



EFFECT OF 2% CHLORHEXIDINE SURFACE TREATMENT ON BONDING MECHANISM OF 5TH GENERATION DENTINE BONDING AGENT-INVITRO STUDY

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ABSTRACT studies suggested that host derived proteinases in the form of MMP's present in the saliva and released from the dentin matrix plays an important role in degradation of collagen in hybrid layer. Hasimoto et al in 2005 reported that Chlorhexidine inhibits dentin MMP activities and there by increases the durability of resin-dentin bonding. Application of 2% chlorhexidine on the dentin surface may prevent wetting of dentin bonding agents resulting in low bond strength values. The purpose of this study is to evaluate the effect of chlorhexidine on microshear bond strength of 5th generation bonding agent by means of microshear testing method. Conclusion : Chlorhexidine can be used in Primer, Etchants or as an additive to adhesive co-monomer, as stated by San (2006) as it does not affect the bond strength of adhesive resins.

KEYWORDS : chlorexidine, antiMMP, dentine bond strength, 5th generation

INTRODUCTION

In dental clinical procedures. The ability to preserve the tooth structures depends on the micromechanical retention achieved by means of resin infiltration into demineralised tooth structure (Bunocore, 1995& Nakabayashi et al 1982).The adhesion to enamel has been reached with relative ease and proved to be durable and most reliable procedure for routine applications in modern adhesive dentistry, but adhesion to dentin is not as reliable as adhesion in enamel¹.The dentin adhesion involves conditioning or etching followed by the primer and finally by application of bonding agents.

The incomplete resin infiltration was associated with reduced etching potential of acidic monomers towards base of hybrid layers. The presence of acidic but non polymerizable hydrolytic adhesive components creates potential sites for the degradation. Recent studies suggested that host derived proteinases in the form of MMP's present in the saliva and released from the dentin matrix plays an important role in degradation of collagen in hybrid layer. Incomplete resin impregnation in the collagen network leaves an exposed demineralised dentin zone at the base of the hybrid layer. Hence it has been speculated that the region of the exposed collagen fibrils is susceptible to hydrolytic degradation over a long period leading to reduction in bond strength^{2,3,4}.Inadequate molecular interaction, wetting and infiltration with hydroxy- apatite depleted collagen result in incomplete hybridization leaving collagen unprotected and vulnerable to proteolytic degradation.⁵ Hasimoto et al in 2005 reported that Chlorhexidine inhibits dentin MMP activities and there by increases the durability of resin-dentin bonding. In vivo and in vitro studies showed that less degradation of hybrid layer after application of 2% Chlorhexidine on acid etched dentin⁶.If Chlorhexidine can be used in Primer, Etchants or as an additive to adhesive co-monomer, it may block the degradation and there by preserves dentin hybrid layer. Application of 2% chlorhexidine on the dentin surface may prevent wetting of dentin bonding agents resulting in low bond strength values⁶. The purpose of this study is to evaluate the effect of chlorhexidine on microshear bond strength of 5th generation bonding agent.

AIM AND OBJECTIVES

The aim of this study is to determine the effect of 2% Chlorhexidine gluconate on bond strength between dentin and 5th generation adhesive systems by means of microshear testing method.

MATERIALS AND METHODOLOGY

Sixty recently extracted human mandibular first molars due to periodontal reasons, which are free of caries, restorations, cracks, fractures or other structural defects, were collected for this study. Teeth were cleaned with ultrasonic scaler and disinfected with 10% formalin and stored in distilled water until start of the procedure.

SPECIMEN PREPARATION

The occlusal enamel of all teeth were removed under water cooled high

speed airtor turbine with diamond bur. The complete elimination of enamel was confirmed by Optical Microscope. 2mm thickness disc of coronal mid dentin were prepared from each tooth by using Isomet saw (Isomet - model 650, South Bay technology, Sun Clemente, CA, USA) at low speed with water coolant was used to section the specimens. All specimens were attached to the 1cm x 1cm acrylic resin blocks with the help of cyanoacrylate adhesive paste. In order to standardize smear layer, teeth were polished with 220 300 and 600 grit Silicon Carbide polishing disc with water cooling for 60 seconds. Teeth were divided into three groups (CONTROL group A) and EXPERIMENTAL GROUP (with chlorhexidine GROUP B)

Table 1

Bonding Agent	Manufacturer	Composition
TE-econom	Ivoclar- Vivadent Batch No 20512	Dimethacrylate, ethanol, phosphoric acid acrylates. HEMA SiO ₂ , initiators, stabilizers

STANDARDISATION OF BONDING SURFACE

Prefabricated polytetrafluoroethylene sheet with iris of 0.7mm internal diameter and 1mm thickness was placed on all the specimens with the help of double side adhesive sticker to get a standardized bonding surface of area of 0.7mm diameter on dentinal disc before bonding procedure.

BONDING PROCEDURES FOR MICRO SHEAR BOND STRENGTH EVALUATION

GROUP A: 0.7mm of exposed dentin surface was etched with 37% phosphoric acid (eco etch ivoclar vivadent) for 15 seconds and washed with water gently dried with absorbent paper. Then 5th generation bonding agent (TE-ECONOM –ivoclar- vivadent) was applied on dentin with applicator tip and light cured for 20 seconds.

GROUP B: 0.7mm dentin surface was etched with 37% phosphoric acid, rinsed dried and 2% Chlorhexidine applied on the dentinal surface and left for 30 seconds without rinsing. Then the surface was blotted with absorbent paper. Then 5th generation bonding agent Te-econom was applied on the dentinal surface and cured with visible light curing unit for 20 seconds

COMPOSITE PLACEMENT

Composite (Filtek Z 350 3M ESPE) buildup of 0.7mm diameter and 1mm height was done in all the groups and cured for 40 seconds and the bonded specimen was immersed in the water at 37 ° C for 24 hrs and thermocycled for 500x cycle in 5°C and 55°C water bath with the dwell time of 30 seconds in each bath.Each specimen with acrylic mould was attached to jig of universal testing machine. A thin wire of 0.2mm diameter orthodontic ligature wire was embedded in the 1cmx1cm acrylic resin block was looped around composite cylinder and gently held flush against the dentin- resin interface and tested. The wire loop

and the center of load cell were aligned as straight as possible to ensure correct application of the shear force. Shear force applied at cross head speed of 1mm/minute by LLOYD universal instron testing machine.

LLOYD INSTRON MACHINE WITH SPECIMEN BEFORE FRACTURE

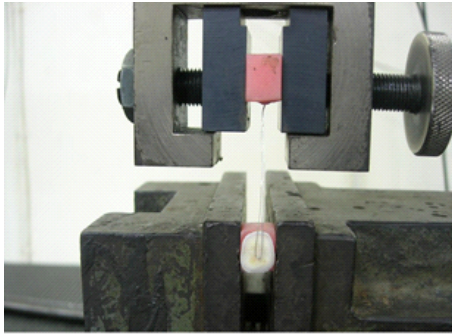


Fig 1

RESULTS

MICROSHEAR BOND STRENGTH STUDENT T TEST was used to compare the Microshear bond strength

MICRO SHEAR BOND STRENGTH VALUES

TABLE 2

Specimen No	Group A Without Chlorhexidine In Mpa	Group B With Chlorhexidine In Mpa
1.	22.40	18.00
2.	20.80	30.40
3.	26.00	28.40
4.	28.60	26.40
5.	26.50	28.40
6.	16.90	22.60
7.	20.40	24.40
8.	28.20	26.60
9.	24.40	28.40
10.	22.40	22.40

MEAN AND STANDARD DEVIATION

TABLE 3

Subgroups	MEAN	SD	P Value Between Sub Groups
GROUP A	23.22	4.15	0.044*
GROUP B	26.60	2.68	

* denotes significant at 5% level

STUDENT T-TEST was used to compare between control GROUP A and experimental group B for all the groups.

GROUP I

On comparing subgroup A and subgroup B shear bond strength there is significant differences with the p value of 0.044 which is < 0.05

DISCUSSION

The fundamental principle of adhesion to tooth structure based on an exchange process by which the inorganic tooth substrate is exchanged for synthetic resin after acid etching. The hydrophilic adhesive resin penetrates and adapts to the demineralised intertubular dentin and exposed collagen fibrils. The resulting inter-diffusion zone is termed as hybrid layer. Incomplete resin impregnation in the collagen network leaves an exposed demineralised dentin zone at the base of hybrid layer. Hence it was speculated that the region of exposed collagen fibrils was susceptible to hydrolytic degradation over a long period of time, leading to reduction in bond strength. Inadequate infiltration, wetting and molecular interaction of resin monomer with hydroxyapatite depleted collagen is challenging. This results in incomplete hybridization leaving collagen unprotected and vulnerable to hydrolytic degradation. (Hashimoto et al 2000)⁸.

Autodegradation of collagen matrices occurs in resin-infiltrated dentin by slow action of host derived Matrix Metalloproteinases⁹. Matrix Metalloproteinases (collagenases) are a family of zinc dependant proteolytic enzymes, which are capable of degrading the dentin

organic matrix after demineralization. Lehmann (2007) reported that metalloproteinases synthesized by odontoblast may get in to the hybrid layer through tubules and could contribute damage to incompletely infiltrated collagen in hybrid layer¹¹.

Matrix Metalloproteinases contain Zn²⁺ at the catalytic site and in addition require Ca²⁺ for stability and for its activity. Of several types of MMPs, MMP-2 has been reported to be present in both mineralized and demineralised dentin. Low pH and heat treatment can alter the conformation of propeptide and induce cysteine switch which represents a critical step in activation process. Decrease in pH and acidic environment can activate host derived proMMPs in dentin¹². The latency of the enzyme is maintained by unpaired cysteine sulfhydryl group in the propeptide domain, which interacts with the active zinc ion. Activation requires cysteine-zinc interaction to be perturbed by normal proteolytic removal of propeptide domain. This liberates active zinc site to bind a water molecule then attack the peptide bonds of collagen¹³.

Collagen degradation also occurs in endodontically treated teeth and which are restored with post. Host derived MMPs contribute to degradation of collagen fibrils in root dentin¹⁴. Increasing concentration of ionic or hydrophilic resin monomer are included in both etch and rinse for bonding to hydrophilic dentin substrate and in the self etching primer adhesives for simultaneous etching and bonding to dentin. Highly simplified one step adhesives and etch and rinse adhesives are highly prone to water sorption. Water may permeate these hybrid layers and allows the activated MMPs to exert their hydrolytic activity and deteriorates long term bond strength. Since hybrid layer of both etch and rinse are prone for hydrolytic degradation, 5th generation bonding systems were used in this study.

Endogenous hydrolytic degradation activity of matrix metalloproteinases can be arrested by the use of potential non toxic inhibitors such as chlorhexidine or doxycycline (Grenier et al., 2002). It can be safely applied intra orally, unlike highly toxic protease inhibitors¹⁵. Since in vitro and in vivo studies demonstrated preservation of hybrid layers and bond strength with 2% Chlorhexidine treatment on acid etched dentin (Hebling et al 2005). 2% chlorhexidine digluconate solution was used in this study. Solution used contains 2% of chlorhexidine in 98% of aqueous solution.

Chlorhexidine is a cationic bis- bigunaide. It possesses antimicrobial properties, substantivity¹⁶ and it is nontoxic. It is widely used as an antimicrobial agent including for the disinfection of cavities before placement of restorations and does not affect bond strength of etch and rinse and self etching adhesive resins. Chlorhexidine does not have any significant effect on the microleakage. Along with the antimicrobial action it has anti matrix metallo proteinase activity. Anti-MMP activity of Chlorhexidine is due to interaction with essential sulfhydryl group and or cysteine present in the active site of MMP¹⁷.

Mandibular first molars were selected in this study to obtain flat 2mm mid coronal dentinal surface using slow rotating diamond disc under water irrigation. Since smear layer has influence on bond strength, smear layer was standardized with 220,300,600 grit silicon carbide disc. The specimens were randomly selected and divided into control group and experimental group. Prefabricated Polytetrafluoroethylene mould with 0.7 mm diameter iris and with the height of 1mm thickness was used to get standardized 0.7mm dentinal surface for bonding procedure. Teflon sheet was held on the surface of the dentin disc with help of double side sticker. 2% Chlorhexidine surface treatment was done on etched dentin surface for experimental group 5th generation bonding agent All the specimens were checked under an optical microscope for bonding defect. Since the buildup was 0.7mm diameter and 1mm height there was no curing defect found.

The critical factor is that the shear force must be applied exactly at dentin-adhesive interface. In wire loop method, the shear force was applied more evenly by rapping the 0.2mm wire around the circumference of the composite cylinder at adhesive dentin interface. All the specimens were stored at room temperature for 24 hours.

All the specimens were thermocycled for 500 cycles in 5°C and 55°C water both with the dwell time of 30 seconds in each bath before testing. Shear force applied to each specimen at cross head speed of 1mm/min. STUDENT – T TEST was used to compare group A (control) and group B (experimental group). The p value is 0.044

which is less than 0.05 shows significant statistical increase in bond strength t at 5% level this may be explained due to (i) Chlorhexidine which has strong positive ionic charge, ready binding to phosphate group has a strong affinity to the tooth surfaces that is increased by acid etching. This may increase the surface free energy could lead to assumption that the application of after etching would increase the wetting of primers there by improving bond strength¹⁸ (ii) Secondly, the bonding procedure adopted after application of chlorhexidine. Since introduction of wet bonding concept to dentin (Kanca 1992) desiccation of dentin is no longer indicated. The main reason is that the spatial alteration of collagen occur upon drying demineralized dentin which may prevent monomer penetration into collagen network .

CONCLUSION

Chlorhexidine can be used in Primer, Etchants or as an additive to adhesive co-monomer, as stated by San (2006) as it does not affect the bond strength of adhesive resins. However further evaluation has to be done to evaluate the long term effect of chlorhexidine surface treatment in vivo 2% chlorhexidine surface treatment does not have detrimental effect on microshear bond strength of generation bonding agents used in this study.

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