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COMPARATIVE EVALUATION OF DIFFERENT PRECONDITIONING AGENTS ON IMMEDIATE AND DELAYED BOND STRENGTH OF DENTIN TO COMPOSITE USING SINGLE BOTTLE SYSTEM: AN EX VIVO STUDY

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ABSTRACT **Objective:** This study was aimed to assess the dentinal bond strength following the selective application of Tetracycline, Proanthocyanidin, and Chlorhexidine (CHX) to the dentin, tested immediately (24 hours) and after six months of aging in artificial saliva. **Materials And Method:** Eighty extracted single-rooted human teeth were randomly selected and divided into four groups: Group I (control, no preconditioning), Group II (preconditioned with 2% CHX), Group III (preconditioned with Tetracycline), and Group IV (preconditioned with Proanthocyanidin). Each tooth was sectioned to obtain a flat surface, and a smear layer was created. Preconditioning was done for 30 seconds, followed by the application of a self-adhesive bonding agent and composite buildup. Samples were stored in artificial saliva and divided into two subgroups: immediate testing after 24 hours and delayed testing after six months. **Results:** The Proanthocyanidin group showed statistically higher bond strength values compared to the 2% CHX, Tetracycline, and control groups for both immediate and delayed tests. All groups exhibited higher bond strength values at six months compared to 24 hours. **Conclusions:** Dentin pretreated with Proanthocyanidin significantly increased micro-tensile bond strength at both 24 hours and six months compared to other groups.

KEYWORDS :

INTRODUCTION

Composite resins are being popular due to their low invasiveness and high aesthetic value. Over the decades, composite technology has improved, making resin composites the material of choice for both anterior and posterior restorations.[1]

Dentin is a mineralized tissue consisting of inorganic apatite crystals embedded in an extracellular organic matrix (EOM). Type I collagen is the primary component, making up about 90% of the organic material. Additionally, various proteins known as non-collagenous proteins constitute approximately 10% of the matrix, including proteoglycans, phospholipids, and enzymes. Among these enzymes, matrix metalloproteinases (MMPs) have recently gained significant attention due to their potential roles in various physiological and pathological processes in dentin.[2] MMPs are zinc- and calcium-dependent endopeptidases involved in tooth development, the progression of dentin caries, and the degradation of the hybrid layer in composite resin restorations.[3]

Under physiological conditions, these proteases are secreted by odontoblasts during dentinogenesis and remain entrapped within the extracellular matrix of dentin in an inactive form after matrix mineralization. The acidic environment resulting from caries processes, including demineralization with phosphoric acid or acid resinous monomers from self-etching adhesives, activates various dentin MMPs like MMP 2 and MMP 9 present in the dentin[4,5]. These activated MMPs degrade components of the extracellular matrix, primarily type I collagen[6], which are not properly infiltered by resin monomer during hybrid layer formation thus compromising the bond strength, which directly affects the longevity of adhesive restorations.[7] Therefore, inhibiting MMPs may be a key strategy in improving dentin adhesion.

Various materials have been shown to hinder or degrade the activity of MMPs, chlorhexidine (CHX) is one of the most commonly studied MMP inhibitors[8] has found to function as a potent MMP inhibitor, by preventing or delay the interfacial degradation of the dentin-resin bond, preserving the bond strength.[9]

In addition, PA also have MMP inhibitory effect, by the dehydration of collagen, consequently enhancing adhesive penetration and reduced water sorption. These could contribute to the stability of the hybrid layer and its resistance to MMPs' activity.[10]

Tetracycline and their semi-synthetic analogues doxycycline and minocycline are a family of broad-spectrum antibiotics with cationic chelating proprieties that also have been reported to serve as effective MMPs inhibitors. In addition to zinc chelation, they can down-regulate MMPs mRNA expression. Doxycycline, the only clinically available tetracycline with MMPs inhibiting properties, strongly decreases dentin matrix degradation.[11-12]

Microtensile bond strength (μTBS) is a critical parameter used to evaluate the adhesive performance of dental materials, particularly the bond between dentin and composite resins, The authors currently recommend the micro-tensile bond strength test (μTBS), especially after subjecting specimens to a durability challenge, as the best surrogate measure for evaluating the retention of dental composite restorations.[13]

Thus, the present study was done to evaluate and compare the different agents (chlorhexidine, proanthocyanidin, and tetracycline) used for the degradation on MMPs activity thereby enhancing the bond strength of dentin to composite.

MATERIALS AND METHOD

The study was performed in single rooted eighty extracted noncarious, human teeth. The teeth were examined under stereomicroscope

(Olympus, Tokyo, Japan) and teeth free of caries, cracks, or any developmental defects were included. Teeth were cleaned of debris. Calculus was removed using ultrasonic scaler and then the teeth were stored in 0.5% Chloramine T Trihydrate (Sigma Aldrich, Bangalore, India) for no more than 3 months. Tooth crowns were flattened using a low-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under water irrigation unless superficial dentin was visible and a standardized smear layer was created with 600-grit silicon-carbide (SiC) paper. The samples were embedded in an autopolymerizing resin at the level of cementoenamel junction with long axis perpendicular to the acrylic resin surface. Teeth were randomly divided into four groups according to different agents used for preconditioning of dentin.

1) Group I (n=20): no preconditioning was done.
2) Group II (n=20): preconditioning done with 2%CHX.
3) Group III (n=20): preconditioning done with Tetracycline.
4) Group IV (n=20): preconditioned done with Proanthocyanidin.

Each group were further, subdivided into immediate test group (A) and delayed test group (B). In the immediate test group, micro tensile bond strength test was performed after 24 hours and in delayed test, after 6 months bond strength was evaluated.

Each tooth was sectioned 3mm from the occlusal surface to exposed the dentin using a low-speed saw under copious water irrigation, such that a flat surface, was obtained.

A 600-grid silicon carbide paper was used to rubbed on exposed dentin surface to create smear layer. All the test material was prepared in solution form, for pre-treatment [mixed with distilled water].

Teeth were divided into four groups depending on agents used for preconditioning.
I. Control group - no preconditioning was done (n=20)
II. Test group – preconditioning done with 2% CHX (n=20)
III. Test group – preconditioning done with Tetracycline (n=20)
IV. Test group –preconditioning done with Proanthocyanidin (n=20)

Preconditioning was done for 30 seconds followed by rinsing with distilled water for 10 seconds and air drying.

With the help of applicator tip a self-adhesive bonding agent (CLEARFIL TRI-S BOND UNIVERSAL- KURRARY) was then applied on each sample and light cured for 20 seconds. Subsequently, composite buildup of 2x2 mm dimension was done in incremental method and each increment was cured for 40 seconds.

The samples were then be divided into two subgroups depending on the time of storage.
1. Subgroup A (Immediate test group) – samples were stored in artificial saliva for 24 hours.
2. Subgroup B (Delayed test group) – samples were stored in artificial saliva for 6 months.

All the samples were then stored in artificial saliva in a closed container.

Sample Testing:
After storage for either 24 hours for subgroup A and 6 months for subgroup B all the samples were subjected to micro tensile bond strength using a universal testing machine (ASIAN, India). The samples were tested at a crosshead speed of 1mm/min. The tensile load, at which the debonding of composite occur with dentin was evaluated, and the micro tensile strength was calculated according to the area of the fractured surface.

Statistical Analysis
The data were collected and statistical analysed using the SPSS statistical software 23.0 Version. The level of the significance for the present study was fixed at 5%.

RESULT
The intergroup comparison was done using the One Way ANOVA followed by post Hoc Analysis. The intragroup comparison between the different time intervals was done using Paired t test. depending upon the normality of the data.

Intergroup Comparison Of Microtensile Bond Strength At 24 Hours

Table 1 One Way ANOVA Test With P Value Less Than 0.05 Is Significant.

	Mean	Std. Deviation	Std. Error	Minimum	Maximum
Group I	23.94	15.430	6.041	9.81	78.32
Group II	43.94	25.430	8.041	9.81	78.32
Group III	47.51	10.538	3.332	33.34	63.71
Group IV	77.04	38.539	12.187	43.86	143.11

Table 2 Post Hoc Analysis

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	7722.896	3	2574.299	7.563	0.001 (Sig)
Within Groups	26007.820	36	722.439		
Total	33730.716	39			

Table 3

		Mean Diff	Std Error	P value	
Group I	Group II	20.000	12.020	0.012	Significant
Group I	Group III	-23.569	12.020	0.009	Significant
Group I	Group IV	-53.102*	12.020	.009	Significant
Group II	Group III	-3.569	12.020	0.768	Non-Significant
Group II	Group IV	-33.102*	12.020	0.009	Significant
Group III	Group IV	-29.533*	12.020	0.019	Significant

The above tables show that the bond strength was significantly higher in the Group IV as compared to other three Groups. The bond strength was lowest in the Group I and significantly lower than all other three groups. The bond strength of Group II and Group III was higher than Group I and lower than Group IV but the difference in bond strength of Group II and Group III was not statistically significant.

INTERGROUP COMPARISON OF MICROTENSILE BOND STRENGTH AT SIX MONTHS

Table 4 One Way ANOVA Test With P Value Less Than 0.05 Is Significant

	Mean	Std. Deviation	Std. Error	Minimum	Maximum
Group I	76.60	13.015	4.115	61.68	96.11
Group II	119.46	7.513	2.376	100.51	128.36
Group III	131.35	8.637	2.731	109.83	139.11
Group IV	151.85	9.043	2.859	139.25	169.06

Table 5 Post Hoc Analysis

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	30269.208	3	10089.736	105.579	0.001 (Sig)
Within Groups	3440.375	36	95.566		
Total	33709.583	39			

Table 7

		Mean Diff	Std Error	P value	
Group I	Group II	-42.857	4.371	0.001	Significant
Group I	Group III	-54.751	4.371	0.001	Significant
Group I	Group IV	-75.249	4.371	0.001	Significant
Group II	Group III	-11.894	4.371	0.010	Significant
Group II	Group IV	-32.392	4.371	0.001	Significant
Group III	Group IV	-20.498	4.371	0.001	Significant

The above tables show that at 6 months time interval (Delayed test) the bond strength was evaluated using the same method. At 6 months time interval the bond strength was highest in the Group IV (151.85), followed by Group III (131.35), Group II (119.46) and least in the Group I (76.60). The comparison of bond strength between the four groups was done using the One-Way ANOVA test.

The result of the statistical test revealed that the bond strength was significantly higher in the Group IV as compared to other three Groups. The bond strength was lowest in the Group I and significantly lower than all other three groups. The bond strength of Group II and Group III was higher than Group I and lower than Group IV and the difference in bond strength of Group II and Group III was also statistically significant with higher bond strength in the Group III as compared to Group II.

Intragroup Comparison Of Microtensile Bond Strength At 24 Hours And Six Months In Each Group

Table 8 Paired T Test With P Value Less Than 0.05 Is Significant

	Immediate		Delayed		P value
	Mean	Std. Deviation	Mean	Std. Deviation	
Group I	23.94	15.430	76.60	13.01	0.001 (Sig)
Group II	43.94	25.43	119.46	7.51	0.001 (Sig)
Group III	47.51	10.53	131.35	8.63	0.001 (Sig)
Group IV	77.04	38.53	151.85	9.04	0.001 (Sig)

The table depicting that at 24 hours' time interval the micro-tensile bond strength was highest in the Group IV (77.04), followed by Group III (47.51) and least in the Group I (23.94). At 6 months time interval the bond strength was evaluated using the same method, the bond strength was highest in the Group IV (151.85), followed by Group III (131.35), Group II (119.46) and least in the Group I (76.60). Between 24 hours to 6 months there was significant increase in the bond strength in all the four groups (p=.001) when analysed using Paired t test.

DISCUSSION

Several substances are known to inhibit MMPs, including chlorhexidine (CHX), ethylenediaminetetraacetic acid (EDTA), tetracycline compounds (minocycline and doxycycline), ammonium compounds, and proanthocyanidin (PA).

Chlorhexidine, used in this study, has shown good adhesive performance at the resin/eroded-dentin interface. Kim et al. (2010) suggested that CHX remains trapped within the interfibrillar spaces, resulting in a significant increase in CHX uptake by demineralized matrices.[14]

It was also suggested that CHX prevents the binding of ions such as Zn2+ and Ca2+ to the MMPs structure, thereby inhibiting its catalytic activity.[15]

Breschi et al. (2010) proposed that applying CHX to the etched dentin layer before adhesive or resin placement increases bond strength and extends the shelf life of dental adhesives.[16]

Other agents, such as PA and tetracycline, have also been studied, but there are limited comparative studies evaluating their effects on micro tensile bond strength after pretreatment with CHX, PA, and tetracycline as dentin pretreatment agents. In the present study, the micro tensile bond strength was evaluated and compared after application of these preconditioning agents at 24 hours and six months. In our study it was found that samples pretreated with PA at one month had higher μTBS compared to those pretreated with tetracycline and CHX. The tetracycline group at 24 hours, and in delayed test at six months showed the second highest bond strength, which was more than CHX (at 24 hours, and at six months). The control group (no preconditioning) showed the least bond strength at both 24 hours and six months.

The superior performance of the PA group may be due to the fact that PA is cross- linking agent which promotes the chemical bond with the collagen fibrils, based on a high affinity and specificity for interaction with collagen proteins. This is achieved through several chemical mechanisms, e.g. hydrogen bonding, hydrophobic interactions, covalent and ionic bonds, which produce collagen cross linking.[16] Liu et al. (2014) suggested that PA biomodification effectively inhibits proteolytic activity on demineralized dentin matrix and stabilizes the adhesive/dentin interface against enzymatic degradation.[17]

Epasinghe et al (2013) who demonstrated that PA can inactivate more than 90% of soluble recombinant MMP-2, 8 and 9 and approximately 70%–80% of cysteine cathepsin B and K and these results were significantly better than those derived from the use of CHX.[18]

Comparing the tetracycline group to the CHX group, tetracycline showed better bond strength preservation, statistically non-significant at 24 hours but significant after six months. Tetracycline acts as a non-competitive inhibitor of MMPs by interacting with zinc or calcium atoms in the catalytic domain. De Carvalho et al. (2020) also suggested that doxycycline, a semi-synthetic tetracycline antibiotic, positively affects MMP-2 inhibition and μTBS.[19]

In this study, CHX increased dentin bond strength compared to the control group but was the least effective compared to PA and tetracycline. Carrilho et al. (2007) reported significantly less failure and higher micro tensile bond strength in the hybrid layer with CHX, suggesting its usefulness in preserving dentin bond strength.[9]

This study also showed that μTBS increased significantly over time. The bond strength for the PA group was highest after six months, followed by the tetracycline group and the CHX group. Even in the control group, bond strength increased significantly over time.

This finding aligns with Ming Fang et al. (2012), who stated that Collagen cross-linking treatment by PA-based preconditioners presented a concentration- and time-dependent increase in dentin bond strength even in reduced, clinically applicable treatment duration.[20]

Singh et al. (2017) also found that dentin biomodifiers like carbodiimide (EDC), epigallocatechin 3-gallate (EGCG), and minocycline (MI) significantly preserved resin-dentin bond strength after six months.[21]

Bharti et al. (2017) suggested that treatment of dentinal surfaces with collagen cross-linking agent increases the shear bond strengths. As these agents are considered to be inhibitors of MMPs and cysteine cathepsins. Thus, pretreatment can promise a significant improvement in the bonding mechanism preserving the integrity of collagen hybrid layer which was also, confirmed in this study, where the pretreatment of dentin with all the preconditioning agents significantly improved the micro tensile bond strength immediately as well even after six months.[22]

Incorporation of MMP inhibitors at any step of the dental adhesive system reduces the rate of bond strength degradation, which contributes to the longevity of dental resin-based restorations. Thus, incorporation of matrix metalloproteinase inhibitors in adhesive systems could be a viable option, as the incidence of secondary caries development shall be reduced considerably.

CONCLUSION

The present study concluded that, all the three tested pretreatment agents increased the μTBS. Maximum μTBS was seen in PA followed by Tetracycline and CHX. The μTBS values increased with time (24 hours versus six months) in all study group. Thus, MMP-inhibiting agents could be considered for the pretreatment of teeth.

However, the study had certain limitations like long term testing of bond stability was not done and the present study was an invitro study. Also, only tensile bond strength was taken into account. Furthermore, future clinical studies are required to assess its effectiveness as a preconditioning agent in the long term and under the challenge of proteolytic agents in the clinical scenario.

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