Impact of Various Cross linking agents on Dentin Adhesive Systems - an in-vitro study.

ABSTRACT

Aims and Objectives: The main aim of this study is to compare and evaluate the shear bond strength of composite resinusing a total etch adhesive and self-etch adhesive after treatment with different collagen cross-linking agents.

Materials and methods: Forty freshly extracted human maxillary central incisors were taken. The proximal dentin was exposed, cavities were prepared on mesial and distal of each tooth. The specimens were randomly divided into two groups based on bonding agent applied-Group A and Group B i.e universal bonding agent and all in one bonding agent respectively and are subdivided into four groups based on the surface treatment of dentin. Group I A (n=10) Control-total etch(CTE);Group II A (n=10) Sodium Ascorbate-total etch(STE);Group III A (n=10)- Proanthocyanidin total etch(PTE); Group IV A (n=10)- Chitosan total etch(CHTE); Group I B (n=10) Control-self etch(CSE);Group II B (n=10) Sodium Ascorbate-self etch(SSE); Group III B (n=10)- Proanthocyanidin self-etch(PSE); and Group IV B (n=10) - Chitosan self-etch(CHSE). Shear bond strength of the specimens are tested with universal testing machine, and the data was statistically analysed with one way ANOVA.

Results: Significantly higher shear bond strength to dentin was observed in teeth treated with 6.5% Proanthocyanidin Total etch, 10% Sodium Ascorbate Total etch and 1% Chitosan acetate compared to the control group. No significant difference was seen with self-etch treated groups.

Conclusion: Dentin surface pretreatment with 6.5% Proanthocyanidin, 10% Sodium Ascorbate and 1% Chitosan acetate results in significant enhancement in bond strength of composite resin to deep dentin.

Keywords: Chitosan; Sodium Ascorbate; Dentin bonding agents; Proanthocyanidin; Shear bond strength.

1. INTRODUCTION:

Various advancements in the adhesive dentistry have been proposed on the improvement of bonding agents and techniques, but a very limited investigation has been done on the relation of collagen structure and stability to bond strength.

Many challenges are made to achieve adequate and predictable adhesion of resin composite to tooth structure. The stability of the restoration, higher bond strength and less microleakage are the main objectives of reliable bonding. [1]

Dentin is made up of Type I collagen which provides tensile strength, form, and cohesiveness properties by endogenous covalent intermolecular crosslinking.[2] Various studies have revealed that bond strength reduction occurs, when composite resin is bonded to deep dentin unlike superficial dentin.[3,4] Dentin as such is not uniform along its depth because of its structural complexities, such as increase in the number of tubules and their diameters with much less intertubular dentin matrix in deep dentin. Higher water content is present in deep dentin (22 % volume) as compared to superficial dentin (1% volume) which may dilute the organic solvents of some bonding agents, resulting in separation of monomers from the soluble phase and leading to the formation of resin globules in water .[5]

Successful bonding to dentin depends mainly on proper resin infiltration and the formation of resin tags. [6,7] The durability of the bond between dentin and adhesives depends on the structural integrity and mechanical properties of acid-demineralized collagen fibers thus stabilizing the collagen and enhances the bonding [8]. Mechanical properties of collagen can be improved by an increase in the formation of intramolecular, intermolecular and intermicrofibrillar crosslinks.

Biomodification of collagen with exogenous cross-linking agents alters the structure of collagen fibrils and improving their degradation resistance,which is critical for increasing the stability of hybrid layer and durability of bonded restorations. [1,4]. Cross-linking agents

decreases the enzymatic degradation by matrix metalloproteinase inhibitors (MMP-I) which help in improving the bond strength. A more resistant and insoluble collagen provides a stable substrate for dental adhesive restorations [9,10].

In the context of pretreatment of biological tissues, synthetic collagen cross linking agents namely glutaraldehyde, formaldehyde, carbodimide, epoxy compounds were reported to have high cytotoxicity and incompatible mechanical properties unlike naturally synthesized grape seed extract which consists of Proanthocyanidin, Sodium Ascorbate and Chitosan to induce exogenous cross links [10].

SA is an antioxidant and helps in the synthesis of hydroxyproline and hydroxylysine in collagen. Hydroxyproline stabilizes the collagen triple helix and hydroxylysine forms intermolecular collagen crosslinks.

Proanthocyanidins (PA) are bioflavonoids that are available in maritime pine bark, cinnamon, *Aronia*fruit, cocoa beans, grape seed, grape skin (procyanidins and prodelphinidins), PA has ability to bind to proline-rich proteins, such as collagen via covalent, ionic, hydrophobic interactions[10]. PA facilitate the enzyme proline hydroxylase activity that is essential for collagen biosynthesis[3].

Chitosan is a cationic polysaccharide formed through the deacetylation of chitin, a polysaccharide found in the exoskeleton of crustaceans, through an alkalinization process under high temperatures.Chitosan is a hydrophilic biopolymer (2-amino-2-deoxy-β-d-glucopyranose) with more number of free hydroxyl and amino groups that have the capability to form crosslinks with other reactive molecules. The free reactive groups present in the chitosan interacts with collagen to form chemical bonds [11].

The main aim of my study was to evaluate the impact of collagen stabilizing agents namely Sodium Ascorbate, Proanthocyanidin and Chitosan on the shear bond strength of composite to dentin.

2. MATERIALS USED IN THIS STUDY:

1) Proanthocyanidin Grape Seed Extract (Puritans Pride Inc, Oakdale, NY, USA).
2) Sodium Ascorbate (Sigma Aldrich, India)
3) Chitosan Powder (Panvo Organics Ltd, Chennai, India)
4) Etchant: DeTrey Conditioner (DentsplyDeTrey, GmbH,Konstanz, Germany)
5) Tetric N Bond Universal(Vivapen,IvoclarVivadent)
6) 3M Adper Single Bond(3M ESPE ,St.Paul, MN,USA)
7) 3M EspeFiltek Z350 XT(3M ESPE ,St.Paul,MN,USA)
8) Acetic Acid

3.METHODOLOGY

3.1) PREPARATION OF SOLUTIONS:

Three solutions for dentin pretreatment used in the current study were prepared as described below:

10% Sodium ascorbate solution was prepared by dissolving 10 grams of Sodium ascorbate powder in 100 mL of distilled water.

6.5% Proanthocyanidin solution was prepared by dissolving 6.5 ml of grape seed extract liquid (Natures plus, USA) is dissolved in 100 mL of distilled water.

Chitosan solution (Panvo Organics Ltd, Chennai, India) with a degree of acetylation >85% was prepared by dissolving 10 mg in 1 ml of 1% acetic acid (Sisco Research Laboratories-SRL, Mumbai, India) using a magnetic stirrer for 2 h at the rate of 50 rpm at room temperature to bring to a concentration of 1% Chitosan acetate [11].

3.2 SPECIMEN PREPARATION:

Forty freshly extracted, sound human permanent maxillary central incisors free of fractures, craze lines, discoloration, caries were collected for the study. The teeth were cleaned of debris and stored in 0.2% thymol until use. By using a diamond disc, the dentin on both mesial and a distal half of the crown from incisal to the cementoenamel junction was removed until the remaining dentin thickness (RDT) was approximately 1 mm. Each proximal half was treated as a specimen, hence sample size counts to 80. All the specimens were immersed in an ultrasonic bath to get rid of the smear layer formed due to sectioning after which the roots of the specimens were then mounted in self-cure acrylic resin.

These specimens were randomly divided into two groups based on bonding agent applied i.e Group A- universal bonding agent and Group B- all in one bonding agent respectively, and are further subdivided into four groups based on the surface treatment of dentin prior to bonding.

Group IA (n=10) - CONTROL TOTAL ETCH (CTE): The prepared proximal surfaces of the specimens were acid-etched with 37% ortho-phosphoric acid (DeTrey Conditioner 36, DentsplyDeTrey GmbH, Konstanz, Germany) for 15 seconds, rinsed with water for 15 seconds and blot dried.

Adhesive application and composite build up was done according to the bonding protocol as described below. Two successive coats of universal bonding agent TETRIC N BOND were applied on the prepared proximal dentin surface of the specimens according to the manufacturer's instructions, and light curing was done (Astralis 3 light curing unit (530 mW/cm2), IvoclarVivadent, Schaan, Liechtenstein) for 40 seconds. Composite build-up was done by placement of two increments of 2-mm-thick composite resin (3M ESPE FILTEK

Z250 XT, DentsplyDeTrey GmbH) with each increment being light cured for 40 seconds, using a 2.5-mm diameter plastic tube as a matrix.

Group IB (n=10): CONTROL SELF ETCH (CSE): On the prepared proximal surfaces of the specimens all in one bonding agent was applied and cured for 40 seconds. Composite build up was done by placing the increments of 2-mm-thick composite resin.

Group IIA (n=10)-SODIUM ASCORBATE TOTAL ETCH (STE): The etched dentin surface was treated with 10% Sodium Ascorbate solution for five minutes and rinsed with water for 15 seconds, followed by the bonding/ buildup procedure as described above application in group IA.

*Group IIB (n=10)-SODIUM ASCORBATE SELF ETCH (PSE):*The prepared proximal surfaces were treated with 10% Sodium ascorbate solution for five minutes and rinsed with water, followed by the bonding and composite build-up procedure as described above application in group IB.

Group IIIA (n=10)-PROANTHOCYANIDIN TOTAL ETCH (PTE): The etched dentin surface was treated with 6.5% proanthocyanidin solution for five minutes and rinsed with water for 15 seconds, followed by the bonding/ buildup procedure as described above application in group IA.

Group IIIB (n=10)-PROANTHOCYANIDIN SELF ETCH (PSE): The prepared proximal surfaces were treated with 6.5% proanthocyanidin solution for five minutes and rinsed with water, followed by the bonding and composite build-up procedure as described above application in group IB.

Group IV A (n=10)- CHITOSAN TOTAL ETCH (CTE): The etched dentin surface was treated with 1% chitosan acetate solution for five minutes and rinsed with water, followed by the bonding/ buildup procedure as described above application in group IA.

Group IV B (n=10)- CHITOSAN SELF ETCH (CHSE)**:** The etched dentin surface was treated with 1% chitosan acetate solution for five minutes and rinsed with water, followed by the bonding/ buildup procedure as described above application in group IB.

Shear Bond Strength Testing: All the specimens were then stored in distilled water at 37℃ for 24 hours. Shear bond strength was determined using a universal testing machine (LR 100K, Lloyd Instruments, Largo, Florida, USA) by using knife shaped indenter. Sample is kept at an angle of 45° at a crosshead speed of 1 mm/min until fracture .

4. STATISTICAL ANALYSIS:

The shear bond strength were analysed using a SPSS statistical software, version 22. Descriptive statistics presentation of shear bond strength data in Bar chart and values are expressed as mean, SD and CV. One way ANOVA test is done to calculate the p-value ($P < 0.05$) and compare intergroup comparision and the Tukey post hoc test for pair-wise comparisons. In all analysis, $P < 0.05$ was considered to be significant.

5. RESULTS:

The mean shearbond strength (SBS) and standard deviations for all groups were calculated and are shown in Table 1. The highest bond strength was observed for proanthocyanidin total etch group i.e Group IIIA (PTE) 28.7 ± 0.89 MPa ($p < 0.05$) which was significantly higher than all other groups. Proanthocyanidin, Sodium Ascorbate and chitosan acetate pretreatment resulted in statistically significant increase in shear bond strength when compared to control

groups. There was no statistically significant difference between the values of self-etch treated samples.

Table 1: Mean and Standard Deviation (Mean±SD) Of Experimental Groups

EXPERIMENTAL GROUPS	SAMPLE SIZE	SHEAR BOND STRENGTH IN MPa Mean±SD	Coefficient of variation	P value*
Group IA (CTE)	n = 10	18.4 ± 0. 36	1.96	0.65NS
Group IIA (STE)	n = 10	22.3 ± 0.67	3.00	0.031S
Group IIIA (PTE)	n = 10	28.7 ± 0.89	3.10	0.033S
Group IVA (CHTE)	n = 10	24.3 ± 0.60	2.47	0.034S
Group IB (CSE)	n = 10	16.2 ± 0.27	1.67	0.70NS
Group IIB (SSE)	n = 10	16.25 ± 0.27	1.66	0.71NS
Group IIIB (PSE)	n = 10	17.2 ± 0.30	1.74	0.09NS
Group IVB (CHSE)	n = 10	16.3 ± 0.40	2.45	0.77NS

*Statistical significance was determined at *- P<0.05; S- Significant; NS- Not Significant. Mean shear bond strength values are statistically significant between experimental Groups by using one-way ANOVA and Tukeyposthoc test.

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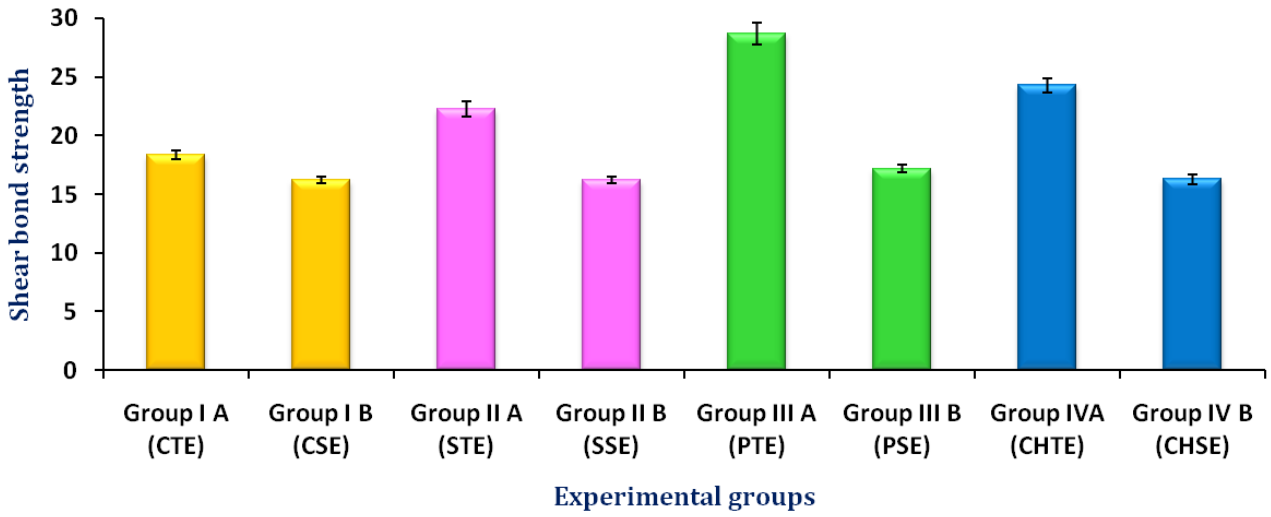
Below are the bar charts representing the comparisions of various groups shown in figure 1.

Figure 1 - Bar chart depicting mean shear bond strength of all experimental groups.



CTE-Control total etch group; CSE-Control self-etch group;

STE- Sodium ascorbate total etch group;SSE- Sodium ascorbateself-etch group;

PTE-Proanthocyanidin total etch group; PSE-Proanthocyanidin self-etch group;

CHTE-Chitosan total etch group; CHSE-Chitosan self-etch group;

6. DISCUSSION:

Bonding to dentin, is a great challenge due to difference in structural and chemical composition of dentin. Some of which include eradication of smear layer, formation of hybrid layer, enzymatic breakdown of collagen [11]. The percentage of collagen fibrils decreases as we proceed from superficial to deep dentin. Large dentinal tubules have less collagen-rich intertubular dentin matrix which make bonding to deep dentin questionable [4, 12, 13].

Post-etching, the collagen fibrils in the peritubular dentin get exposed, and their structural integrity and mechanical properties play an important role in the determination of bond strength and its durability [10].

In the present study, group I (control) recorded a mean shear bond strength value of 18.4± 0.5 MPa. to deep dentin, which is less than the optimal bond strength of resin composite to superficial dentin (20–23 MPa) [14,15]. Macedo et al stated that application of chemical cross linking agents to etched dentin prior to bonding procedures significantly enhanced the dentin bond strengths of caries affected and sound dentin[16]. This is in accordance with previous studies done by Yazici and others [17] who showed that bond strength of resin composite to deep dentin can be as low as 10.3-16.7 MPa.

Results of this in vitro study showed an increase in shear bond strength after pretreatment with 6.5% Proanthocyanidin, 10 % Sodium Ascorbate and 1% chitosan acetate, as compared to control group. This can be attributed to improved dentin collagen stability, due to increase in the number of collagen cross-links, achieved by the use of these collagen cross-linkers [18].

Proanthocyanidin interacts with proteins and increases the Type I collagen cross-linking by promoting hydrogen bond formation between protein amide carbonyl and the phenolic hydroxyl groups [11].

Walter and others showed that 0.5% proanthocyanidin efficiently stabilized collagen and increased its resistance to caries compared to 0.625% genipin and 5% gluteraldehyde [19]. Bedran-Russo and others found that naturally occurring cross-linking agents such as 6.5% Proanthocyanidin and 0.625% genipin are capable of stabilizing demineralized dentin collagen more effectively compared to 5% gluteraldehyde [20].

Srinivas et alfound that when deep dentin was treated with sodium ascorbate, the bond strength reached values (22.12–23.05 MPa), comparable with the optimal bond strength

values of superficial dentin. The results of this study are concurrent with the present study. After surface treatment with proanthocyanidin, the bond strength was increased to levels (27.57-27.85 MPa) that are higher than the optimal bond strength values of superficial dentin [18].

However, Proanthocyanidin presents a disadvantage of discoloration on prolonged duration i.e when used for more than one hour, but clinically as the time of application is only five minutes, this disadvantage is ruled out [21].

Chitosan has showed higher bond strength due to presence of large number of free hydroxyl and amino groups, which interact ionically with amino acids of collagen thus forming a microfibrillar and nanofibrillar network resulting superior mechanical properties including higher resistance to collagen degradation.[22] The organic component of demineralized dentin which is composed of collagen and GAGs forms an electrostatic interactions with the chitosan, which increases the stability of the hybrid layer [23].

Hence, it is recommended that collagen cross-linkers could be employed as safe and effective chairside procedure to overcome the disadvantage of reduced bond strength of composite resin to deep dentin.

7. CONCLUSION:

Within the limitations of the current study, the following conclusions can be drawn;

1. Dentin surface pretreatment with 6.5% Proanthocyanidin, 10% Sodium Ascorbate and 1% Chitosan acetate resulted in significant improvement in the bond strength of resin composite to deep dentin compared to the control group.
2. The present study observed the effect of cross-linking agents for 5 minutes, which is not a time consuming treatment and hence much more clinically feasible.

3. Crosslinking of collagen takes place only when collagen was exposed during etching, but in case of self-etch treated groups, there is no cross linking as it is modified by the resin layer.

CONSENT

It is not applicable**.**

ETHICAL APPROVAL

Before carrying out the present in vitro study, institutional ethics committee approval was obtained from the college.

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COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist.

AUTHOR CONTRIBUTIONS:

This work was carried out in collaboration among all authors. Author VP designed the study,performed the statistical analysis, conductedthe protocol. Author VR, SB analysed the study. Author CG managed the literature searches. Authors VP drafted thefinal manuscript. Author BK, SN conducted the protocol and performed the statistical analysis. All authorsread and approved thefinal manuscript.

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