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Effect of pomegranate peel extract on the shear bond strength of composite resin to dentin using etch& rinse adhesive system

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Abstract
Matrix metallo proteinases are a group of calcium, zinc dependent host derived enzymes which are trapped within the mineralized dentin matrix during tooth development. These enzymes are thought to induce the degradation of bonding interphase in the composite restorations. A recent review suggested that various exogenous MMP inhibitors, can inhibit dentin MMPs, preserve the dentin hybrid layer, and stabilize the adhesive interface and increase bonding strength. However the synthetic inhibitors have the disadvantage of high cytotoxicity, mismatched mechanical properties, or unsatisfied long-term stability. Proanthocyanidins (PAs), as natural cross-linking agents, overcome some of these drawbacks and have been successfully used in the pretreatment of biological tissues to improve their mechanical properties. Recent studies have shown them to have the capability to control the MMPs activity. The objective of this study was to evaluate the effect of pretreatment with pomegranate peel extract on the shear bond strength of a two-step etch and rinse system. Study was carried out in two main groups of teeth samples (control and PPE treated). Half of of the samples in the both groups were subjected aging process. The shear bond strength was tested for all the samples. Results were compared with Immediate and aged samples. Even though mean shear bond strength was slightly higher for PPE treated 2nd groups, it showed that there was no statistically significant difference in bond strength for four groups
Keywords: *Matrix metalloproteinase, Proanthocyanidin, Hybrid layer, Dentin biomodification.*

Introduction

The major short come in the adhesive dentistry is time related loss of resin –dentin bond strength. It is mainly due to the hydrolytic degradation of collagen matrix present in the hybrid layer(1).

According to Nakabayashi, Kojima, and Masuhara (1982), the hybrid layer is the zone of contact between the restorative material and the solid dentin(2). Nakabayashi and team were the first to demonstrate that, resins could infiltrate the etched dentin to form a new structure composed of resin matrix reinforced by collagen fibers. They named it as biocomposite "hybrid layer" (3). In the dentin, efficacy of bonding depends on hybrid layer and resin tags in dentin tubules(4).

Hybridization is the key phenomenon in the bonding of resin-based composite restorations to the dentin. Resultant hybrid layer is unstable and

permeable[5]. One of the major reason for this weakened hybrid layer is incomplete infiltration of resinous monomers in demineralized dentin matrix. It may facilitate permeability to external fluids as well as bacterial enzymes. It also permeable to the endogenous proteolytic enzymes which are slowly released from etched dentin surface after adhesion. All these can impair the performance of the attached interface [5, 6]. Matrix metallo proteinases (MMP) and cysteine cathepsins are the examples for proteolytic enzymes. They may cause degradation of exposed and denuded collagen fibrils at the bottom of hybrid layer. These kind of degradation is more significant in etch and rinse system[3].

Preservation of the collagen matrix integrity by inhibition of endogenous dentin proteases is the key to improve dentin bonding durability. Several approaches to retain the integrity of the hybrid layer and to improve the long-term dentin bond strength have been tested. Komori et al. in 2009 revealed that MMP inhibitors have a role in protection of degradation of collagen matrix by strengthening the hybrid layer[7]. The hybrid layer can be mechanically strengthened by dentin bio modification procedures. It can be categorized in to physical and chemical methods. Physical methods include ultra violet radiation and photo oxidative method with riboflavin. Chemical methods can be done either using synthetic or natural compounds. Synthetic bio modification agents are CHX 2%, Gluteraldehyde, and Carbomide. They are having the limitations like toxicity, mismatched mechanical properties and sub optimal long term stability. Natural bio modification agents are Proanthocyanidin (PA), Genipinetc[8]. Proanthocyanidins are a class of bioflavonoids that are naturally present plant metabolites. They are available in fruits, vegetables, nuts, seeds, flowers and barks.

Pomegranate is an easily available fruit which is having high medicinal value. It contains flavanols, flavonols, anthocyanins, proanthocyanidin and hydrolysable tannins. Normally available pomegranate contain 60-70% of proanthocyanidine in its peel whereas Kashmiri varieties showing 124 GAE of proanthocyanidine content. In current study we choose pomegranate peel for making biomodification agent because making its peel extract is comparatively easy procedure. Pomegranate is cheaper and easily available all year around too [9].

This study used pomegranate peel extract (PPE) with dilution 20% and application time was kept at 1 minute as it is possibly acceptable for most clinicians. Its effect on the shear bond strength of composite bonded with etch& rinse adhesive system was evaluated.

Aims & Objectives

The aim of this study was to assess and evaluate the action of pomegranate peel extract on resin–dentin bond strength using 2 step etch and rinse system. This study also compared resin – dentin bond strength of immediate as well as aged teeth pretreated with pomegranate peel extract.

Materials and Methods

After obtaining the approval for study design, this in vitro study was conducted in Department of Conservative dentistry & Endodontics, Sri Sankara Dental College, Varkala in collaboration with SCTIMST, Poojappura, TVPM. It assesses the effect of pomegranate peel extract (PPE) on resin – dentin bond strength especially using etch & rinse (E& R) system. PPE acts as a natural dentin biomodification agent and for the present study PPE obtained from Arjuna Natural pharmaceuticals, Aluva with dilution set at 20% (i.e. 2gm in 10 ml distilled water) (Figure 1&2).

Figure 1- Pomegranate peel extract

Figure 2: Pomegranate Peel Extract with 20% dilution

The study was carried out in recently extracted premolar teeth, twenty in number. Samples were prepared and dentin was exposed for study by removing 2 mm of coronal region using diamond cutting disk (Figure 3).

Figure 3: Decoronated teeth sample (20 in number)

Then they were mounted on acrylic block. Samples were divided in to two main groups and then subdivided in to two subgroups.

Group I A (Control) --- etching, application of bonding agent, composite placement

Group I B --- etching, application of bonding agent, composite placement, aging process

Group II A--- etching, pretreatment with PPE, application of bonding agent, composite placement

Group II B--- etching, pretreatment with PPE, application of bonding agent, composite placement, and aging process

All the 20 teeth samples were etched using 37% phosphoric acid gel (Eco etch) for 15 seconds and rinsed off.

Group I samples (10 in number - including both A&B sub groups) directly subjected to bonding process. Adhesive (Adper 3M ESPE) was applied and light cured for 20 seconds. Composite (Filtek Z 250 3M ESPE) was placed and cured for 20 seconds. Light curing was done with LED unit (Blue LED, 430-490nm, 11.7 watt).

Group II samples (10 in number –including A& B subgroups) subjected to pretreatment with 20% PPE for 1 minute using applicator tip and rinsed off for 15 seconds, and continued with bonding process. Adhesive applied and cured for 20 seconds followed by composite placement and curing for 20 seconds. All the 20 samples were incubated for 24 hours at 37^0C and Group I B and Group II B samples underwent aging process (i.e. thermal cycling 5^0 -55^0 C for 500 cycles). Samples were collected and subjected to shear bond strength testing (Figure 4) using Universal testing machine at cross head speed 1mm/minute (Instron 3365 UK).(Figure 5).

Figure 4: Measuring shear bond strength in UTM machine

Figure 5: Universal testing machine (Instron)

Statistical Analysis: Comparison of mean resin bond strength between 4 groups was made. Statistical analysis was done with non-parametric Knuskal-walli's test. Analysis was done using SPSS version 23(Statistical package for social science software). Statistically no significant difference was present between groups.

Results

Table 1 shows the maximum and minimum values of shear bond strength obtained for each group. The mean shear bond strength was slightly higher for PPE treated 2nd groups. It showed that there was no statistically significant difference in bond strength in the four groups (p value 0.548> 0.05).

Table 1: Mean value of shear bond strength for each groups with standard deviation

Group	No. of samples	Minimum shear bond strength value(MPa)	Maximum shear bond strength value(MPa)	Mean value of shear bond strength (MPa)	Standard deviation
GP1A (control)	5	17.40	21.30	19.10	1.73
GP1B	5	16.41	19.70	17.98	1.53
GP2A	5	18.42	20.31	19.22	0.80
GP2B	5	16.90	20.10	18.78	1.22

Graph 1 clearly shows aging reduces the mean value of shear bond strength (Figure 6).

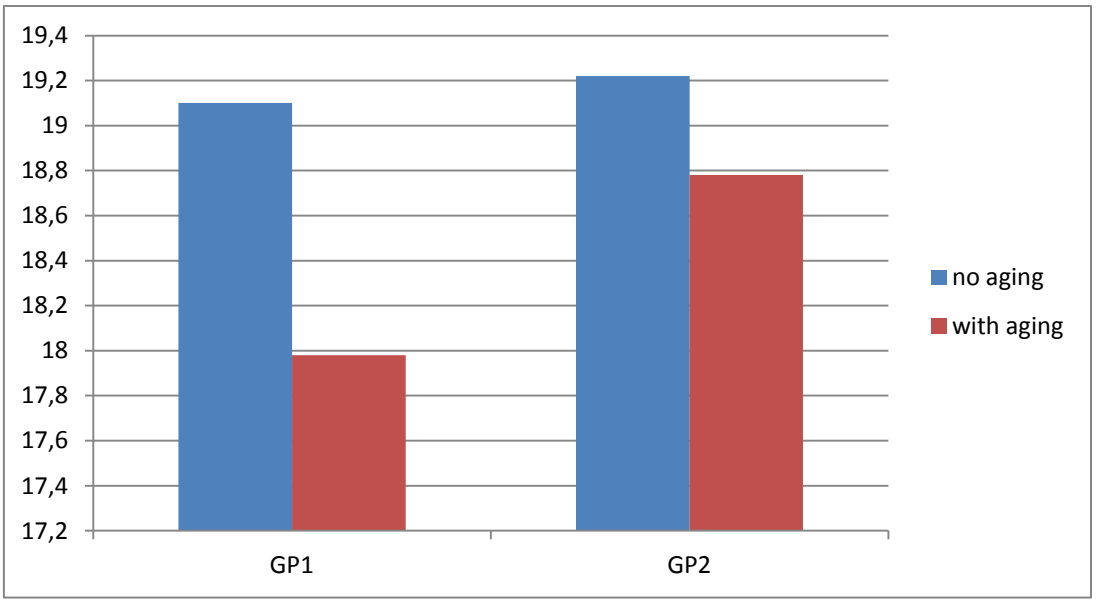

Figure 6 Graphical representations of mean shear bond strength values for various groups

Table 2 shows the mean rank for shear bond strength obtained through Kruskal Wallis Test. The mean rank bond strength for the control group was 11.90 and least for group 1B.The value was highest for PPE treated second group which doesn't undergo aging process. Aging may be the reason for the reduced the mean rank value for 2B group.

Table 2 Kruskal Wallis test showed that there was no statistically significant difference in bond strength in the four groups (p= 0.548).

Kruskal-Wallis Test	Ranks		
	Groups	No of Samples	Mean Rank
Bond strength	1A (control)	5	11.90
	1B (control+aged)	5	7.30
	2A (PPE treated)	5	12.10
	2B(PPE treated +aged)	5	10.70
	Total	20	

Discussion

Studies have shown that reduction in bond strength occurs when resin composite bonded to deep dentin compared to superficial dentin. It may attribute to higher water content in deeper dentin, improper resin infiltration[10]. Integrity of the hybrid layer is also a critical parameter for bond strength and weakened hybrid layer constitute reduced resin - dentin bond strength. Degradation of hybrid layer can occur in a variety of ways in which slow nonbacterial enzymatic degradation occurs by host derived enzymes.

MMPs are important example for host derived enzymes. They are otherwise called matrixins, are calcium dependent zinc c containing endopeptidases. They are normally present in dentin and get activated during low PH created by etchant, once activated it will lead to collagen matrix breakdown in the hybrid layer.

The term biomodification is used for agent-induced effects on dentin. Dentin biomodification procedures are using mainly synthetic and natural chemicals. In order to strengthen the resin dentin bond it is very important to strengthen the hybrid layer. Strengthening of hybrid layer can be achieved by dentin biomodification, especially use of exogenous cross linkers. They are substances that increase number of inter and intra molecular cross linkages in collagen. It will increase mechanical properties and resistance to enzymatic degradation of collagen. eg. Proanthocyanidin, Gluteraldehyde, Genipin.

Proanthocyanidin (PA) which is an exogenous cross linker of collagen molecule ,that establishes cross links through various types of bonding-hydrogen bonds between the protein amide carbonyl and the phenolic hydroxyl groups (Hagerman &Klucher, 1986),covalent , ionic and hydrophobic bonding are also involved (Figure7)[11].

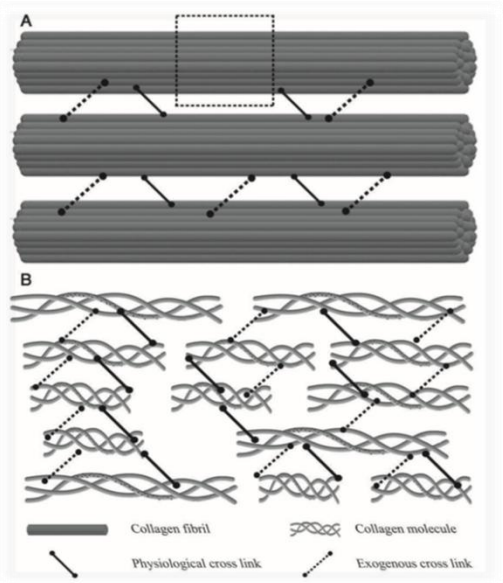

Figure 7: Structure of collagen with exogenous cross linkages

Thus increases thermal stability and resistance to degradation of collagen. It also improves modulus of elasticity thereby increase the stiffness of collagen fibrils. PA will mask the cleavage sites of collagen matrix and make them resistant to collagenase enzyme. They also inhibit collagen molecules from sliding past each other under mechanical stresses. PA also plays role in non-specific MMP inhibition causing chelation and reduced mobility of MMP through cross linkage.

PPE can be used for dentin biomodification as etchant, pre conditioner, or PA incorporated adhesives[12]. PA pre conditioners were preferred for this study (with 20% PPPE for 1 minute application), because it can deliver higher concentration of PA and it will not interfere with curing behavior of the adhesive. Bedran Russo et

al 2007 reported that PA as well as gluteraldehydeis capable of increasing mechanical properties of collagen and thereby stabilizing hybrid layer [13]. As PA is natural component, it is biocompatible and the effect of PA was concentration as well as time dependent[14].

In this study Etch and Rinse system used as adhesive due to its very low PH (0.7) and can elute calcium and zinc ions from matrix and leading to activation of MMPs. The choice of adhesive for the current study was Adper single bond 2 which practice ethanol wet bonding. It is mainly due to the following reasons. Ethanol wet bonding promote replacement of water within demineralized dentin with hydrophobic resin infiltration and formation of hybrid layer. Ethanol wet bonding increases resin infiltration and reduce nanoleakage compared to conventional water wet bonding[15].

Restorations are experiencing heavy occlusal forces and especially the masticatory forces which are similar to compressive and shear force generated at interface of restorations .So shear bond strength was analyzed to get an idea regarding the resin dentin bond strength at interface.

The results of this study showed that biomodification with PPE followed by aging showed slightly higher values to non-pretreated samples. PPE treated samples with immediate testing showed comparable values to non-pretreated samples. It's due to the action of PA present in PPE. These results were in accordance with Radhikaverma et al (2013), Bedran –Russo AK et al (2009)[16, 17].

Conclusion

The present study had limitations like small sample size , reduced pretreatment time and concentration of PPE used, aging not mimic the exact long term oral environment, and the test used for analyzing bond strength was not focusing on molecular mechanism. Within these limitations, this study showed that bio modification with PPE had a positive influence on the resin- dentin bonding. So a conclusion can be drawn that proanthocyanidin is having action on collagen to make additional cross linkages and act as a non-specific MMP inhibitor. This will helps to avoid the major challenge that experienced in field of adhesive dentistry i.e. loss of bond strength. Further research that overcoming current study's limitations is needed to provide scientific data to explore the mechanism in molecular levels in resin-dentin interface.

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