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Effect Of 3% Riboflavin on the Adhesion of Dental Composite Resin to Etched Dentin.

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ABSTRACT

Use of photo-oxidative cross-linking agents, like riboflavin has been suggested as a method to modify the dentin substrate to improve the bonding of dental composite resin. The purpose of this study was to assess the adhesion of dental composite resin to etched dentin surface treated with 3% riboflavin. Twenty prepared samples were randomly divided into two groups. Group I was the control group, where the etched dentin surface was not treated with the cross-linking agent. Group II was the experimental group, where the etched dentin surface was subjected to photo-activated oxidation using 3% riboflavin and visible blue light. Two 2-mm thick micro hybrid composite cylinders were bonded to each sample. The specimens were then evaluated for the strength of bonding between composite and dentin surface. Shear bond strength was analyzed for each sample and the means were calculated. Statistical analysis was done using independent T-test. The level of significance was set at $P < 0.05$ value. The shear bond strength of dental composite resin to etched dentin was shown to be statistically significantly higher in the experimental group (Group II), than that of the control group (Group I). The pretreatment of the etched dentin surface with 3% riboflavin activated with blue light can improve the immediate bond strength of composite resin.

Keywords: Composite resin, Collagen, Dentin, photosensitizer, Riboflavin, Shear bond strength, Visible blue light

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INTRODUCTION

True adhesion has been the holy grail of dental restorative materials for many decades. As we have progressed into the twenty-first century, we have seen an exponential growth in the development of adhesive materials and technique.[1]Dental adhesion has made marked progress recently as a result of advances in dentin bonding technology. Dentine is a complex mineralized tissue that consists of inorganic and organic components along with water.[2] Collagen constitutes 90% of the dentin organic matrix. The organic components of dentin mainly consists of type I collagen.[3]

Bonds formed between the adhesive resin and dentin forms a layer that contains both hydrophilic and hydrophobic chemical groups.[4] This resin-impregnation creates a "hybrid" layer, that is neither resin nor tooth, but a hybrid of the two.[5] Hybrid layer formation is the most established theory of dentin bonding mechanism. In the two step etch and rinse technique, the hybrid layer results in a porous interface. The hybrid layer acts as permeable membrane resulting in water sorption and collagen degradation which is a likely mechanism for bond deterioration.[6] The phosphoric acid used for etching is regarded to have an aggressive effect on the dentine and denuding the dentine collagen.[7] Hence it is essential to develop a strategy for minimal collagen degradation and to improve resin bonding.[8]

Recently a number of natural cross linkers including proanthocyanidins, genipin, and tannic acid, have been shown to increase dentin stiffness and strengthen the resin-dentin adhesion are known to have the ability to increase the number of intramolecular collagen crosslinks.[9- 11] This low elasticity modulus permits rotational and lateral movements of the adjacent collagen peptides.[12] The strengthening of collagen fibrils by crosslinking agents to increase the mechanical properties and decrease the enzymatic degradation may be an important application in restorative dentistry.[13]

For collagen cross-linking method, the presence of singlet oxygen is required, and riboflavin [RFV] (vitamin B2) is one of the most potent producers of these oxygen radicals.[14] The effect of photo-activated RFV on dentin collagen has been known to increase the biodegradation resistance, the mechanical properties, and the stability of the dentin matrix.[15].Additionally, RFV acts as a photosensitizer, thus promoting the formation of new cross-linkages. This treatment is also advocated in inactivating dentin matrix metalloproteinases, thus to improve the durability of resin adhesion. [16] .The application of RFV on dentin resin adhesive aimed at improving the stability of resin–dentin bond. Therefore the aim of this study were to modify a two-step, experimental, etch-and-rinse model dentin adhesive with RFV and to study its effects on the bond strength and the durability of dentin.

MATERIALS AND METHOD

Sample selection

Twenty extracted human posterior teeth was selected based on the inclusion criteria; i.e. no evidence of caries, restorations and lack of any cracks or fractures in the crown. They were stored in distilled water at room temperature.

Sample preparation

The teeth was sectioned occlusally 3mm above the cementum-enamel junction by passing the diamond disk attached to a slow speed micro motor headpiece parallel to the occlusal surface under water coolant. The sectioned teeth were embedded in auto polymerizing acrylic resin and a flat dentin surface was exposed. The exposed dentin surface was further flattened with a 600-grit silicon carbide abrasive paper to standardize the smear layer. The teeth were rinsed with distilled water to remove any debris. The prepared teeth were randomly divided into two groups and 10 teeth was assigned to each group (Figure 1)

Preparation of riboflavin solution

Three grams of riboflavin powder (Loba Chemie Pvt Ltd, Mumbai, India) was weighed and was dissolved in 100ml of distilled water to prepare the 3% solution. The solution was stored in an amber colored bottle.

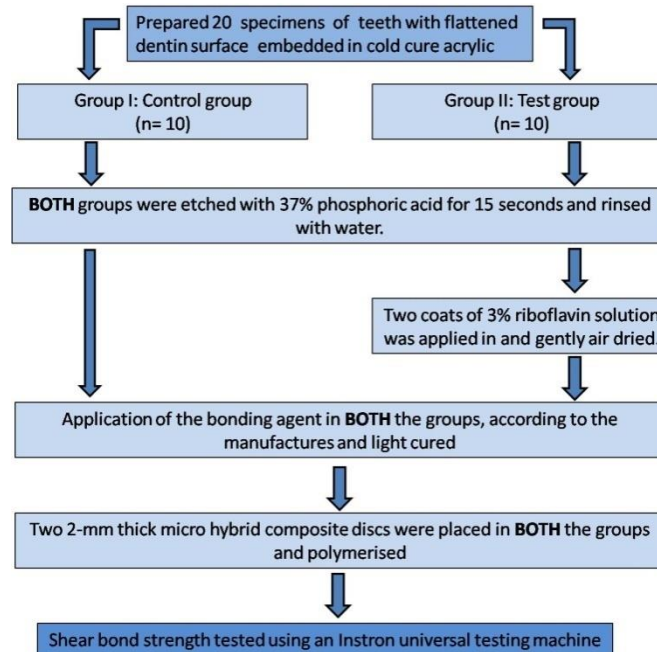
Groups

The two groups are as follows;

Group I- No riboflavin [NR] (control group) application on etched dentin.

Group II- 3% riboflavin [RFV] (test group) application on etched dentin.

Figure 1: Schematic diagram representing the experimental procedure in this current study



Bonding procedure

Both groups were etched with 37% phosphoric acid (3M ESPE scotch bond multi-purpose etchant) for 15 seconds and rinsed with water. Two coats of 3% riboflavin solution was applied in Group-II and gently air dried. Application of the bonding agent in both the groups, according to the manufactures instructions (3M ESPE Adper Single Bond 2 adhesive). Each bonded specimen was light cured for 20 seconds. Two 2-mm thick micro hybrid composite discs were placed in both the groups and polymerised individually for 20 seconds. The specimens were then evaluated for the strength of bonding between composite to the treated dentin surface.

Storage of the samples

After bonding to the dentinal surface, was stored under 37°C in distilled water for 24 hours in 100% humidity.

Measurement of shear bond strength (SBS)

Ten samples from each group was used to assess the SBS. Samples are placed into a positioning jig and tested in shear with an Universal Testing Machine (3366, Instron Corporation, Canton, MA) at a crosshead speed of 0.5 mm/min. The shear bond strengths of the sample were calculated and expressed in MPa (Table 1).

Data management and analysis

The mean and standard deviation of the shear bond strength (SBS) in both the groups were calculated. Statistical analysis was done using independent T-test (SPSS 17.0 version, Chicago, IL, USA). The level of significance was set at $P < 0.05$ value.

RESULTS

The shear bond strength (SBS) of dental composite resin to etched dentin was shown to be statistically significantly higher in the experimental group (Group II), i.e. the dentin surface treated with 3% Riboflavin than that of the control group (Group I). (Table 1).

Table 1: Shear bond strength (SBS) of dental composite resin to etched dentin.

GROUPS	n	MEAN	Std. Deviation	P- VALUE
GROUP- I (CONTROL)	10	5.16	1.46	.011
GROUP- II (TEST)	10	7.68	2.39	

DISCUSSION

In the current study, macro-shear bond-strength method was used for testing the effect of a collagen cross linking agent, i.e. riboflavin (RFV), on the adhesion of dental composite resin. It is the most commonly used method for bond-strength testing. It is highly popular as it is easy, fastest and requires no further specimen processing after the bonding procedure.[17] In the present study, efforts were taken to reduce the influence of confounding factors such as the tooth surface variability by standardizing the depth of the flat dentine surface as well as the thickness of smear layer. The specimens were finished using a 600- grit silicon carbide paper to produce a thicker and a uniform smear layer on the dentinal surface.[18] As the purpose of this study was solely to assess the bond strength of dental composite resin after treatment of etched dentin with a collagen cross linking agent in the absence of any additional stresses, the specimens were not subjected to thermocycling.

In dentin adhesive systems, the stability and integrity of collagen fibrils within the hybrid layer are crucial for the maintenance of bond effectiveness over time. Photo-oxidative cross-linking agents to strengthen the collagen network has been suggested as a method to modify the of dentin substrate to improve the bond durability. [19, 20]Even though many photosensitizing chromophores such as porphyrins, rose bengal and methylene blue were shown to strengthen collagen fibrils, the pale yellow color of riboflavin makes it more appropriate for dental use.[21] For the photo-oxidative collagen cross-linking method, the presence of singlet oxygen is required, and riboflavin (vitamin B2) is one of the most potent producers of these oxygen radicals. Moreover RFV is labeled ‘generally recognized as safe’ by the FDA and is also used as a food dye.[22] Ultra Violet A (UVA) radiation of photosensitive riboflavin has been reported as a successful treatment for human ophthalmic diseases such as keratoconus, wherein the recovery of collagen stiffness is necessary.[22]The same principal of stiffening of collagen can be applied to the field of dentistry.

A study by Daood *et al.*[23] used the various concentrations of riboflavin (m/m, 0, 1%, 3%, 5% and 10%) along with an etch-and-rinse dentin adhesive to study its effect on the bond strength, degree of conversion, along with resin infiltration within the demineralized dentin substrate. As per the results of the study it was concluded that incorporation of riboflavin at 3% (m/m) improved the immediate bond strengths and bond durability after 9-month storage in artificial saliva without adversely affecting the degree of conversion of the adhesive monomers and resin infiltration. Hence a concentration of 3% RFV was used in the present study. Although most of the studies have used ultraviolet (UVA) to activate RFV, in the current study RFV was simultaneously photo-activated using visible blue light (VBL) along with the curing of the dentin adhesive. This was used as per the conclusion made in the study by Fawzy *et al.*[20]where they stated that VBL can be a promising substitute for UVA as it is clinically more applicable and acceptable. Their study showed that the use of VBL managed to increase the biodegradation resistance, enhance the mechanical properties of dentin collagen and improve and maintain the bond strength and interface integrity after short-term water storage.

From the results of the present study it can be observed that the etched dentin surface subjected to RFV photo-activation can improve the immediate bond strength. This is in accordance with the study by Cova *et al.* [22] as well as Chiang *et al.*[21]In this study, an improved bond strength was observed when the etched dentin surface was pretreated with RFV. It is known that irradiation of RFV during curing produces active

oxygen species that can induce formation of covalent cross links between collagen fibrils through oxidization.[24] Effects of cross linking agents on stabilizing dentin matrix degradation have been attributed to their capacity to increase the stiffness of dentin collagen.[25] RFV treatment is also said to enhance the resin-dentin bond possibly by maintaining the expanding collagen matrix in the hybrid layer.[21]The stabilized fibrillar network is said to enhances resin infiltration and thus the hybrid layer formation.[26]

The activation of riboflavin with the use of blue LEDs is said to generate reactive oxygen species (ROS).[27] These ROSs is shown to cause bacterial cell death either by oxidative damage to cell membrane or to deoxy-ribonucleic acid (DNA). Photosensitization of bacteria has shown to be independent of the antibiotic resistance spectrum, non-mutagenic or genotoxic. There appears to be no induction of resistance to PDT even after multiple treatments.[28] The same principle can be translated to dentistry as well. Thus photo-oxidation of riboflavin apart from collagen cross-linking can disinfect the dentin surface prior to the placement of the restoration. Hence further investigations are required to substantiate the above mentioned claims in the present study.

CONCLUSION

Considering the experimental conditions of this in vitro study and the results achieved, we can conclude that the pretreatment of the etched dentin surface with 3% riboflavin activated with blue light can improve the immediate bond strength of composite resin restoration

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