Comparative evaluation of the effect of chlorhexidine and Aloe barbadensis Miller (Aloe vera) on dentin stabilization using shear bond testing

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Abstract

Introduction: The main objective of adhesive dentistry is to create an effective, durable union between the tooth structure and restorative material. However, degradation of adhesive dentine interface remains largely responsible for the relatively short lifetime of tooth colored resin restoration.

Aim: The aim of the study is to compare the dentin collagen stabilization property of Chlorhexidine (CHX) and Aloe barbadensis Miller using shear bond strength testing.

Materials and Methods: Occlusal reduction was done in sixty extracted human mandibular molars to expose the mid coronal dentin and divided into three groups \( n = 20 \). Following the surface pretreatment (Group 1 = control, Group 2 = CHX, Group 3 = Aloevera), dentine bonding agent and composite resin were applied and cured. The specimens were then subjected to shear bond strength testing.

Results: From the results analyzed, it was noted that there was statistically significant difference between the groups Control and CHX and Control and A. barbadensis Miller (\( P < 0.05 \)). However, there was no statistically significant difference between CHX and A. barbadensis Miller (\( P > 0.05 \)). Hence, the following result for the shear bond strengths to dentin was obtained: Control < CHX ≈ A. barbadensis Miller.

Conclusion: CHX and A. barbadensis Miller, as pretreatment agents of acid demineralized dentin collagen, has no adverse effect on the immediate shear bond strength of a two-step etch and rinse adhesive to dentin.

Keywords: Collagen cross-linking agents; matrix metalloproteinases; shear bond testing

INTRODUCTION

The success of restorative procedures depend on effective removal of infected dentin prior to the placement of the restorative material.[1] Failure to mechanically remove any carious portion and not being able to achieve complete disinfection may lead to microleakage, increased pulp sensitivity, pulpal inflammation, and eventually may cause secondary caries, thereby making it necessary to replace the restoration.[2] Therefore, application of cavity disinfectant before tooth restoration is gaining acceptance.

The main objective of adhesive dentistry is to create an effective, durable union between tooth structure and the restorative material.[3] Resin-dentin bonds obtained with contemporary adhesive systems can deteriorate over time.[4] The resin to dentin adhesion occurs through the infiltration into and polymerization of hydrophilic resins within the collagen matrix exposed through acid demineralization.

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decalcification of dentin, thus forming a hybrid layer. The durability of the dentin adhesive interface is directly related to the quality of hybrid layer and the hydrophilic nature of the adhesive. In case of etch and rinse adhesives, it is pertinent that the adhesive resin monomer penetrates the acid-etched exposed dentine collagen fibrils. But studies have revealed that this goal is seldom achieved.

Certain mechanisms have been proposed to improve dentin adhesion i.e., adjunctive collagen pretreatment, matrix metalloproteinases (MMP) inhibitors, etc. Different types of MMPs have been identified from human dentin, including MMPs 2, 3, 8, 9, 20. Moreover, cathepsin B is identified from sound and carious dentin. Both MMPs and cysteine cathepsins contribute to the degradation of denuded collagen within the hybrid layer.

Chlorhexidine (CHX) has been widely used as a cavity disinfectant because of its antimicrobial property. It also has an inhibitory effect on the MMPs (against MMPs 2, 8, 9) in the dentin. This effect can be useful in preventing collagen degradation and disintegration of the bonding interface over time.

In recent years, the potential for the inhibition of MMPs by substances derived from natural products has gained increasing attention. A. barbadensis Miller (Aloe vera) is a short succulent herb resembling a cactus, with green fleshy, spiny, and well margined leaves filled with a clear viscous gel. A. vera has potent antibacterial, antifungal, and antiviral properties. A. vera has been used to relieve thermal burn, sunburn, and promote wound healing and has antimicrobial activity and can help stimulate the body's immune system. The total leaf extracts contain anthraquinones. Recent study has revealed A. vera exhibits MMP inhibitory effect against MMP 2 and 9. Prabhakar et al. conducted a randomized clinical trial in 2015 and advocated the use of A. vera as cavity disinfectant.

There have been many researches to prove antimicrobial efficacy A. vera against caries causing microorganisms, i.e., Streptococcus mutans. Therefore, we intended to use A. vera as a cavity disinfectant in our study.

In order to use these agents for extending the longevity of resin-dentin bonds, it is necessary to first evaluate whether these agents interfere with the dentin bond strength following their use to pretreat the acid-etched dentin. The aim of our study was to evaluate A. vera for resin-dentin bond stabilization. This was the first study of its kind to test A. vera for resin-dentin bond stabilization.

**MATERIALS AND METHODS**

Sixty freshly extracted non carious human mandibular molars were thoroughly cleaned and stored in 0.1% thymol until use. The teeth were randomly divided into three groups n = 20. They were horizontally cut using diamond disk (Markus Ink., Michigan, USA) in a high-speed hand piece under air and water spray; the long axes of the teeth were perpendicular to the surfaces cut. After the removal of enamel, the mid coronal dentin was exposed. The dentine surface was examined for the lack of enamel or pulp tissue under stereomicroscope (Olympus; zoom type, Japan). The sections of the teeth including the roots were embedded in auto polymerizing acrylic resin to form cylinders 2.5 cm in diameter and 5 cm high. Dentin surfaces were flattened with 1200 grit silicon carbide paper under running water, so that a very smooth surface and to obtain a standardized smear layer.

Acid etching of the exposed dentine was performed for 15 s with 37% phosphoric acid gel (Scotchbond Etchant, 3M ESPE, St. Paul, MN, USA). In Group 1, the specimens were not treated with any cavity disinfectant and served as control. The teeth in experimental groups were treated with one of the following cavity disinfectants i.e.

**Group 2:** 2% CHX solution was prepared from dilution of 20% CHX solution using distilled water (Basic Pharma, Gujrat, India).

**Group 3:** A. barbadensis Miller (A. vera) solution was prepared using A. barbadensis powder of 99% purity and dissolving 20 mg of A. vera powder in 10 ml of distilled water.

The acid etched dentin was pretreated with 2% CHX in Group 2 and A. barbadensis Miller solution in Group 3 for 30 s, active application with a brush applicator (Microbrush International, WI, USA) and the excess removed with cotton pellet prior to the application of bonding agent (Adper single bond 2, 3M ESPE, St. Paul, MN, USA). Adhesive tape with a 3 mm diameter hole in it was used to define the bonding agent. The dentin surfaces of the teeth were then dried with air for 10 s, resin composite was applied in 5–6 increments (Filtek Z 350, 3M ESPE, St. Paul, MN, USA) with the aid of polyethylene tubes (3 mm diameter, 2 mm height and 0.5 mm thickness) and individually light cured for 40 s using light curing unit Spectrum 800 (Dentsply, Caulk, Milford, USA) with an output of 600 mW/cm². The tubes were then removed. The teeth were then stored in distilled water at room temperature for 24 h.

For shear bond strength testing, each tooth was secured in a specially designed attachment jig to hold the specimens to the universal testing machine (Instron, ADMET, Enkay Enterprises, New Delhi). Load was applied by the testing machine through a wire loop adjusted to the bonded interface at a cross head speed of 0.5 mm/min. Shear bond strength in MPa was calculated from the peak load at failure divided by the specimen surface area.

After testing, the fracture modes were evaluated under a stereomicroscope (Olympus, Zoom type) and classified according to the predominant mode of fracture as adhesive...
Table 1: Shear bond strength to dentin (mean±standard deviation)

| Dependent variable | n  | Mean  | SD    | SE    | 95% CI for mean Lower bound | Upper bound | Minimum | Maximum |
|--------------------|----|-------|-------|-------|-----------------------------|-------------|---------|---------|
|                     |    |       |       |       |                             |             |         |         |
| Control            | 20 | 18.9095 | 0.07742 | 0.01731 | 18.8733 | 18.9457 | 18.73  | 19.01   |
| CHX                | 20 | 20.6610 | 0.04667 | 0.01044 | 20.6392 | 20.6828 | 20.59  | 20.73   |
| Aloe               | 20 | 20.6615 | 0.04511 | 0.01009 | 20.6404 | 20.6826 | 20.59  | 20.76   |
| Total              | 60 | 20.0773 | 0.83472 | 0.10776 | 19.8617 | 20.2930 | 18.73  | 20.76   |

SD: Standard deviation, SE: Standard error, CI: Confidence interval, CHX: Chlorhexidine

Table 2: Comparison of immediate mean shear bond strength values (MPa) of all groups

| TR (I) | TR (J) | Mean difference (I−J) | SE | Significant | 95% CI | Lower bound | Upper bound |
|--------|--------|-----------------------|----|-------------|-------|-------------|-------------|
| Control | CHX    | −1.75150* 0.01845     | 0.00 | −1.7959    | −1.7071 |
| Aloe    | −1.75200* 0.01845 | 0.00 | −1.7964    | −1.7076 |
| CHX     | Control | 1.75150* 0.01845     | 0.00 | 1.7071     | 1.7959  |
| Aloe    | −0.00050 0.01845 | 1.00 | −0.0449   | 0.0439  |
| CHX     | 0.00050 0.01845 | 1.00 | −0.0439   | 0.0449  |

*The mean difference is significant at the 0.05 level. SE: Standard error, CI: Confidence interval, CHX: Chlorhexidine

Table 3: Failure modes of all test groups

| Description failures | Adhesive % | Cohesive % | Mixed % |
|----------------------|------------|------------|---------|
| Control              | 75         | 15         | 10      |
| CHX                  | 80         | 5          | 15      |
| Aloe vera            | 85         | 5          | 10      |

CHX: Chlorhexidine

Fracture at the resin-cement dentin interface, cohesive fracture in the resin cement, cohesive fracture in dentin, or mixed adhesive and cohesive fracture in the resin cement.

RESULTS

Data were analyzed using one-way ANOVA and post hoc Tukey test for multiple comparison between the groups. The test was done at a 0.05 level statistical significance [Tables 1 and 2].

From the results analyzed, it was noted that there was statistically significant difference between the groups Control and CHX and Control and A. vera (P < 0.05), specifically values of Control < CHX and Control < A. vera (P < 0.05). However, there was no statistically significant difference between CHX and A. vera (P > 0.05). Hence, the following result for the shear bond strengths to dentin was obtained: Control < CHX ≈ A. vera.

Fracture analysis indicated that most specimens showed adhesive fracture after 24 h [Table 3].

DISCUSSION

Acid etching creates a low pH which activates the dentin MMPs in the presence of zinc and calcium ions. It has been advocated that to improve the durability of restorations, pretreatment of acid-etched dentin with MMP inhibitors should be carried out.

CHX increased the immediate shear bond strength in this study, which was in support of the study by Brackett et al., 2009. In total etch adhesive resins, the use of CHX after etching prevents collagen fiber degradation and preserves the hybrid layer due to its inhibitory effect on MMPs, thereby increasing the bond strength. Yet few studies have showed that applying CHX before acid etching did not significantly affect the bond strength. This increase in the immediate bond strength can be attributed to the MMP inhibitory action of CHX.

The present study was conducted from agents derived from natural products that has been recently reported to possess anti-MMP potential, especially against MMP-2 and MMP-9. Aloe contains aloins and barbadoins as main chemical constituents. Its bactericidal activity is because of anthraquinone. The results of this study revealed that the application of A. barbadensis Miller to acid-etched dentine improves the longevity of resin dentin bonds by inhibiting MMPs.

The purpose of this study was to introduce new herbal product with possible potential for the inhibition of MMPs in order to maintain the dentin adhesive interface. The parameters compared were the control group (with no pretreatment) and CHX. The shear bond testing was performed only after 24 h of storage as this was the first study of its kind and it aimed to determine whether the tested concentration of the herbal product would be really effective and thereby conduct longitudinal studies. As if immediate bond strength was negatively affected by the use of A. vera, there would have been no point for conducting long duration studies.

Although the use of A. barbadensis Miller on acid-etched dentin prevents the degradation of collagen and improves the longevity of composite restorations, it is imperative that they do not
adversely affect adhesive bonding to dentin. As pretreatment with the herbal used was able to maintain immediate dentin bond strength, there is not enough evidence to reject the null hypothesis. The ability of this agent to improve the durability of resin-dentin should be evaluated in future studies.

**CONCLUSION**

Within the limitations of this *in vitro* study, it may be stated that the use of CHX or *A. barbadensis* Miller, as pretreatment agents of acid demineralized dentin collagen, has no adverse effect on the shear immediate bond strength of a two-step etch and rinse adhesive to dentin. Further, *in vitro* and *in vivo* studies are still warranted to evaluate the effect of *A. barbadensis* Miller for cavity disinfection.

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**Conflicts of interest**

There are no conflicts of interest.

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