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Ethanol based vs Aqueous based Proanthocyanidin pre-treatment and its effect on micro-tensile bond strength of composite restorations - An in vitro study.

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I. Introduction:

Aesthetic dentistry has tremendously transformed patients' perspectives of dentistry. Resin composites have been extensively employed in restorative dentistry to meet the patients' demands for aesthetics. There has been an evolutionary development in composite resins to best suit the clinical challenges and requirements. But polymerization shrinkage and hydrolysis of hybrid layer have been the perennial difficulties compromising the longevity and integrity of composite restoration leading to post-operative sensitivity, secondary caries formation and finally failure of restoration[1]. Human dentin matrix contains MMP-2, MMP-8, MMP-9, MMP-14, and MMP-20 as inactive zymogens. As a result of the local acidic pH (etching), these MMPS are activated and released from dentin upon the dissolution of inorganic compounds. These proteases act on the collagen fibres that are incompletely enveloped by the resin monomers during bonding process. Degradation of collagen fibres by the matrix-metalloproteases (MMPs)leads to degeneration of hybrid layer. The hybrid layer can be stabilized either by inhibiting the activity of MMPS or making certain that excess moisture around the collagen in the demineralised matrix is completely replaced by resin monomer. Natural substances that mimic the action of biological tissue inhibitor of MMP's have been used to inhibit the collagenolytic activity or to stabilize collagen [2]. In comparison with the common synthetic crosslinkers such as glutaraldehyde and carbodiimide, proanthocyanidin (PA) has shown to be effective cross linker. Cytotoxicity of glutaraldehyde and carbodiimide material limits their use as collagen linkers[3]. Proanthocyanidine is an extract obtained from grape seed and is capable of MMP inhibition and collagen stabilization of acid etched dentin[4]. PA pretreatment also increases the elastic modulus and hardness of resin-dentin interface structures [5].Ethanol and acetone have been commonly referred to as water chasers.The ethanol wet-bonding concept is based on the chemical property of dehydration. Thus during the dentin bonding process, the use of ethanol promotes hydrophobic resin infiltration by gradually replacing water within the demineralized dentin matrices with resin monomers into a resulting into a hybrid layer[6,7]. Most of the studies in the earlier literature have used the aqueous preparations PA. Thus it was hypothesized that PA solution prepared with ethanol would have synergistic effect in protecting the hybrid layer from degradation. The objective of this paper was to test if the bond formed by ethanol-based PA was stronger than the aqueous based solution when used for pretreatment of acid etched dentin. This study tested the null hypotheses that the micro tensile bond strength of resin-dentin bond is not altered by ethanol or by aqueous-based PA preparations.

II. Methodology:

30 non-carious maxillary and mandibular premolars that were extracted for orthodontic reasons were collected. The roots of the teeth were removed 2 to 3 mm below the cement- enamel junction. Teeth were vertically embedded in self- cure resin up to 1mm below Cemento–Enamel Junction.

Preparation of specimens:

Conventional MOD cavities were prepared with a depth of 3mm (floor in dentin) and width 1.5mm to reduce the 'C' factor and to emulate the clinical conditions. The tooth preparations were acid etched with 37% phosphoric acid for 15 seconds, followed by water rinse for 1 minute and blot dried. According to the bonding procedure, the specimens were randomly allocated to 3 groups (n = 10), i.e,

Group 1: without any cross linker (control)

Group 2: PA with ethanol (99.9%)
Group 3: PA with distilled water

Preparation of Proanthocyanidin solution: Purified proanthocyanidin(PA) from grape seed extract was obtained from USP sigma Aldrich. 2% solution was prepared by mixing 2g of PA in 100 ml of distilled water and 100 ml of 99.9% ethanol, respectively.

Bonding procedure: Both the test solutions were applied for one minute on the individual specimens and the excess was blotted (Group 2 and 3). Later, ethanol based bonding agent (Tetric N Bond, Ivoclar) was applied on all the specimens, agitated with a microbrush for 10 secs followed by gentle air stream to evaporate the excess solvent and Blue phase N LED light(Ivoclarvivadent) cured for 20 seconds. All the tooth preparations were restored with composite in incremental layers (Tetric N Ceram, Ivoclar). Teeth were stored in distilled water at room tempperaturefor a 24 hours after composite restoration.

Bond strength (μTBS**) testing:** At the completion of the storage period, teeth were sectioned longitudinally to obtain composite dentine beams of 1×1 mm thickness with the help of diamond disc. Two beams were retrieved from widest slabs of each tooth. Thus 20 specimens were obtained for each group (n = 20). The cross-sectional area of each specimen was measured. Bonded specimens were mounted on a custom-made jig and stressed to failure under tension with a Universal testing machine with a speed of 1mm/min. At the separation of resin dentin interface, bond strength was calculated taking the cross sectional area into consideration. Values were expressed in MPa.

Statistical analysis: The bond strength data were analyzed using a Statistical Package for Social Sciences (SPSS) software. All the data collected was first checked for normal distribution with Kolmogorov- Smirnov test. Thus the data was analysed with the parametric, ANOVA significance test. Post hoc multiple comparisons were performed using the Tukey difference test to compare the specific group means.

III. Results

The mean micro-tensile bond strength obtained in Mpa for each group and standard deviations are presented in Table No 1. The application of PA affected the mean bond strength and showed a significant difference (p=<0.001*) in ANOVA analysis. The bond strengths produced by Group 3 specimens (*i.e*. water based PA) gave μTBS values of only 38.91± 0.89 MPa, significantly lower than those of specimens of Group 2 pretreated with ethanol based PA (41.87 ± 1.10 MPa).

Table 2 represents the pairwise comparison between study groups. There was a significant difference among all the groups. The negative control group (Group 1) showed a momentous difference with both the test groups (p=<0.001). The ethanol based and water based PA groups showed a mean difference of 2.95 with a statistically significant difference (p=<0.001).

Table 1 :- Comparison between the study groups

	N	Mean	SD	ANOVA	
				F	p-value
With out Proanthocyanidine	20	34.79	0.93	264.52	<0.001*
Proanthocyanidine with ethanol	20	41.87	1.10		
Proanthocyanidine with water	20	38.91	0.89		

Table 2 :- Pairwise comparison of between the study groups

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	p-value	95% CI	
					Lower Bound	Upper Bound
With outproanthocyanidine	**Proanthocyanidine with ethanol**	-7.08	0.31	<0.001*	-7.82	-6.34
	Propanthocyanidine with water	-4.13	0.31	<0.001*	-4.87	-3.38
Proanthocyanidine with ethanol	**Propanthocyanidine with water**	2.95	0.31	<0.001*	2.21	3.70

IV. Discussion:

The collagenolytic activity in dentin was first reported by Dayan et al. [8], whereas further clarification and attribution of this activity by the matrix metalloproteinases (MMPs) was provided by Tjäderhane et al.[9]. Dentin matrices contain MMPs that were developmentally secreted as inactive proenzymes[10,11].They represent a family of zinc/calcium-dependent endopeptidases that are capable of degrading extracellular matrix components, including collagen. MMP expression is regulated by growth factors such as transforming growth factor-beta (TGF-b) and bone morphogenetic protein-2 (BMP-2). This regulation is essential in dentin matrix

formation and remodelling during physiological and pathological processes. But these proteases get activated during the acid etching step in adhesive procedures [12].The incompletely infiltrated collagen fibres are exposed to the collagenolytic activity of these MMPs, resulting in the degradation of a hybrid layer, thus influencing the quality and mechanical properties of the bonded interface [13]. The bond strength gradually deteriorates, and resulting in the failure of composite restoration. Biomodification of collagen using natural cross linkers has been a novel approach to maintain collagen stability [14].Proanthocyanidin (PA), a plant flavonoid and a potent antioxidant has been demonstrated to be capable of not only inhibiting MMPs but also stabilize collagen on acid etched dentin. PA consists of highly hydroxylated structures and interacts with proline-rich proteins like collagen by covalent linkages, ionic interaction, hydrogen and hydrophobic bonding interactions [15]. Hence, it plays a role of a dentin collagen stabilizer, thereby improving its mechanical properties (increases the modulus of elasticity and nano-hardness of dentin [16]) and increased resistance to biodegradation [17]. PA mixed with the bonding agent and distilled water have proven to improve the micro-tensile bond strength[4]and collagen stability [18].

In the Ethanol wet bonding technique, ethanol instead of water is used to support the demineralised dentin collagen matrix. Ethanol is a better solvent of resin and promotes intimate coating of the collagen with adhesive. It slowly replaces residual water in the dentin matrix by chemical dehydration, thus preventing collagen hydrolysis and plasticization of resin [19]. It is also proven to decrease the collagen fibrillar diameter and increase the inter fibrillar spaces in the hybrid layer, facilitating better resin infiltration [20]. Consequently, it can be hypothesized that ethanol provides better resin sealing of the collagen matrix by decreasing residual moisture, thereby decreasing the collagenolytic activity of the MMPs and increasing the resin dentin bond durability.

Micro tensile bond strength is an indirect measure of anti MMP activity. More the anti-collagenolytic activity of the test materials, better the μTBS. It was introduced to dentistry by Sano et al. to measure the ultimate tensile strength and modulus of elasticity [21]. It permits testing of very small areas and facilitates SEM/TEM examinations of the failed bonds since the surface area is approximately 1mm [22].

In the current study, Group 1 specimens, without pre-treatment, served as a negative control and showed the least mean μTBS (34.79 ± 0.93 MPa) when compared to the groups that were pre treated with PA. The specimens that were treated with aqueous-based PA, Group 3, had higher bond strengths when compared to Group 1, but less when compared to those treated with ethanol-based PA. The improvement in bond strength with aqueous-based PA is in accordance with most of the previous studies [4,18]. So as to have an added advantage of both ethanol and PA, Group 2 specimens were chosen to be pretreated with ethanol-based PA. This permutation proved successful and was evident in the results. Hagerman and Klucher reported that ethanol stimulates PA and collagen interactions and enhance the stability of hydrogen bonds [23]. Ethanol replaces water from interfibrillar and intrafibrillar spaces of collagen also preventing the phase separation. These factors explain the increase in bond strength values for ethanol compared to distilled water. Thus the null hypothesis was rejected. The results of this study indicate that bonding with ethanol-based instead of distilled water facilitates higher bond strengths, primarily when relatively hydrophobic resins are used. The ethanol-based bonding concept may create more hydrophobic hybrid and adhesive layers that absorb less water over time [24]. More research is required to determine if this strategy will lead to more durable resin-dentin bonds.

V. Conclusion:

1. During composite restorations, pre-treatment of tooth preparation (before bonding) with PA improves bond strength.
2. PA mixed with ethanol has shown an increase in the micro-tensile bond strength compared to when mixed with distilled water. (Benefits of collagen cross linker and removal of residual moisture)

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