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SEM EVALUATION OF RESIN DENTINE INTER DIFFUSION ZONE ON CHLORHEXIDINE SURFACE TREATMENT OF DENTINE -IN VITRO STUDY

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ABSTRACT Inadequate molecular interaction, wetting and infiltration with hydroxy- apatite depleted collagen result in incomplete hybridization leaving collagen unprotected and vulnerable to proteolytic degradation.3Hasimoto 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 dentin ,.

Aim: to evaluate the effect of chlorhexidine on microshear bond strength of 7th generation bonding agents and to evaluate the resin infiltration on the chlorhexidine treated dentin surface by scanning electron microscopic study.

RESULTS: Hybrid layer measuring about 3µm to 5 µm and resin tag measuring about 2µm to 3µm were present. Apex of resin tags was observed in all the specimens.

KEYWORDS : chlorexidine, dentine bonding , SEM , hybrid layer

INTRODUCTION:

Collagen fibrils in acid etched dentin are susceptible to in vivo degradation.Theoretically, the use of self-etch adhesives in which infiltration of resin occurs simultaneously with the self etching process, the risk of discrepancy between both collagen and resin infiltration will be non existent. But morphological evidences were already provided showing discrepancies between the depth of demineralization and depth of resin infiltration in some mild self-etch adhesives [1]. 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. 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]. Inadequate molecular interaction, wetting and infiltration with hydroxy- apatite depleted collagen result in incomplete hybridization leaving collagen unprotected and vulnerable to proteolytic degradation.[3] 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 dentin [4].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[5] The purpose of this study is to evaluate the effect of chlorhexidine on microshear bond strength of 7th generation bonding agents and to evaluate the resin infiltration on the chlorhexidine treated dentin surface by scanning electron microscopic study.

AIM AND OBJECTIVES

The aim of this study is to evaluate 7th generation bonding agents adhesive resin-dentin interface after 2% Chlorhexidine surface treatment by scanning electron microscope.Procedural flow chart

SPECIMEN PREPARATION

The occlusal enamel of all teeth were removed under water cooled high speed airotor 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 slow speed with water coolant was used to section the specimens. All specimens were attached to the 1cmx 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.

MATERIAL USED

TABLE 1

(7th generation bonding agent)	G-bond	GC-Corporation Japan batch no 0703081	4-MET, UDMA, Phosphate -monomer, fumed silica filler, photoinitiator, acetone, water

SPECIMEN PREPARATION FOR SCANNING ELECTRON MICROSCOPIC STUDY

Eighteen freshly extracted non carious mandibular teeth were selected and stored in distilled water with 0.2% thymol until start of procedure. All teeth were grounded to get flat mid coronal dentinal surface using diamond bur under water irrigation. Then the surface was polished with 220, 300 and 600 grit silicon polishing disc.

BONDING PROCEDURE FOR SCANNING ELECTRON MICROSCOPIC STUDY

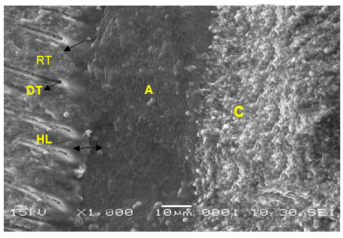
7th generation bonding agent was applied on the dentinal surface of SPECIMEN according to manufacturer's instruction. 2% Chlorhexidine applied on the dentinal surface and left for 30 seconds without rinsing. Then the surface was blotted with absorbent paper and briefly dried with oil free air. Then 7th generation bonding agent as applied on the dentinal surface and cured with visible light curing unit for 20 seconds. . The specimens were stored in water for 24 hrs. After thermocycling procedure all the specimens were chiseled to expose resin dentin interface and prepared for electron microscopic study. Each specimen was mounted on metal stubs and sputtered with 15 nm platinum in sputtering machine (JEOL JFC 1600, Japan electronics limited). Specimen was attached with metal bolt with double sticker and examined with scanning electron microscope (JEOL JSM 6360 Japan electronics limited) using acceleration voltage of 15 Kv. Dentin resin interface was viewed at 1000x magnification.

SPUTTERING MACHINE
JEOL JFC 1600 Japan electronics limited
FIG 1

SCANNING ELECTRON MICROSCOPE
JEOL JSM 6360 Japan electronics limited
SCANNING ELECTRON MICROSCOPIC RESULTS

Scanning electron microscopic analysis shows that the bonding agent was well adapted to the dentin in all specimens. In some cases voids were present in the adhesive layers this may be due to dehydration or dessication in the high vaccum chamber during specimen processing for scanning microscopic study. Large defects were seen below the resin surface. Hybrid layer measuring about 3µm to 5 µm and resin tag measuring about 2µm to 3µm were present. Apex of resin tags was observed in all the specimens. Some smear layer occluded dentinal tubules without resin infiltration was seen .

SCANNING ELECTRON MICROSCOPIC PICTURE
FIG 2



A- adhesive layer. C- composite.

HL –hybrid layer measuring about 2µmto 5µm, DT dentin tubule . RT- resin tag measuring about 5µm to10µm with the apex in the tubule

DISCUSSION

The formation of hybridized dentin greatly depends upon the permeability of the dentin substrate to which the dentin bonding agent is applied as well as diffusion potential of the applied adhesive monomer [6].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) .Autodegradation of collagen matrices occurs in resin-infiltrated dentin by slow action of host derived Matrix Metalloproteinases[8] .Matrix Metalloproteinases (collagenases)[1] are a family of zinc dependant proteolytic enzymes, which are capable of degrading the dentin organic matrix after demineralization [9]. 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 layer [10]. Matrix Metalloproteinases contain Zn^{2+} at the catalytic site and in addition require Ca^{2+} 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 dentin [11].

Chlorhexidine gluconate solution which contains 98% of water and 2% of chlorhexidine when applied to the dentin may leave the surface over wetted. Taking this in to consideration the influence of chlorhexidine on adhesion could be more related to wetness control than the intrinsic properties of this material. In SEM study Apex of resin tags was observed in all the specimens. Some smear layer occluded dentinal tubules without resin infiltration was seen in all the specimens of 6th and 7th generation. This shows that the hybrid layer formation was not affected by 2% Chlorhexidine surface treatment on 7th generation bonding agents. Since Chlorhexidine application was done on dentin surface in the case of self etching adhesives, it may be speculated that it could prevent adhesive wetting of dentin, leading to low bond strength. However results in this SEM study provided the resin infiltration on dentin was not affected by 2% Chlorhexidine application.

CONCLUSION

From this study it was concluded that Scanning microscopic analysis shows that resin infiltration on dentin was not affected by 2% Chlorhexidine surface treatment. Chlorhexidine can be used an an anti MMP inhibitor in bonding procedures as it does not affect the bond strength of adhesive resins. However further evaluation has to be done to evaluate the long term bond strength of Chlorhexidine surface treatment in vivo.

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