Original Article

Comparative evaluation of antibacterial activity of total-etch and self-etch adhesive systems: An *ex vitro* study

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Abstract

Aim: The aim of this *ex vivo* study was to compare the antibacterial activity of total-etch and self-etch adhesive systems against *Streptococcus mutans, Lactobacillus acidophilus,* and *Actinomyces viscosus* through disk diffusion method.

Materials and Methods: The antibacterial effects of Single Bond (SB) and Adper Prompt (AP) and aqueous solution of chlorhexidine 0.2% (positive control) were tested against standard strain of *S. mutans*, *L. acidophilus*, and *A. viscosus* using the disk diffusion method. The diameters of inhibition zones were measured in millimeters. Data was analyzed using Kruskal-Wallis test. Mann-Whitney U test was used for pairwise comparison.

Result: Of all the materials tested, AP showed the maximum inhibitory action against *S. mutans and L. acidophilus*. Aqueous solution of chlorhexidine 0.2% showed the maximum inhibitory action against *A. viscosus*. Very minimal antibacterial effect was noted for SB.

Conclusion: The antibacterial effects observed for the tested different dentin bonding systems may be related to the acidic nature of the materials.

Keywords: Antibacterial activity; *actinomyces viscosus*; dentin bonding systems; *lactobacillus acidophilus; streptococcus mutans*

INTRODUCTION

The bonding of resin-based composite to dentin can be accomplished by means of etch-and-rinse or self-etch adhesive systems. The etch-and-rinse technique has been considered sensitive.[1] Incomplete resin infiltration and evidence of phase separation within resin-dentin interfaces and its detrimental effects have been demonstrated.[1,2] Self-etching dental adhesives have been developed to simplify bonding procedure and to make their application less time-consuming. In two-step systems, the primer and adhesives are combined into one solution unlike the one-step systems — the so-called all in one adhesives — the etchant, primer, and adhesives are combined into one solution. The bond strength of the all-in-one adhesives may be lower than those of the two-step systems.[3] Despite the recent advances in adhesives, polymerization shrinkage, and resultant contraction gaps at the tooth-restoration interface continue to be a significant problem in restorative dentistry. Gap formation can be observed between the adhesive resin and the primed dentin or between the adhesive resin and the hybrid layer. The cariogenic bacteria in dental biofilm may invade along the microgaps and generate secondary caries, which have been considered as the most common reason for the replacement of restoration.[4,5] Residual bacteria in the oral cavity may also increase the risk of developing recurrent caries. This is due to the contamination of smear layer, which is partially incorporated into the hybrid layer.[2,3,6] Therefore, the antibacterial properties of adhesive materials are beneficial in the eradication of residual bacteria in the oral cavity.[7] Root caries is a growing problem in older adults as the incidence of lesion increases due to root exposure. Roots are susceptible to acid dissolution and the reduced saliva flow in seniors reduces the buffering and acid neutralization capacity via saliva in the oral environment. Therefore, antibacterial restorations are especially needed and beneficial for seniors to combat biofilm growth, acid production, and recurrent caries. Hence, the aim of the

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present study is to assess the antibacterial activity of total-etch and self-etch adhesive systems.

MATERIALS AND METHODS

The present study is a laboratory investigation to determine the antibacterial efficacy of etch-and-rinse and self-etch adhesive systems against *Streptococcus mutans, Lactobacillus acidophilus*, and *Actinomyces viscosus.* The study was conducted in the Department of Microbiology, Father Muller's Medical College, Mangalore.

Tested materials

Materials used

Two commercially available dentin bonding agents were used in the study [Table 1]. All materials were handled and polymerized in strict compliance with the manufacturer's instructions.

The organisms selected for sensitivity tests in the present investigation were lyophilized stock cultures of *S. mutans, L. acidophilus* and *A. viscosus*, obtained from Department of Clinical Microbiology, Christian Medical College, Vellore.

Method

Freeze-dried stock cultures of bacteria were subcultured in Robertson's Cooked Meat (RCM) medium to provide anaerobic conditions.

The purity and viability of the bacteria were confirmed by inoculating them into brain heart infusion blood agar supplemented with vitamin K and hemin and incubating for 24-48 h under anaerobic conditions.

Anaerobic environment was created using Anaerobic System Mark II and Anaero Pack (Hi Media Pvt Ltd) (Anaero – 3.5 L is a disposable oxygen-absorbing and carbon dioxide generating agent for use in anaerobic jars) and incubating for 48 h at 37°C.

After incubation, isolated bacterial colonies of the stock culture were suspended in sterile Brain Heart Infusion Broth until the turbidity was compatible with 0.5 Mac Farland. This scale allowed the estimation of the bacterial concentration of the suspension by its turbidity (0.5 corresponding to a concentration of 1.5 × 108 (bacteria/ml) at an opti density of 550 nm.

Three Mueller Hinton Blood Agar plates were swabbed with 100 µl of *S. mutans*, *L. acidophilus*, and *A. viscosus*, respectively. Sterile Whatman no. 1 filter paper disks (6 mm diameter, 1.5 mm thick) were saturated with 50 µL of each bonding agent, after which photo polymerization was performed using a light source (3M ESPE) for 20 s.

The specimen disks were placed on a plate using sterile tweezers. 0.2% aqueous solution of chlorhexidine (added onto paper disk and tested as described for bonding agent) was used as the positive control in the same plate.

After anaerobic incubation of the plates at 37°C for 48 h, the diffusion of antibacterial components was determined using the inhibition zone produced around the paper disks.

The diameter of the inhibition zone was measured (in millimeters) in three locations and the average was calculated. The antibacterial activity of each dentin-bonding agent was tested by Kirby-Bauer disk diffusion technique.

The reliability of the result was confirmed by repeating the experiment 12 times using each bacterial strain.

Criteria for evaluation

The antibiotic sensitivity pattern, represented as the zone of inhibition, appeared around each disk.

Hi antibiotic zone scale is used for this purpose and measurement of each zone is recorded in millimeters.

A total of 36 experiments were carried out to evaluate the result.

Statistical analysis

The antibacterial activity of each dentin-bonding agent was analyzed statistically using the Kruskal-Wallis test, with a level of significance set up at $P < 0.05$.

Intermaterial comparisons in conjunction with each bacterial species were statistically analyzed using the Mann-Whitney U test with the same level of significance.

Table 1: Dentin bonding agents used in the present study

Dentin bonding system	Code	Composition	Type
Single Bond (3M Dental Products Division, St Paul, MN)	SB	Priming resin Bis-GMA, HEMA, polyalkenoic acid, water, ethanol, dimethacrylates	3 step, total etch, pH-4.5
Adper Prompt (3M ESPE, Germany)	PR	Liquid 1 Methacrylated phosphoric esters, Bis-GMA, initiator based on camphorquinone, stabilizer Liquid 2 Water, HEMA, polyalkenoic acid, stabilizer	Two bottle, one step, self-etch, pH-0.4

Bis-GMA: Bisphenol A-diglycidyl methacrylate, HEMA: 2-hydroxyethyl methacrylate

Type of statistic software version used for analysis was Statistical Package for Social Sciences (SPSS) version 17 (SPSS Inc, Chicago, IL, USA).

RESULTS

In the present study all the six experimental groups showed positive inhibiting zone against the microorganisms tested which is demonstrative of the antibacterial property of the bonding agents.

Among the material tested, Single Bond (SB) did not demonstrate any noticeable antibacterial action against *S. mutans* and *A. viscosus*.

Adper Prompt (AP) demonstrated higher antibacterial effect than 0.2% chlorhexidine for all microorganisms tested ($P < 0.05$) except *A. viscosus.*

AP produced the largest zone of inhibition against *S. mutans* and *L. acidophilus* and 0.2% chlorhexidine produced the largest inhibition zone for *A. viscosus.*

According to Kruskal-Wallis test when comparing SB and AP with the positive control, there is highly significant difference in the mean inhibition zone among the three groups in relation to *S. mutans* as seen in Table 2.

When comparing SB and AP with the positive control, there is highly significant difference in the mean inhibition zone among the three groups in relation to *L. acidophilus* as seen in Table 2.

When comparing SB and AP with the positive control, there is highly significant difference in the mean inhibition zone among the three groups in relation to *A. viscosus* as seen in Table 2.

According to Mann-Whitney U test, as seen in Table 2 it is clearly seen that the difference in the antibacterial activity is very high when we compare control vs SB and SB vs AP; whereas, control vs AP is found to be not significant in relation to *S. mutans*.

In relation to *L. acidophilus* the difference is very highly significant when we compare SB vs AP and significant with SB vs control; whereas, AP vs control is found to be not significant as seen in Table 2.

In relation to *A. viscosus*, a very highly significant difference is seen when comparing SB/AP and SB/control and significant difference is seen with AP/control as seen in Table 2. The antibacterial activity of the materials tested and the control against *S mutans*, *L acidophilus* and *A viscosus* respectively.

DISCUSSION

A large body of *in vitro* and *in vivo* data supports the belief that caries only occurs in the presence of microorganisms. However, which bacteria are the primary etiologic agents in various types of dental caries is not completely clear.

Pit and fissure caries are the most common human lesions. Of several species of bacteria isolated from these lesions *S. mutans* and Lactobacilli are suspected as etiologic organisms. The smooth surface lesion has a well-characterized microbiology. The most consistently found organisms in this lesion are gram positive facultative cocci, specifically *S. mutans* and *S. salivarius*.[2,5]

Dentinal caries exhibit somewhat different microbial ecology related to its location. Organisms growing here are more anaerobic. The most commonly found pathogens in this region are Lactobacilli.[5] An important ecological feature of these bacteria is their ability to produce lactic acid and to grow in a relatively acidic environment created by their fermentation. There are studies that have reported significant correlation of Lactobacilli in root surface plaque and root caries lesion. Histological analysis of deep dentinal caries also has shown the presence of Lactobacilli.[8,9]

Plaque sampling studies have identified gram positive filamentous Actinomyces species as the predominant bacteria for the development of root caries. Animal studies in rodents have supported this finding and bacteria such as *A. viscosus* were known to cause cemental caries in these animals.[8-10] The Federation Dentaire Internationale (1962) defined secondary caries as a 'positively diagnosed carious lesion, which occurs at the margins of an existing restoration'. The lesion usually consists of two carious

Table 2: Group and intergroup comparison

	S. mutans Mean (SD)	*L. acidophilus* Mean (SD)	*A. viscosus* Mean (SD)
SB	2.75 (4.115)	9.000 (5.96962)	3.1667 (4.70654)
AP	13.92 (4.055)	19.9167 (6.81520)	12.5000 (1.78377)
Control	10.33 (4.924)	13.4167 (5.08935)	15.2500 (3.27872)
Kruskal-Wallis test	18.859	18.25	25.92
P-value	0.001	0.001	0.001
SB vs AP	*P*=0.001	*P*=0.001	*P*=0.001
SB vs control	*P*=0.001	*P*=0.05	*P*=0.001
AP vs control	*P*=0.518	*P*=0.004	*P*=0.031

SD: Standard deviation, SB: Single bond, AP: Adper prompt

regions: An 'outer lesion' formed in the enamel or cementum of the tooth surface, similar in histology to a primary lesion, and a wall lesion which is a narrower defect in the enamel and or dentine along the cavity wall-restoration interface.[11]

Secondary caries is one of the major reasons of restoration failure. Accumulated data from numerous studies have demonstrated that the durability of dental restorations is shorter than generally expected and secondary caries is a major reason for the replacement of resotratrions.[11-13] It has been estimated that the removal and replacement of restorations because of secondary caries occupies as much of the dentist's time as the treatment of primary lesions;[11] at present the prevalence of secondary caries could be higher than the prevalence of primary caries among adults.[11] Secondary caries is responsible for the failure of more than 50% of amalgam and approximately 40% of composite restorations.[14]

Microleakage is considered to be one of the most important factors which results in secondary caries and thus responsible for clinical failure of restorations.[15] Microleakage is the passage of bacteria and their toxins between restoration margins and tooth preparation wall.[16] Several studies have demonstrated that after insertion of restorations, bacterial infiltration is common.[14] Similar observations have been made by other investigators using new adhesives materials such as glass ionomer cements and dentin-enamel bonding agents under composites and amalgams. Bacteria have been detected along restoration interfaces and inside dentin.[14]

In the present study very minimal antibacterial effect was noted for SB against all the bacteria tested. However, acid etching accomplished before using this adhesive in clinical conditions and the antibacterial effects of the acid may compensate for the inadequacy of SB. This is supported by a study in which phosphoric acid etchant was shown to produce zones of inhibition against *S. mutans*.[17] On the other hand, this antibacterial effect works as a so-called "contact disinfectant", that is, it destroys the bacteria that come in contact with the surface.[18] The pH value of SB is 4.5, and this may explain why SB showed minimal inhibitory effects against the bacteria used in our study. In another study, SB showed no inhibition zones against *S. mutans* and *L. casei*. This was mainly due to the pH which was not acidic enough to prevent *S. mutans* and *L. casei* to maintain their metabolism.[19] This observation is in line with the results reported by Atac A S *et al*.,[20] (2001) and Baseren *et al*.[18]

Self-etching adhesive has been introduced in an attempt to simplify the clinical application procedure and to reduce the technique sensitivity and risk of the primed surface being contaminated. In self-etching adhesive systems, the pH of self-etching primer solution is sufficiently low to demineralize the smear layer and underlying dentin surface, so that etching and priming of the cavity can be accomplished simultaneously.[21] Therefore, separate acid-etching step is generally omitted. Because of the non-rinsing procedure, residual bacteria may remain at the interface between the tooth and the restorative material.[2] The dentin primer is the component that comes into contact and reacts 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 the secondary caries. So the antibacterial activity of these adhesive systems primers, which are directly applied to the dentin, plays an important role in the longevity of the restoration.[2,18]

In the present study, AP showed antibacterial effect to all the bacteria tested. AP displayed statistically significantly higher antibacterial effect than chlorhexidine ($P < 0.05$), against *S. mutans* and *L. acidophilus*. The inhibitory action of AP has previously been confirmed in conjunction with *S. mutans* and *L. acidophilus* by similar studies and they have reported that the pH value of the adhesive is 2.0 or lower.[18] Addition of acidic monomers in large amounts for self-etching adhesive systems to promote adhesion, decreases the pH values of these materials.[5,22] 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.[18] But AP has displayed a lower antibacterial activity than chlorhexidine against *A. viscosus*. As the present *ex vivo* study is a limited one, the variable antimicrobial activity exhibited by the tested materials against *A. viscosus* is uncertain. According to a study, Lactobacilli are the most acid-tolerant species.[23] In our study both the materials showed zones of inhibition against *L. acidophilus*. Further research is required in this field regarding the mechanism of antimicrobial action of these agents and the virulence factors in these oral pathogens incorporating significant number of samples.

The pH values of dentin adhesive required to completely kill the organisms in 3 h period, were 2.3 for the *L. casei* and 3.0 for *S. mutans*.[6,24] Bactericidal effects observed for primers mainly arise from their acidic properties.[22] So the growth inhibitory effect of this adhesive could be attributed to its low pH. On the other hand, the pH value of the bonding systems increases after contact with dentinal substrate due to buffering action. The solution is diluted by dentinal fluid as it works its way into the deeper areas of the lesion and subsequently reduces its antibacterial effect.[6] Therefore, for successful restoration, it is beneficial to provide a bonding system with intrinsic antimicrobial properties such as a primer that contains methacryloyloxydecylpyridinium bromide (MDPB) or a cationic monomer, methacryloxylethyl cetyl dimethyl ammonium chloride (DMAE-CB).[1,3,4,7,19,25,26]

They are incorporated into the dental adhesives without affecting the bonding efficiency and biocompatibility of the parental adhesives.

A 0.2% chlorhexidine was included as a positive control material as it a very potential antibacterial agent and also because of its widespread clinical use it serves as a common point of reference for comparisons with other studies.[1,5,19,26,27] In this study, 0.2% chlorhexidine showed a very highly significant antibacterial activity against *A. viscosus* compared to AP and the mean inhibition zone was less than AP in relation to *S. mutans* and *L. acidophilus*.

Different methods have been used to investigate the antimicrobial effects of dental materials. The agar-diffusion method was used in this study because it is a simple and easy way to determine the antibacterial effects of a liquid substance and also it has been used in previous studies.[22] The zone of growth inhibition provided by the materials depends on the toxicity of the material against the bacteria tested, and on the diffusibility of the material across the culture medium used. So, although the agar diffusion test is easier to perform, the inhibitory properties of solid materials placed on agar surfaces are dependent upon the hydrophilicity of the material to wet the agar surface.[5] A material that diffuses more easily, in addition to its direct cytotoxicity, will provide larger zones of inhibition. This facilitates comparison of our data with those from the literature.[1,3,19,22,26,28-31]

CONCLUSION

Under the limitations of this *ex vivo* study, AP displayed the highest bacterial inhibition than 0.2% chlorhexidine and SB against *S. mutans* and *L. acidophilus*. Chlorhexidine showed better antibacterial effect than AP in relation to *A. viscosus*. However, it needs to be determined whether the growth inhibiting effects of dentin adhesives observed in this study would be similar *in vivo*.

Long-term effect might be useful in case of future microleakage and bacterial infiltration. Therefore, long-term clinical studies are necessary to determine whether antibacterial effects of the bonding systems observed *ex vivo* are sufficient to increase the longevity of dental restoration.

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