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Evaluation of the Effect of Grape Seed Extract, Propolis and Ascorbic Acid on Shear Bond Strength of Deproteinized Dentin to Composite Resin: An In Vitro Study

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ABSTRACT

Aim: The need for this study was to evaluate the effect of Propolis on the shear bond strength of deproteinized dentin to composite compared with that of grape seed extract and sodium ascorbate.

Materials and Methods: Group I: dentin surface of specimens pretreated with 5.25% NaOCl for 60 s; Group II: dentin surface of specimens pretreated with 5.25% NaOCl followed by 10% Sodium ascorbate solution for 60 s; Group III: dentin surface of specimens pretreated with 5.25% NaOCl followed by 5% proanthocyanidin solution for 60 s; Group IV: dentin surface of specimens pretreated with 5.25% NaOCl followed by propolis solution for 60 s. The dentin surfaces of all specimens were then coated with self-etch adhesive, Adper Easy One and light cured for 40 s. After adhesive application, nano hybrid composite (Tetric N-Ceram Bulk fill) was placed and light cured for a period of 40 s. Instron universal testing machine was then used to determine the shear bond strength.

Results: Statistical analysis showed that 5.25% NaOCl caused significant reduction in the bond strength ($P<0.05$) which can be reversed by 5% PA, 10% sodium ascorbate and propolis. No statistical significant difference in bond strengths was found between Group II and Group IV.

Conclusion: Dentin surface pre-treatment with propolis, proanthocyanidin prior to application of bonding agent over deproteinized dentin significantly increased the bond strength of dentin to composite

Keywords: Deproteinised dentin; dentin bond strength; propolis; resin cement.

INTRODUCTION

Restoration of posterior teeth with resin-based materials continues to gain popularity among dentists and the demand for such aesthetic restorations is being increased.[1] Residual bacteria on the dentin surface under restorations due to incomplete removal of caries may result in weakening of bond strength, post-operative sensitivity, development of recurrent caries, pulpal inflammation and necrosis. Therefore, the adjunctive use of disinfectant solutions after cavity preparation may be considered.[2] Sodium hypochlorite (NaOCl) is widely used in chemomechanical caries removal and as an endodontic irrigant, because of its tissue dissolving properties and antimicrobial effect.[1] It has been previously established that treatment with sodium hypochlorite is an effective dentin deproteinization method which has also been proposed as a method

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to improve wettability of an adhesive. Although it is an effective protein denaturant, NaOCl is also a potent biologic oxidant.[3] Earlier studies have shown that 5.25% sodium hypochlorite (NaOCl) significantly reduced the bond strength of resin cements.[4,8] Restoration of reduced bond strength is necessary for resin cements which are widely used in restoration of posterior teeth. Previous studies have also shown that antioxidants such as 10% sodium ascorbate, 5% proanthocyanidinwere used to reverse the negative effects of NaOCl.[5,8]

Propolis is a natural resinous material, which honey bees collect from various plant species. They contain flavonoids which are well-known to have free radical scavenging activity is shown to be more potent than sodium ascorbate.[6,7] Hence, the aim of the study was to evaluate the effect of Propolis on the shear bond strength of deproteinized dentin to composite as compared with that of grape seed extract and sodium ascorbate.

MATERIALS AND METHOD

Preparation of solutions:

• 5% Proanthocyanidin solution

5 grams of grape seed extract (Nutrija Lifescience, Nagda, MP, India) in the form of powder was dissolved in 100 ml of distilled water to prepare 5% proanthocyanidin solution.

• 10% Sodium ascorbate solution

10 grams of sodium ascorbate (SDFCL, Sd fine-chemicals limited, Mumbai, Maharashtra, India) powder was dissolved in 100 ml of distilled water to make 10% sodium ascorbate solution.

Specimen preparation:

In the present study, 40 freshly extracted human molars free of caries, cracks, fractures, without previous restorations were collected. The teeth were thoroughly cleaned for debris and rinsed under running water for a period of 15 minutes each. They were then stored in 0.1% thymol solution until preparation. The teeth were mounted in plastic rings of 1 inch diameter with cold cure acrylic resin. The occlusal surfaces of teeth were then ground flat using a diamond disc with a water coolant at low speed to expose the dentin. Dentin surfaces were flattened using 600-800 grit and 1200 grit water proof polishing papers.

The specimens were then divided into four groups with 10 specimens in each group according to the dentin surface pretreatment, as follows:

Group I (n=10): The dentin surface of specimens were treated with 5.25% NaOCl using a disposable brush tip and left undisturbed for 60 s. The dentin surface was then rinsed with water for 5 s and dried with absorbent paper.

Group II (n=10): The specimens were treated with NaOCl as in group 1 followed by 10% Sodium ascorbate solution for 60 s. The dentin surface was then rinsed with water for 5 s and dried with absorbent paper.

Group III (n=10): The specimens were treated with NaOCl as in group 1 followed by 5% proanthocyanidin solution for 60 s. The dentin surface was then rinsed with water for 5 s and dried with absorbent paper.

Group IV (n=10): The specimens were treated with NaOCl as in group 1 followed by propolis solution for 60 s. The dentin surface was then rinsed with water for 5 s and dried with absorbent paper.

The dentin surfaces of all specimens were then coated with self-etch adhesive, Adper Easy One (3M, Deutschland GmbH, Carl-Schurz-Str. 1, 41453 Neuss- Germany). The adhesive was left in place undisturbed for 20 s, then gently air dried and light cured for 40 s. After adhesive application, nano hybrid composite (Tetric N-Ceram Bulk fill) was placed and light cured for a period of 40 s vertically to ensure complete polymerization of the resin. For all the specimens, the curing tip was placed as closely as possible to composite and dentin surface.

The shear bond strength was then determined using Instron universal testing machine, which was constructed to direct the shearing force on the dentin to composite interface at a cross head speed of 1mm/min.

Statistics

The results were performed using one way analysis of variance test and post hoc tukey test. One way

analysis of variance was applied to the mean retentive strengths of various bonding agents. The post hoc tukey multiple comparison tests were used to identify means that are significantly different from each other. All statistical analysis of the data were performed by using a statistical software package SPSS 15.0 software and decisions were made at a level of significance of 0.05%. Results were regarded as significant if *P<0.05*.

RESULTS

The effects of the various pretreatments on the shear bond strength of dentin to composites are shown in the Table I. The mean shear bond strength is maximum in Group III (23.8013± 1.51), followed by Group IV (21.3757± 1.20) and Group II (20.3376±1.29). Group I (13.4618±1.90) exhibits the least bond strength among all the other groups. Statistical analysis showed that 5.25% NaOCl caused significant reduction in the bond strength (P<0.05) which can be reversed by 5% PA, 10% sodium ascorbate and propolis. No statistical significant difference in bond strengths was found between Group II and Group IV.

Table 1: Comparison of mean shear bond strength (MPa) of different study groups after various pretreatments.

Group	Mean ± SD	Significant groups
I	13.4618± 2.01	IV, III, II vs. I
II	20.3376± 1.36	II vs. III
III	23.8013± 1.59	III vs. IV
IV	21.3757± 1.27	

DISCUSSION

The residual bacteria on the surface of dentin due to incomplete removal of caries has proved to be the main causative factor for replacement of restorations.[2] Because of its antimicrobial and tissue dissolving properties, sodium hypochlorite is widely used in chemomechanical caries removal, as an endodontic irrigant and in dentin bonding techniques.[1] In the present study, NaOCl-treated group demonstrated significantly lower bond strength. NaOCl being a strong oxidizing agent causes superficial oxidation thereby leaving behind an oxygen rich layer on the dentin surface which inhibits the interfacial polymerization of resin-based materials.[2,3] NaOCl also removes organic matrix from the dentin leaving a less receptive bonding surface.[4] Reducing agents such as sodium ascorbate, proanthocyanidin and propolis reversed the negative effects of NaOCl by restoring the altered redox potential that changes the oxidized dentin back to a reduced substrate which allows free radical polymerization of the resin.[4,8] In addition to the anti-oxidant property, sodium ascorbate, proanthocyanidin and propolisalso have the ability to increase the collagen cross-links in biological tissues.[11]

Sodium ascorbate has been investigated in various studies to reverse the negative effects of NaOCl.[3,4,8,9] Sodium ascorbate plays an important role in the synthesis of hydroxyproline which stabilizes the collagen triple helix and hydroxylysine in collagen which helps in the formation of intermolecular cross-links in collagen.[11] On the other hand, a naturally occurring compound Proanthocyanidin, which is widely present in nuts, seeds, flowers, vegetables and fruits improves the mechanical properties of demineralized dentin by its crosslinking and anti-oxidant properties.[10,11,12] Proanthocyanidin facilitates the enzyme proline hydroxylase that catalyzes the hydroxylation of proline which is essential in collagen biosynthesis.[11] Ascorbic acid, proanthocyanidin and propolis are nontoxic and its use intraorally will not produce any adverse effects.

In contrast, Propolis used in the present study has better anti-oxidant property than vitamin C and E due to its radical scavenging ability. The main chemical classes in propolis are phenolics, flavonoids and other various aromatic compounds. Flavonoids are plant compounds that have antioxidant, anti-inflammatory, antibacterial, antifungal and antiviral properties.[6,13] Flavonoids, which contain an ortho-dihydroxy (catechol) structure for electron delocalization and multiple hydroxyl sites (electron donor sites) that allow it to bind to unstable free radicals by donating its hydrogen atoms contribute to the anti-oxidant property.[13,14] In addition to that property, flavonoids also increase collagen synthesis and also accelerates its conversion from soluble collagen to insoluble collagen which enhances collagen stabilization that is necessary to achieve optimum

bond strength. Flavanoids interacts with proteins to induce collagen cross-links by four different mechanisms: ionic interaction, covalent interaction, hydrophobic interaction and hydrogen bonding interactions thereby producing fibrillar and collagen stabilization. Covalent inter and intramolecular crosslinks form the basis for stability, viscoelasticity and tensile strength of the collagen fibrils.[11] Caffeic acid and flavonoids in propolis play an important role in the cell metabolism, circulation, stimulation of various enzyme systemsand collagen formation thereby contributing to dentin stabilization by propolis.[15]

Propolis can be considered as one of the pre-treatment regimes prior to application of bonding agents over deproteinized dentin. This new collagen stabilizing agent seems to have a promising future required to achieve a restoration with stable bonding abilities.

CONCLUSION

Dentin surface pre-treatment with propolis, proanthocyanidin prior to application of bonding agent over deproteinized dentin significantly increased the bond strength of dentin to composite. There was no statistically significant difference between ascorbic acid and propolis groups.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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