

Effect of 2% Chlorhexidine on the Bond Strength of Direct Composite Restoration to Dentin: An *in vitro* Study

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ABSTRACT

Context: Etching of dentin results in demineralization and activation of the dormant matrix metalloproteinases (MMPs). If the dentin bonding agent subsequently applied fails to penetrate the full depth of this demineralized zone, their collagenolytic effect results in deterioration of the resin–dentin bond over time. Anti-MMP agents like chlorhexidine (CHX) have a potential role in preventing this degradation.

Aim: To evaluate the effect of 2% CHX as a rewetting agent on the bond strength of direct composite restoration to dentin.

Settings and design: *In vitro* study.

Materials and methods: Superficial dentin of the samples was exposed, etched, and divided into two groups. Group I specimens were rewet with water while CHX was used in group II. Composite cylinders of uniform dimension were built up and the specimens were subjected to shear bond strength testing immediately (at 24 hours) and after 6 months.

Statistical analysis: Independent t-test to determine whether differences if present were significant.

Results: Although the immediate shear bond strength values were higher for the CHX group, the difference was not statistically significant. After 6 months, the higher shear bond strength values were obtained for the CHX group and the difference was statistically significant.

Conclusion: Rewetting with 2% CHX has a beneficial effect on the resin–dentin bond over a 6-month storage period.

Keywords: Adhesion, Chlorhexidine, Composite resin, Matrix metalloproteinase, Shear bond strength.

Key messages: Reduction in bond strength subsequent to the time bound deterioration of the resin–dentin interface has been well documented. This study validates the role of CHX as a rewetting agent in addressing this problem. Modifications to the composite bonding protocol by integrating this philosophy have a promising role in future developments in adhesive dentistry.

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INTRODUCTION

Modern dental practice has shown a perceptible shift toward adhesive dentistry, with the advent of newer dental materials and a better understanding of the underlying mechanisms. The introduction of composites and the development of reliable dental adhesives have indeed marked the beginning of a new era with a reduced need for cavity extension, facilitating better conservation of healthy tooth structure. However, one of the main problems in that plaque adhesive restorations is the decrease in the resin–dentin bond strength over time.^{1,2} This results in a progressive increase in microleakage, marginal staining, and weakening of the restoration, eventually leading to failure.

Matrix metalloproteinases (MMPs) are members of a large subfamily of zinc and calcium-dependent proteolytic enzymes (proteinases) responsible for remodeling and degradation of ECM components. The acid-etching procedure activates endogenous MMPs present within dentin.^{3,4} A decreasing gradient of resin monomer diffusion within acid-etched dentin⁵ results in incompletely infiltrated zones along the bottom of the hybrid layer. These zones contain denuded collagen fibrils⁶ and are sites susceptible to degradation by the activated MMPs. It is well known that chlorhexidine (CHX) is an inhibitor of MMP activity *in vitro*.^{7,8} Hence, the concept of applying a layer of CHX to the dentin surface emerged during the course of tooth preparation for a direct composite restoration.

AIM

To evaluate the effect of 2% CHX as a rewetting agent on the bond strength of direct composite restoration to dentin immediately and after 6 months of storage in artificial saliva.

MATERIALS AND METHODS

The present *in vitro* study was conducted at Government Dental College, Thiruvananthapuram, Kerala in collaboration with the Biomedical Technology (BMT) Wing, Sree Chitra Tirunal Institute for Medical Sciences and

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Technology (SCTIMST), Poojapura, Thiruvananthapuram, Kerala, India. The duration of the study was 6 months. Institutional ethical committee clearance was obtained before proceeding with the study.

Sixty sound, caries-free human premolars, devoid of developmental anomalies, fluorosis, noncarious lesions, and other surface defects extracted for orthodontic purpose were collected from Department of Oral and Maxillofacial Surgery, Government Dental College, Thiruvananthapuram, Kerala. The samples were collected from patients in the 15 to 30 years age group.

Teeth Selection and Preparation

All the tooth samples were collected, stored, sterilized, and handled as per Occupational Safety and Health Administration (OSHA) and the Center for Disease Control and Prevention (CDC) recommendations and guidelines. Teeth samples were cleansed of saliva, blood, and visible debris and soaked in hydrogen peroxide to remove the connective tissue. They were then washed thoroughly with deionized water and used within 6 months of extraction. A flat and superficial dentin surface was exposed on each tooth after wet grinding the buccal enamel with a diamond disk. The enamel-free, exposed dentin surfaces were further polished with wet #600-grit silicon carbide paper for 60 seconds to standardize the smear layer.

Restorative Procedure

Sixty samples were divided into two groups of 30 samples each and prepared as follows:

Group I was taken as the control. The exposed dentin surface was acid-etched with 37% phosphoric acid for 15 seconds. After rinsing with water for 10 seconds and drying the etched surface, rewetting with water for 60 seconds was done, followed by gently blotting away any excess moisture present using a cotton pellet, resulting in a glistening surface. Adper Single Bond 2 (3M ESPE, St. Paul, MN, USA) was applied using an applicator tip. Two coats of dentin bonding agent were applied and gently air thinned for 5 seconds to evaporate the solvent. The adhesive was light cured with a halogen light set at 600 mW/cm² for 10 seconds.

Group II was taken as the experimental group. The same protocol as above was followed, but for the difference in rewetting agent 2% CHX gluconate [Aseprc, Anabond Stedman Pharma Research (P) Ltd.] was employed during the rewetting procedure.

After tooth sample preparation, composite resin (Filtek™ Z350 XT Universal Restorative, 3M ESPE) build-up was done on the bonded surfaces. Two increments of 1.5 mm each were placed into a brass ring of uniform dimensions (3 mm radius and 3 mm height)

placed onto the prepared tooth surface. Each increment was individually light cured for 40 seconds. All the bonding procedures were carried out by a single operator in a standardized environment.

From each group, half of the specimens were immediately subjected to a shear bond strength test after storage for 24 hours at 37°C and the other half after long-term storage of 6 months in artificial saliva.

Shear Bond Strength Testing

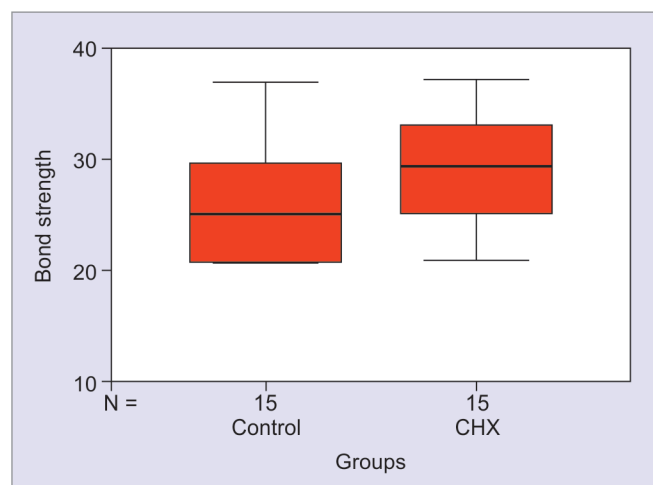
Shear bond strength was determined using a universal testing machine (INSTRON 3365, UK) at a cross-head speed of 1.00 mm/minute. The tooth sample was placed such that the tooth substrate–adhesive interface lay parallel to the direction of application of force. The force was applied by means of pulling a wire looped around the composite cylinder. Bond strength was calculated from the load applied and the area of the restoration as shear bond strength = load/area and expressed in megapascals (MPa).

Statistical Analysis

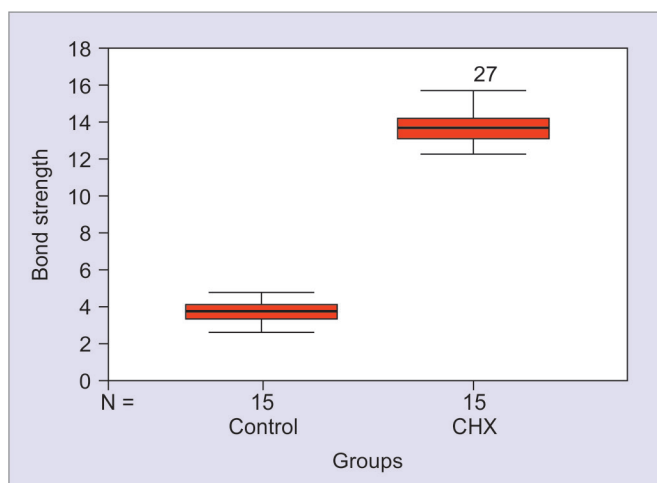
The data was tabulated using Microsoft Excel 2007 and analyzed using Statistical Package for the Social Sciences (SPSS) version 11.0 software. The mean and standard deviations were calculated for each group. The results were analyzed using independent t-test. The difference was considered statistically significant if the p-value obtained was <0.05. The intergroup comparisons were made using independent t-test.

RESULTS

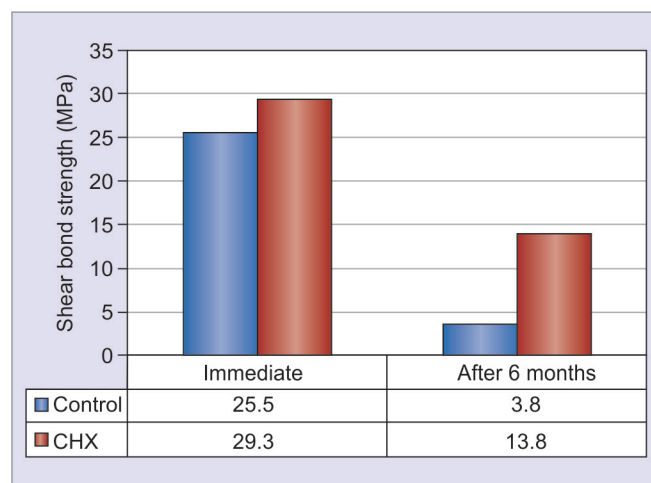
Graph 1 is a box plot showing the comparison between immediate shear bond strength values of the CHX and control groups. The mean immediate shear bond strength values were found to be 25.5 and 29.3 MPa for the control group and CHX group respectively. The difference in the values was not statistically significant.



Graph 1: Box plot of the immediate shear bond strength



Graph 2: Box plot of shear bond strength (after 6 months)



Graph 3: Comparison between shear bond strengths at the two time intervals within each group

Graph 2 is a box plot showing the comparison between mean shear bond strength values of the two groups after the 6-month storage period. The mean shear bond strength values were 3.8 MPa (control) and 13.8 MPa CHX. Considerable reduction in the bond strength values was observed in both groups over the 6-month period. Independent t-test was used to compare the shear bond strengths of the two groups. The difference between values of both groups was observed to be statistically significant.

Comparison of Shear Bond Strength within the Groups

Mean shear bond strength values obtained in each group immediately and after 6 months were found to be different.

Table 1 provides a comparison of mean shear bond strength values within the control group between the immediate and after 6-month storage period.

Table 2 shows a comparison of mean shear bond strength values within the CHX group between the immediate and after 6-month storage period.

Table 1: Comparison of shear bond strength between control groups

Groups	Mean	SD	n	Mean % decrease	Paired t	p-value
Immediately	25.5	6.3	15	85.3	12.87*	0.00
After	3.8	0.6	15			

*Significant at 0.01 level

Table 2: Comparison of shear bond strength within the CHX group after 6 months

Groups	Mean	SD	n	Mean % decrease	Paired t	p-value
Immediately	29.3	5.1	15	52.8	11.02*	0.00
After	13.8	1.1	15			

*Significant at 0.01 level

Graph 3 both the groups showed a reduction in shear bond strength following the 6-month storage period, with the decrease more apparent in the control group at the 6-month storage period. Paired t-test was used to compare these values and the difference was found to be highly statistically significant in the control and CHX groups.

DISCUSSION

Adhesion or bonding is the process of binding two adjoining materials and providing a resistance to their separation. Acid-etching brought about a substantial improvement in bonding to enamel and the bond was predictable. Durable bonding to dentin, however, has remained an elusive goal probably because of the morphological, histological, and compositional differences. Nakabayashi and Pashley (1998) described the nature of the resin–dentin interface as a “hybrid layer” comprising resin and dentin. This layer was found to disintegrate over time,³ primarily by two mechanisms: Hydrolytic and collagenolytic. After etching, the denuded collagen fibrils not enclosed by a protective resin covering stand exposed to the lytic action of endogenous enzymes called matrix metalloproteinases or MMPs. These are a multigene family within the metalloproteinase class of calcium-dependent zinc-containing endopeptidases capable of degrading all extracellular matrix (ECM) and basement membrane components including different collagens in both native and denatured.^{9,10} Mazzoni et al reported that endogenous MMPs are uncovered and/or activated by many, if not all dentin bonding procedures.

Developing new strategies to prevent the degradation of resin–dentin bonds is crucial to increase the longevity of bonded restorations. Due to the role of MMPs in the time-dependent degradation of this interface, it has been suggested that MMP inhibitors be employed to preserve it. Chlorhexidine, a widely used antimicrobial

agent, has been successfully employed to suppress the activities of MMP-2, MMP-8, and MMP-9.⁸ The exact mechanism of anti-MMP action of CHX is still unclear. It was observed that adding calcium chloride to assay mixtures containing CHX almost completely prevented the inhibition of MMP. This observation implied that CHX may act via a cation-chelating mechanism. Also, CHX may affect essential sulfhydryl groups and/or cysteine present in the active site of MMPs. At high concentrations above 2%, the inhibitory action of CHX might be related to protein denaturation rather than by chelation of cations.¹¹ Another advantage of using CHX is its substantivity, since it is able to bind to mineralized dentin for at least 12 weeks.¹² Additionally, it has been shown that demineralized dentin can bind more CHX than mineralized dentin and that it may remain attached to demineralized dentin even after the application of bonding agent.¹³ Given that degenerative changes at the resin–dentin interface occur over a period of time, substantivity of CHX is undeniably an asset. Since the binding mechanism of CHX to demineralized dentin is electrostatic in nature and hence reversible,¹⁴ CHX molecules could be eventually displaced by competing cations derived from dentinal fluid or saliva and leach out of the denuded collagen matrix. For this reason, there is a prevailing notion that CHX binding to demineralized dentin merely postpones rather than permanently arrest bond degradation. Then again, for how long can CHX extend its protective effect is still a question that requires further research. Chlorhexidine proved to be beneficial only for etch-and-rinse adhesives, as it could not bind to the collagen matrix in the presence of an acidic environment as present in self-etch adhesives.¹⁵

In the present study, immediate shear bond strength testing provided values that served as a baseline for comparison with values obtained after the 6-month storage period. Comparison of immediate shear bond strength showed no statistically significant difference between the two groups ($p > 0.05$). Hence, CHX has no effect – favorable or otherwise, on the resin–dentin bond in the immediate time period. This has been a topic of contention since some studies reported a decrease in the bond strength due to CHX application.^{16,17} Ricci et al¹⁸ in their study found that CHX solution application did lead to high bond strength immediately.

The samples were stored in artificial saliva for a period of 6 months. The values obtained after the storage period are the result of both hydrolytic and collagenolytic disintegration. Since the setting was a static one, the changes that occur in dynamic conditions simulating a clinical setting could not be replicated. Since the samples were not subjected to cyclic loading, the marginal gaps and leakage produced thereof would also be minimal.

Also, there was no active intrapulpal pressure leading to fluid leakage from within the tooth into the resin–dentin interface. The hydrolytic degradation in this scenario would possibly be minimal and the effect is uniform in all the samples.

After the 6-month storage period, the shear bond strength values showed a statistically significant difference ($p < 0.01$) between the two groups, with higher values for the CHX group. Hence, CHX has a beneficial effect in maintaining the resin–dentin bond strength over a period of 6-month. Intragroup analysis showed the bond strength values to diminish considerably in both the experimental and the control groups and the differences were statistically significant. Since CHX primarily prevents the collagenolytic degeneration, whatever drop in values present must be due to the hydrolytic changes in the interface, in part contributed by the water from the CHX solution itself. Since a 2% aqueous solution of CHX is used in this study, water could not be eliminated completely from the interface. The persistence of lytic changes at the resin–dentin interface could thus be attributed to the compromised moisture control rather than any ineffectiveness of CHX.

Another issue is the longevity of this protection CHX offers against collagenolysis. From the results of this study, it is seen that CHX maintained its protective action over 6 months. However, since the bonding between CHX and collagen is reversible, it is unlikely that this effect continues indefinitely. Further research and long-term clinical studies are required in this regard.

A previous study has reported that a CHX-based disinfectant did not adversely affect the shear bond strengths of tested resin cements to dentin.¹⁹ In that study, the authors used etch-and-rinse systems in which dentin surfaces were required to be etched, followed by the application of primers and adhesives prior to the luting of the resin cement. Thus, it appears that CHX on etched dentin surfaces would be preferred over the applications of these agents directly on smear layer covered dentin.²⁰ When dentin surfaces are etched, microorganisms and their products will be removed with the smear layer, allowing CHX to infiltrate the underlying demineralized dentin. This has significant implications especially for self-etch adhesives. Since in this case, CHX application has to be done on the smear layer itself, the extent of its penetration into the dentinal structure is questionable. It can be argued that in the case of self-etch systems, resin penetrates the entire depth to which demineralization has occurred. However, whether CHX which has pH range of 5.5 to 7 for optimal action²¹ can exert its protective effect is still debatable. This could possibly be the reason that most studies which used CHX in conjunction with self-etch adhesives, wherein pH values drop to the range

of 1 to 2, showed no significant effect for CHX on the bond strength values. Furthermore, it can also be rightly assumed that CHX application must be done only onto etched dentin from which the acid has been thoroughly removed, for the desired effect.

CONCLUSION

Within the limitations of the present study, it can be concluded that

- 2% chlorhexidine gluconate did not have any immediate effect – favorable or otherwise on the bond strength of resin to dentin.
- Rewetting with 2% CHX gluconate had a definite positive effect on the resin–dentin bond strength over a 6-month storage period.
- There was a definite degradation of resin–dentin bond strength with time as evidenced by the change in bond strength. This held true in both rewetting with water and 2% CHX gluconate.

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