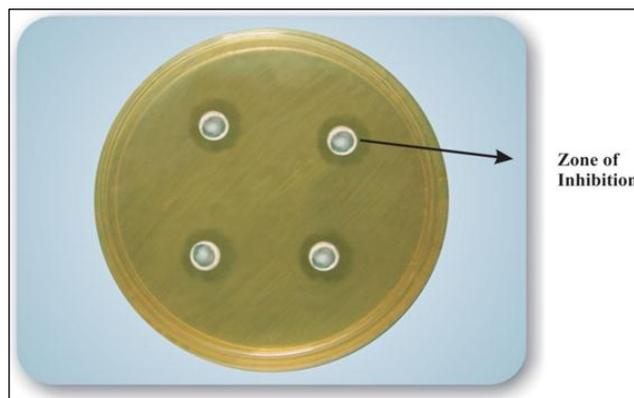


Figure 2 : Showing zone of inhibition in Group B (Adper Bond )



Figure 3 : Showing zone of inhibition in Group C (Xeno 111 )

## DISCUSSION

It is well known that residual bacteria in the prepared cavity can induce recurrent caries and damage to the pulp.<sup>9</sup> In addition, the main cause of unpleasant symptoms including hypersensitivity is, the bacterial invasion through the micro gap between a composite restoration and the cavity wall. Therefore, it is important to avoid the harmful effects of residual and invasive bacteria if a successful restoration is to be obtained. Hence, materials with antibacterial properties have developed a concern.<sup>10</sup>

In self etching adhesive systems, the pH value is sufficiently low in primer solution to demineralize the smear layer and underlying dentin surface, so etching and priming of the cavity can be accomplished simultaneously. Therefore, the separate acid etching step is generally omitted. Because of the nonrinsing procedure, residual bacteria may remain at the interface between the tooth and restorative material. The dentin primer is the component that comes in contact with the dentin substrate at the first stage of restoration in an adhesive system. If Tooth conditioners such as primers, possessed antibacterial activity, these bacteria could be eliminated, thereby preventing secondary caries. Thus the antibacterial activities of these adhesive system primers, play a significant role in the longevity of the restoration.<sup>11</sup>

In the present study Adper prompt and Xeno 111 showed limited antibacterial effect against streptococcus mutans. The inhibitory action of Adper prompt and Xeno111 has been previously confirmed in conjunction with streptococcus mutans. It has been reported that the pH value of this adhesive is 2.0 or lower. As bacteria cannot survive in an extremely low pH environment, the acidic property of the primer might be effective enough to kill or at least inactivate the bacteria.<sup>11</sup> In our study, Clearfill protect bond produced maximum inhibition zones for all samples. Clearfill protect bond is a two-step self-etching adhesive with antibacterial monomer MDPB.

The reason for the prolonged antibacterial effect of Clearfill protect bond can be related to the antibacterial properties of the MDPB molecule, which is a quaternary ammonium derivative synthesized by combining dodecylpyridinium bromide with a methacryloyl group.<sup>12</sup> The mechanism of the antibacterial activity of quaternary ammonium compounds is believed to be due to their cationic and hydrophobic binding to cell wall components that disturbs membrane function and subsequently, induces leakage of cytoplasmic material (Figure 4).

The antibacterial MDPB molecule has a special configuration containing a positive charge of pyridinium ion which can produce loss of electrical imbalance in bacteria, causing cell wall destruction ultimately leading to cell death.<sup>13</sup> The antibacterial properties of MDPB, as well as its biocompatibility with oral tissues, have been scientifically tested and show considerable promise for providing dentistry with the third dimension of restorative dentistry where biological aspects may be considered.

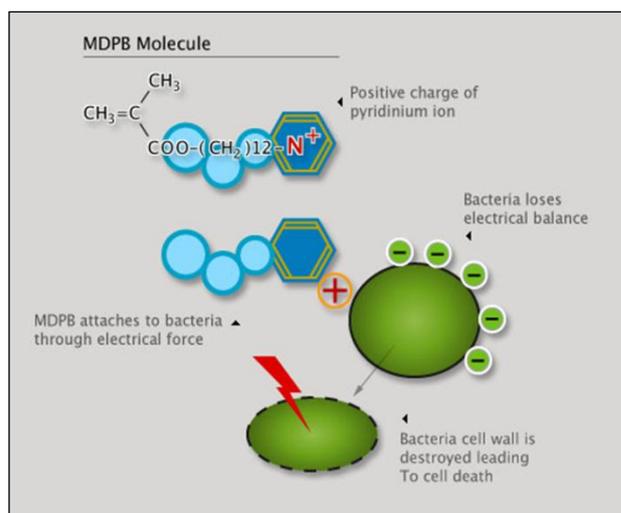


Figure 4: Mechanism of action of MDPB

## CONCLUSION

Elimination of microbes is the main goal in order to prevent and treat pulpal and periapical breakdown.<sup>14</sup> The incorporation of these antibacterial agents, into dentine bonding agents have become an important factor in inhibiting residual bacteria in the oral cavity. Thus these self-etching adhesives which contain MDPB could be recommended in situations where total disinfection of cavity is not accomplished due to their antibacterial activity.

The following conclusions were drawn from the present study:

1. Clearfill protect bond showed the maximum antibacterial activity when compared to Adper prompt and Xeno 111.
2. Both Adper prompt and Xeno 111 showed limited antibacterial activity.
3. The addition of MDPB into self-etching primer exerts potential antibacterial effect against streptococcus mutans.
4. The zone of inhibition against streptococcus mutans was maximum for MDPB containing primer when compared with other self-etching primers.

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