Original Article

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Dentistry Section

Effect of Chitosan and Proanthocyanidin Dentine Biomodifiers on Immediate and Long-term Bond Strength of Composite to Dentine: An In-vitro Study

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ABSTRACT

Introduction: The longevity of restorations depends on the strength of the bond between the adhesive and the adherent. The use of collagen stabilising agents may be of prime importance in achieving this goal, as they have the capability to increase the bond strength of restorations.

Aim: To compare the efficacy of Chitosan and Proanthocyanidin as dentine biomodifiers on the Shear Bond Strength (SBS) of composite to dentin.

Materials and Methods: This is an in-vitro study conducted in the Department of Conservative Dentistry and Endodontics at Bharati Vidyapeeth Dental College, Pune, Maharashtra, India, over a period of three months from July to September 2024. Twenty freshly extracted premolars with mature apices, free of cracks, restorations, or endodontic therapy, were selected for the study. The teeth were decoronated and etched with 37% phosphoric acid. Half of the samples were pretreated with Chitosan, and the other half were treated with Proanthocyanidin solution. A bonding agent was then applied and cured. Using a plastic mold, composite restoration was performed with specific dimensions, and it was cured. Samples were stored in saline until testing. The samples were then secured in resin and tested using a universal testing machine. Half of the samples (n=5) from each group were tested after 24 hours, while the other half (n=5) were tested after 30 days. An independent t-test and paired t-test were used for intergroup and intragroup comparisons, respectively, where $p<0.05$ was considered statistically significant.

Results: The mean value of SBS in the Proanthocyanidin group was 27.18 MPa after 24 hours, whereas for Chitosan, the mean value was 26.94 MPa. When SBS was compared after 30 days, the mean value of SBS in the Proanthocyanidin group was 21.33 MPa, whereas for Chitosan, the mean value was 20.23 MPa.

Conclusion: The results of the study show that the SBS of Proanthocyanidin was better than that of Chitosan; however, this difference was statistically insignificant, and the bond strength values decreased after storage of samples in saline for 30 days.

Keywords: Adhesion, Collagen, Matrix metalloproteinases, Shear bond strength

INTRODUCTION

The goal of minimally invasive dentistry is to preserve natural tooth structure while preventing and treating caries disease as early as possible. The idea of selectively removing carious tissue is the foundation for the success of this therapy [1]. Necrotic dentine is the only part that needs to be removed, allowing the affected or demineralised dentine at the cavity's base to remain, as it can remineralise. However, since caries affected dentine has a disorganised organic matrix and differs structurally from healthy dentin, careful attention is needed to form a bioadhesive interface on the partially demineralised surface [1].

Teeth defects can result from a variety of causes, including trauma, abrasion, and caries. Teeth cannot mend themselves as they lack cellular components. In clinical settings, the most common method of treating enamel and dentine defects involves the bonding and restoration of composite resin materials. Dentine is a complex tissue composed of minerals, water, and organic substances like collagen. The bonding effect between dentine and composite resin is inferior to that of enamel due to the structural properties of dentine [2].

Current restorative techniques include the use of various synthetic polymers and their infiltration from adhesive systems into the partially or fully demineralised collagen fibers that form the dentin's organic matrix [3]. It has been observed that modern dentine adhesive systems often cause resin-dentine bonds to weaken over time [4]. Therefore, it is crucial to have a comprehensive understanding of all the mechanical, physical, and biochemical elements that influence the stability of hybrid layers [4].

Dentine contains a variety of Matrix Metalloproteinases (MMPs) and cysteine cathepsins, which are typically present in their zymogen form. When a tooth is heated, eroded by acid, decayed, or mechanically prepared, the zymogens break down the dentine collagen matrix. These activated enzymes may cause the exposed collagen fibrils in the hybrid layer to break down and disintegrate. Collagen degradation results in the loss of the anchoring function of the hybrid layer, leading to a decrease in bond strength [2].

Applying various collagen cross-linkers, both synthetic and natural, to the dentine substrate prior to the bonding process can help achieve this [5]. Therefore, it is rational to use collagen-stabilizing agents. When chitin is deacetylated, it creates a biopolymer called chitosan, which is naturally present in yeasts, fungi, insect cell walls, and primarily crustacean shells [1]. Due to its amino groups, the chitosan molecule permits chemical substitution reactions and forms cross-links with dentine collagen. Its adhesiveness arises from electrostatic bonding, where the collagen carboxyl group (COO–) attracts the chitosan amine group (NH3+).

Grape Seed Extract (GSE) contains proanthocyanidins (PAs), which have been shown to enhance the mechanical properties of demineralised dentine [6]. PAs are increasingly popular in the fields of nutrition, health, and medicine. Aside from being non toxic, PAs have demonstrated the ability to strengthen and stabilise type 1 collagen fibrils [7].

Bond strength values may be increased before bonding procedures by pretreating the dentine surface with these agents [8]. Using bioactive materials to modify dental hard tissues before the adhesive procedure is one method to enhance the durability of restorations [9].

Currently, there is no study comparing the effect of bond strength after storing samples in saline for 30 days. Hence, this in-vitro study aims to evaluate and compare the efficacy of chitosan and proanthocyanidin as dentine biomodifiers to improve the bond strength of composite to dentine at two different time intervals. The null hypothesis of the study is that there is no difference between chitosan and proanthocyanidin as dentine biomodifiers, while the alternative hypothesis posits that there is a difference between them.

MATERIALS AND METHODS

This in-vitro study was conducted over a period of three months, from July to September 2024, in the Department of Conservative Dentistry and Endodontics at Bharati Vidyapeeth Dental College, Pune, Maharashtra, India. The institutional ethics committee (EC/NEW/INST/2021/MH/0029) approved the research protocol.

Sample sample calculation: The sample size was statistically calculated and estimated using data obtained from a previous study conducted by Nivedita L et al. The samples were selected using a randomised technique. Twenty non carious premolars, extracted for orthodontic and periodontal conditions, were utilised. All tooth samples were sterilised by immersion in a 10% formalin solution and stored in distilled water until use.

Inclusion criteria: Intact, non carious, unrestored teeth devoid of pulpal aberrations.

Exclusion criteria: Teeth with root cracks, restorations, and previous endodontic treatment were excluded.

Strict anonymisation was maintained while the samples were collected. The samples were then randomly divided into two main groups using a computerised randomisation method. [Table/Fig-1] shows the experimental groups of the study.

Groups	Materials	Subgroups
Group I (n=10)	0.2% Chitosan	Subgroup A (n=05)- Shear Bond Strength (SBS) tested on 1st day.
		Subgroup B (n=05)- SBS tested on 30th day.
Group II (n=10)	2% Proanthocyanidin	Subgroup A (n=05)- SBS tested on 1st day.
		Subgroup B (n=05)- SBS tested on 30th day.

[Table/Fig-1]: Experimental groups of the study.

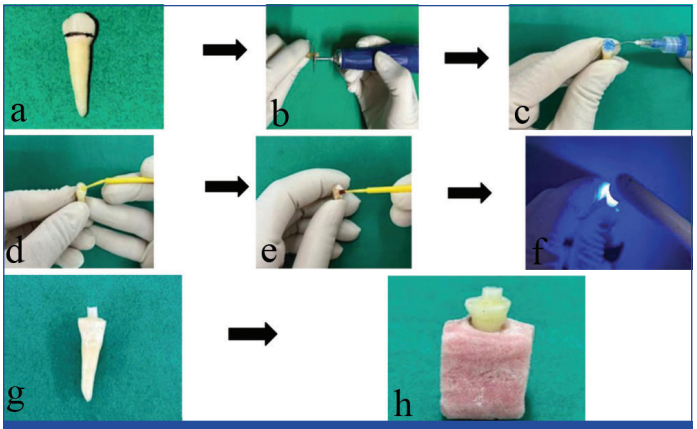
Study Procedure

For sample preparation, all tooth samples were decoronated 2 mm above the cemento-enamel junction using a flexible diamond disc under ample water cooling to maximise dentine exposure [Table/Fig-2a,b]. The dentine was etched for 15 seconds with 37% phosphoric acid (3M ESPE), followed by rinsing and blot drying with absorbent paper pads [Table/Fig-2c]. Pretreatment was done with chitosan and proanthocyanidin.

For the preparation of the 2% Proanthocyanidin solution, 2 g of Grape Seed Extract (GSE) (Inlife) was dissolved in 100 mL of distilled water. A 0.2% chitosan solution (Vedayukt, India) was used [10]. Samples were then divided into two groups:

- Group I: Etching → Rinsing → Pre-treatment with chitosan [Table/Fig-2d]
- Group II: Etching → Rinsing → Pre-treatment with proanthocyanidin [Table/Fig-2e].

Two coats of the respective dentine biomodifiers were applied with a time interval of 30 seconds between each application, and it was allowed to dry. A bonding agent (Adper Single Bond-2, 3M ESPE) was applied and light cured for 30 seconds (Woodpecker Ltd., intensity 1000 mW/cm²) [Table/Fig-2f]. Composite restorations (Filtek Z350, 3M ESPE) were then performed, with a height and diameter of 3 mm, using a cylindrical plastic mold, and cured for 30 seconds [Table/Fig-2g]. Epoxy resin was then used to secure the specimens in position [Table/Fig-2h].



[Table/Fig-2]: a) Line marked 2 mm above CEJ; b) Decoronation of sample; c) Etching of Samples; d) Application Chitosan solution; e) Application of Proantho-cyanidin solution; f) Curing of applied bonding agent; g) Placement of composite; h) Securing samples in resin.

Experimental groups: All the samples were stored in normal saline. Samples in subgroup A were stored for 24 hours, whereas samples in subgroup B were stored for 30 days in saline before testing. The samples were sealed in pouches and submitted to the laboratory, where the laboratory technician was unaware of the grouping.

Fracture testing: A Universal Testing Machine (FIE, UTE, India) was employed to determine the SBS. The specimens were loaded at an average speed of 0.5 mm/min until they fractured. The forces were recorded and then divided by the surface area to determine the amount of SBS in MPa.

STATISTICAL ANALYSIS

Data obtained was statistically analysed using Statistical Package for the Social Sciences (SPSS) v23 software, keeping the level of significance at 5%. An independent t-test was used to conduct the intergroup and intragroup comparisons.

RESULTS

After statistical analysis, it was found that SBS values for the proanthocyanidin group were higher than those for the chitosan group, but the difference was statistically insignificant. The mean value of SBS for the proanthocyanidin group was 27.18 MPa after 24 hours, whereas for chitosan, the mean value was 26.94 MPa. This shows a non significant difference between proanthocyanidin and chitosan as dentine biomodifiers at 24 hours. When SBS was compared after 30 days, the mean value of SBS for the proanthocyanidin group was 21.33 MPa, whereas for chitosan, the mean value was 20.23 MPa.

This shows a non significant difference between proanthocyanidin and chitosan as dentine biomodifiers after 30 days [Table/Fig-3]. The mean bond strength at 24 hours for chitosan was 26.94 MPa, whereas after 30 days it was 20.23 MPa. For the proanthocyanidin group, the mean bond strength at 24 hours was 27.18 MPa, whereas after 30 days it was 21.33 MPa. There was a significant difference between the SBS values at the two different intervals [Table/Fig-4].

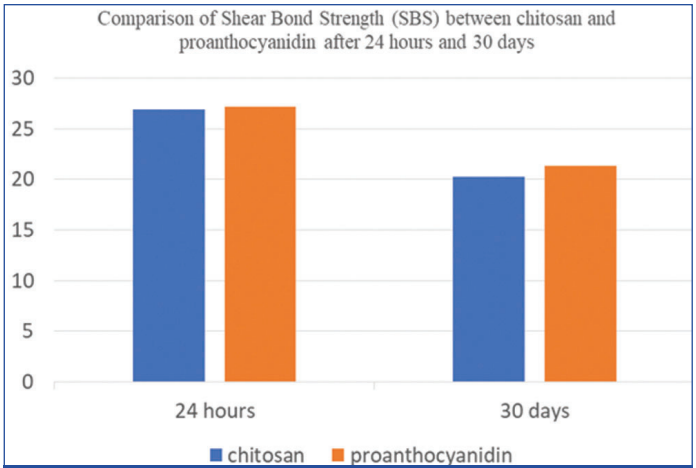
[Table/Fig-5] shows the graphical comparison between the mean values of SBS.

	Chitosan		PAC				
Interval	Mean	SD	Mean	SD	Difference	t-value	p-value
24 hours	26.94	1.44	27.18	0.86	-0.24	-0.318	0.759
30 days	20.23	0.90	21.33	0.84	-1.10	-2.002	0.080

[Table/Fig-3]: Comparison of Shear Bond Strength (SBS) between two groups. Independent t-test, p<0.05 considered as statistically significant,(PAC- proanthocyanidin)

Group	24 hours		30 days		Difference	t-value	p-value
	Mean	SD	Mean	SD			
Chitosan	26.94	1.44	20.23	0.90	6.71	15.009	<0.001*
PAC	27.18	0.86	21.33	0.84	5.85	12.308	<0.001*

[Table/Fig-4]: Comparison of Shear Bond Strength (SBS) within each group at two different intervals.
Paired t-test; *indicates a significant difference at p≤0.05 (PAC: proanthocyanidin)



[Table/Fig-5]: Comparison of Shear Bond Strength (SBS) between chitosan and proanthocyanidin after 24 hours and 30 days.

DISCUSSION

To prevent collagen loss, various strategies are being explored to reduce degradation of the dentine adhesive interface during the bonding process. These strategies include inhibiting collagenolytic enzymes or enhancing collagen resistance to degradation through cross-linking mechanisms [11].

The bonding of adhesive to dentine functions best when the hybrid layer, made of collagen fibrils and resin monomers, is structurally stable [5]. Disaggregation of the hybrid layer, primarily due to the activation of dentine MMP, jeopardizes long-term bonding [12]. The organic framework of dentine is composed of 90% fibrillar Type I collagen and 10% non collagenous proteins, which include proteoglycans and phosphoproteins. Type I collagen imparts viscoelasticity to the tissue and acts as a scaffold for the apatite mineral phase to be deposited [5].

Reports indicate that chitosan is a significant biomaterial that can stabilise the adhesive interface and prevent metalloproteinases from breaking down the dentine organic matrix by creating cross-links with collagen fibrils [1]. The current study assessed the ability of chitosan as a dentine biomodifier. The mechanical performance of the chitosan treated groups in this study can be attributed to chitosan's ability to cross-link with dental collagen, producing a mechanically strong fibril chain, corroborating findings from other studies [1,13]. Chitosan-incorporated and cross-linked dentine collagen is resistant to deterioration [8].

The choice of 0.2% chitosan was based on previous studies, as literature indicates that maximum effects of chitosan are observed at this concentration [14]. The mean bond strength of the chitosan group after 30 days was recorded at 20.33 MPa. The bond strength significantly decreased after storage in saline for 30 days. The primary cause of the gradual decline in bond strength is the degradation of the dentine collagen matrix, following the activation of metalloproteinases with gelatinolytic activity within the matrix [1].

Alahdal K et al. conducted a study to assess the impact of different dentine bio-modifiers, namely Bromelain, Riboflavin Photosensitiser (RFP/Ultraviolet-A), and Chitosan Nanoparticles (CHNPs), on the SBS and microleakage of composite bonded to acid-etched Carious Affected Dentine (CAD). They concluded that the lowest microleakage and highest SBS were observed in Group 2 (Bromelain), while the lowest SBS among the experimental groups was seen in the CHNP group, which was non significant. This was attributed to the capability of the bromelain enzyme to act as a deproteinising agent, effectively enzymatically cleaving proteins into smaller amino acid chains. This deproteinisation process facilitates the removal of exposed collagen fibrils and the organic components within the smear layer [15].

Proanthocyanidin is a Grape Seed Extract (GSE) known to influence the bond strength between composite and dentine [16]. In-vitro and in-vivo models suggest that seed extract inhibits MMP activity and safely cross-links collagen [11,16]. In the study, a 2% concentration of Proanthocyanidin was used, as higher concentrations than 2% may disrupt the formation of linear polymer chains due to the increased density of the molecules. This could lead to insufficient resin polymerization and the creation of microvoids, weakening the resin-dentine interface. It may also prevent the resin's free radical polymerization due to its ability to scavenge free radicals [7].

The mean SBS of the Proanthocyanidin group after 24 hours was found to be 28.17 MPa, which was more than that of the Chitosan group. This can be explained by the fact that even in demineralised dentin, Proanthocyanidin has the capacity to link with proline-rich proteins, promoting instantaneous attachment and stability to collagen fibers [3,17,18]. Strong insoluble bonds are formed between the carbonyl amide group of dentine collagen and Proanthocyanidin's phenolic hydroxyl group [3].

The superior performance of Proanthocyanidin can be explained by the interaction between collagen and Proanthocyanidin, which can make demineralised dentine harder while preserving the integrity of collagen's complex structure. This promotes remineralisation, prevents collagenase activity, and facilitates the interdiffusion of monomers [9]. There are several possible ways that Proanthocyanidin and proteins interact, including ionic, covalent, hydrogen bonding, and hydrophobic interactions [14].

Castellan CS et al. conducted a study to evaluate the long-term resin-dentine bond strength of dentine biomodified by proanthocyanidin-rich (PA) agents. The highest bond strength was observed in the 6.5% GSE group, followed by the 6.5% Cocoa Seed Extract Ethanol-Water (CSE-ET) group, and the least was seen in the 6.5% cocoa seed extract acetone-water (CSE-AC) group. The bond strength also decreased after the storage of the samples for 12 months [19].

Srinivasulu S et al., studied the SBS of composite to deep dentine after treatment with two different collagen cross-linking agents, namely proanthocyanidin and sodium ascorbate, at varying time intervals. They concluded that specimens treated with proanthocyanidin showed significantly higher SBS values than those treated with sodium ascorbate [5]. Studies conducted by Bharati N et al. discovered that pretreatment with proanthocyanidin enhanced the ultimate tensile strength and the SBS [16].

Our results do not align with a study conducted by Nivedita L et al., which compared the efficacy of chitosan and proanthocyanidin, where chitosan performed better than proanthocyanidin. This discrepancy might be due to the differences in the concentrations used in the experimental groups [8]. A study by Al-Ammar A et al. compared the effect of three different cross-linking agents (Glutaraldehyde (GD), Grape Seed Extract (GSE), and Genipin (GE)) on the Tensile Bond Strength (TBS) of resin-dentine bonds. In this study, GSE showed higher micro-TBS, likely due to its greater interaction ability with collagen [20].

As a statistically significant difference was not observed between chitosan and proanthocyanidin in terms of SBS, the null hypothesis was accepted.

Limitation(s)

It is possible that the outcomes of the in-vitro tests will not be directly comparable to in-vivo settings, where additional factors must be

considered. To maximise the benefits of clinical adhesive dentistry, in-vivo research is necessary for evaluating the clinical outcomes of these agents.

CONCLUSION(S)

Under the limitations of the study, it can be concluded that the SBS of proanthocyanidin was found to be better than that of chitosan; however, the difference was statistically insignificant. When comparing time intervals, the SBS values were higher for Subgroup A (i.e., at 24 hours) than for Subgroup B (i.e., after 30 days) for both proanthocyanidin and chitosan groups. SBS decreased after the samples were stored in saline for 30 days.

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