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ORIGINAL ARTICLE

Antibacterial Activity of Dopamine Incorporated Total Etch Adhesive System

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ABSTRACT

Aim: To evaluate the antibacterial efficacy of dopamine incorporated total-etch adhesive against *Streptococcus mutans* (*S. mutans*).

Materials and methods: *S. mutans* were cultured and inoculated in Müeller–Hinton agar plates. Round wells of around 6 mm diameter were created in the center of the agar plates. The experimental groups consisted of agents as follows: group I (DOPA 3%), group II (DOPA 3% + Bonding agent), group III (Bonding agent) and group IV (no material). For each group, 12 plates were used to evaluate the zone of inhibition using agar well diffusion method. All the experimental agents were added to respective wells in agar plates and incubated at 37° C for 24 hours. The diameter of a zone of inhibition around each well was recorded. Results were tabulated and analyzed statistically using the Kruskal–Wallis test. and Mann-Whitney U-test.

Results: Based on the mean diameters, group II showed the maximum zone of inhibition, and it exhibited statistically higher antibacterial activity than group III and group I ($p < 0.01$).

Conclusion: Within the limitations of this study, dopamine incorporated total-etch adhesive system exhibited significantly higher antibacterial activity than conventional adhesive system against *S. mutans*.

Clinical significance: Dopamine can be a promising antibacterial additive to dental adhesive systems to improve both biological seal and bond strength at the resin-dentin interface.

Keywords: Antibacterial, Dopamine, *S. mutans*, Total etch adhesive

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INTRODUCTION

Composite resins are the most commonly used direct tooth-colored restorations. Marginal integrity is one of the factors that decide the longevity of these restorations. Even though the marginal seal is achieved by micromechanical adhesion through etching and bonding procedures, secondary caries at the resin-tooth interface continues to be the most common failure of composite restorations.[1]

Also, polymerization shrinkage and resultant contraction gaps can be associated with the encroachment of cariogenic bacteria at the resin-dentin interface from the saliva and smear layer which also accounts for the occurrence of secondary caries.[2] Even a perfect resin-dentin interface can fail over a period due to hydrolytic and proteolytic degradation of the hybrid layer.[3] Recent research focuses on the development of dental adhesives with antibacterial, water compatible and esterase resistant ability along the resin-dentin interface to enhance both the biological and mechanical integrity of resin-dentin bonding.

The acid component of etch and rinse adhesives with low pH have demonstrated antibacterial activity.[4] However, certain bacteria are acid-resistant, and the buffering capacity of dentin can neutralize the effects of the acid.[5] Various studies have been reported on the inability of total etch adhesives to inhibit bacterial growth or secondary caries.[6]

Literature shows various attempts at incorporation of antibacterial components such as fluorides, antibiotics methacryloyloxy dodecyl pyridinium bromide (MDPB), dimethylamino dodecyl methacrylate in dentin adhesives.[7-9] Tziafas et al. reported that MDBP containing adhesives maintained pulp vitality but interfered with reparative dentin formation in exposed, infected pulp.[10] In addition, these resins do not affect biofilm formation or initial adherence of *S. mutans*.[11-13] Thus, there is a need for a unique adhesive to provide long-lasting antibacterial activity, increased bond strength, and durability.

Dopamine (DOPA) is a synthetic mimetic of mussel adhesive protein. Polydopamine is spontaneously formed by the oxidative polymerization of dopamine in aqueous solutions.[14] It is a small molecule compound that contains both 3, 4-dihydroxy-L-phenylalanine and amine. It shows a good adhesive property to various organic and inorganic substrates under wet conditions.[15] The adhesive property of mussels to the underwater surface is mostly due to the exhaustively repeated units of DOPA

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and amine.[16] Nowadays, it is commonly used as surface modifiers to most of the medical appliance. Catechol-functionalized synthetic polymer of dopamine has been showed to increase the bond strength between dentin and resin material.[17] It also exhibits potent antibacterial activity against most common oral pathogens. Polydopamine Induced-polyethylene glycol coating proved to control and inhibit the cariogenic plaque formation on root surface.[18]

Both the adhisive and antibaterial characteristic of DOPA had lead to the new concept of incorporating this as a potent ingredient to the dental adhesive system. As S. mutans plays a vital role in the initiation and progression of caries, this study aimed to preliminarily evaluate the antibacterial effect of polydopamine incorporated total-etch adhesive system against *S. mutans*.

MATERIALS AND METHODS

Preparation of Experimental Adhesive System

Dopamine (DOPA) powder (Sigma Aldrich H8502,10 G) of 2 mg was added in Tris Buffer solution (1 mM) to 1 mL to form polydopamine. The optimal percentage of dopamine to be added to etch and rinse was determined by a pilot study of using 1, 3, 5 and 10% wt% of dopamine incorporated bonding agent (BA). We evaluated the degree of conversion using Fourier transform infrared spectroscopy and found that addition of upto 3 and 5 wt% of dopamine did not have any effect on polymerization. Hence, 3 wt% was selected to be added to fifth-generation bonding agent (total etch–Te-Econom Bond, Ivoclar Vivadent) vial of 2 mL to obtain a mixture of 3wt%. The vial was kept in a cyclomixer (Sigma scientific instruments, India) for 1 minute to obtain a uniform mix.

Inoculation of *Streptococcus Mutants* and Agar Diffusion Test

Round wells of around 6 mm diameter were created in the center of the agar plates by using a sterile conventional punch. Inoculation was performed by dipping a sterile swab in the *S. mutans* broth which was standardized according to 0.5 McFarland standard and then brushing across the culture media in a zigzag fashion starting from the left top end and ending at the right bottom end. Then turning the plates clockwise and repeating the same so that the inoculation is uniform throughout the culture plate.

The experimental groups consisted of groups as follows: group I (DOPA 3%), group II (DOPA 3% + Bonding agent), group III (Bonding agent) and group IV (no material). For each group, 12 plates were used to evaluate the efficacy. Specimens for each group were prepared by light curing 1 mL of the bioactive[6] (BA) in a dispensing well for 20 seconds, the created wells of each culture plate were filled completely with the experimental cured adhesives. Then, the inoculated agar plates were incubated aerobically for 24 hours at 37°C.

Measurement of the Zone of Inhibition

After 24 hours of incubation at 37°C, the inoculated agar culture plates were analyzed for the zone of inhibition under proper illumination. The zone of inhibition was seen as a round to oval clear area around the central well devoid of any bacterial growth. For measuring the zone of inhibition for each culture plate, the shortest diameter of the inhibition zone was measured as D1, and the longest diameter was measured as D2, and the average of the two was recorded as the "diameter of the zone of bacterial inhibition." The measurements were charted in Table 1 for each group and statistically analyzed using the Kruskal–Wallis test. Mann–Whitney U test was used for pairwise comparison.

RESULTS

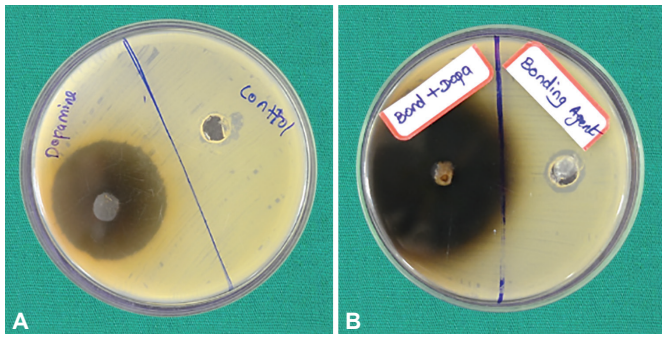
All the groups resulted in a statistically significant difference in the zone of bacterial inhibition. Negative control showed no zone of inhibition. Based on the mean diameters, group II (Fig 1) showed the maximum zone of inhibition, and it exhibited statistically higher antibacterial activity than group III and group 1 ($p < 0.01$).

DISCUSSION

The recent development of adhesives is evolving with antibacterial additives. An antibacterial adhesive should inhibit the biofilm accumulation on the material surface and the restoration interface to prevent the detrimental effects such as pulp damage, hypersensitivity, and recurrent

Table 1: Comparison of mean diameter (zone of inhibition) by various groups

Groups	*Mean Value (SD)*	*p-value*
Group	11 mm (1.44)	p < 0.01
Group II	21 mm (1.03)	
Group III	9 mm (2.03)	
Group IV	0 mm (0)	



Figs 1A and B: Zones of Inhibition of various groups

caries resulting from residual bacteria or microleakage. Thus, enhanced antibacterial action at the tooth-restoration interface will be highly beneficial. A tight biologic seal can improve the longevity of the restoration.

S. mutans was selected in this study for evaluating the antibacterial activity as it is one of the main causative pathogens for dental caries. *S. mutans* can metabolize carbohydrates to acids, leading to demineralization of the tooth structure and the tooth-restoration margins. Also, the bacterium can synthesize extracellular polysaccharides.[19,20] Therefore an antibacterial adhesive system against *S. mutans* could be a promising approach to prevent recurrent caries.

Although the antibacterial activity of adhesive materials is an important factor in terms of reduction of secondary caries, studies have shown that the incorporation of antibacterial agents could impair mechanical properties. Moreover, the release of the antibacterial substance from the material could result in further changes in physical properties of adhesive interface.[21]

The present study is first of its kind on the incorporation of antibacterial wet adhesive DOPA component in the dental adhesive system. The chemical structure of polydopamine lies in its ability to incorporate many functional groups such as catechol, amine, and imine. These functional groups can serve as attachment anchors with desired molecules.[22] Lee et al. evaluated the potential of catechol-functionalized modified polymer with Fe^{3+} additive improved the bond strength of commercial adhesive resin to the saliva contaminated dentin surface.[17]

The results of our study suggest that the total-etch used alone showed markedly low antibacterial effect than polydopamine incorporated. The pH value of Te-Econom (adhisive system) bond is very low (pH 1.7), and this can be attributed to the minimal inhibitory effects against the bacteria used in our study. In another study, a total-etch adhesive system with pH 2.5 showed no inhibition zones against *S. mutans* and L. *casei*.[23] This study result corroborates with the antibacterial activity of adhesive system reported by Atac et al.[24] and Baseren et al.[25]

The DOPA itself has intrinsic antimicrobial activity.[26] This could be the reason for the results of group I which showed 11 mm of the zone of inhibition. Maji et al. showed that antimicrobial activity of various commonly used antibiotics is enhanced against most of the virulent bacteria when combined with dopamine hydrochloride (HCL).[27] The presence of benzene ring in dopamine may be responsible for the antimicrobial activity. The mode of action of dopamine on gram positive and negative bacteria could restrict the growth of bacterial cells and prevent their further multiplication. Bacteria could be encapsulated with a thickness of 120 nm polydopamine shell as indicated by UV and fluorescence spectroscopy.[26]

The DOPA incorporated total-etch adhesive system showed statistically higher antibacterial activity than other groups. Thus, the DOPA-functionalized total-etch adhesive showed synergistic activity when compared to other groups. This could also be due to the local toxic effects of active groups such as phenolic hydroxyl/o-quinone and amino/imino on the outer membrane of bacterial cells by reducing the permeability to specific components that are necessary to bacterial cell survival or preventing metabolic waste from releasing.[28] Mei et al. proved that polydopamine-induced-polyethylene glycol could prevent cariogenic biofilm formation in the form of mouth rinse. He also suggested that it could be an additive to varnish to prevent biofilm adhesion on the tooth.[18]

As studies have proven that DOPA enhances wet adhesion also[29] DOPA incorporated total-etch adhesive system can be a promising antibacterial additive to dental adhesive systems to improve both biological seal and bond strength at the resin-dentin interface.

CONCLUSION

Within the limitations of this study, dopamine incorporated total-etch adhesive system exhibited significantly higher antibacterial action against *S. mutans.* Further, *in vivo* studies are needed to prove the antibacterial effect of this novel adhesive systems to increase the longevity of dental restorations.

CLINICAL SIGNIFICANCE

Dopamine (DOPA) can be a promising antibacterial additive to dental adhesive systems to improve both biological seal and bond strength at the resin-dentin interface.

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