RESEARCH ARTICLE

Application of natural crosslinkers on tooth surface: an in-vitro comparative evaluation of resin-dentin bond strength

Saira Khalid[1], Muhammad Aamir Rafique[2], Abdul Samad Khan[3]

Abstract

Objective: To investigate the effect of natural crosslinkers proanthocyanidin, genipin and glutaraldehyde on shear bond strength at the composite resin-dentin interface.

Method: The in-vitro study was conducted at the Postgraduate Medical Institute, Lahore, Pakistan, from June to September 2018. Exposed dentin surfaces of extracted teeth were conditioned and randomly divided into proanthocyanidin, genipin, glutaraldehyde and control groups according to the type of surface treatment. The dentin surfaces were treated with 6.5% of primers proanthocyanidin, genipin, glutaraldehyde in the relevant groups, while teeth in the control group did not receive any primer application. After thorough rinsing, surfaces of all teeth were restored with a bonding agent and a restorative composite. After 24h, shear bond strength was tested at the Pakistan Council of Scientific and Industrial Research laboratories in Lahore. Pattern of fractures and quality of interface were investigated microscopically at the Lahore campus of COMSATS University, Islamabad. Data was analysed using SPSS 22.

Results: Of the 80 teeth, there were 20(25%) in each of the 4 groups. Surface treatment in the three intervention groups significantly raised the shear bond strength at the composite resin-dentin interface compared to the control group ($p<0.05$).

Conclusion: Chemical modification with collagen crosslinkers improved bond strength at the composite resin-dentin interface.

Keywords: Collagen crosslinkers, Shear bond strength, Resin-dentin interface, Dentin primers.

(JPMA 70: 1363; 2020). **https://doi.org/10.5455/JPMA.17870**

Introduction

Restorative dentistry deals not only with the treatment of diseased tooth, but also looks to principally restore the function as well as aesthetics without compromising the biology.[1] Dental adhesive flows into the microporosities and forms a hybrid layer. The quality of the hybrid layer determines the durability of the bonded interface.[2] Different strategies have been undertaken to improve the durability of the hybrid layer, such as the introduction of bi-functional molecules, which can enhance resin penetration by preventing collagen collapse.[3] Recently, the role of collagen-based cross-linkers has been found to strengthen dentin. They serve to produce new crosslinks between and within the delicate collagen mesh. Moreover, they have also been found to increase the stability of connective tissue, thus enhancing the strength of interface formed between dentin and composite resin.[4]

Glutaraldehyde (GDL) is a synthetic crosslinker[5] which has a great chemical affinity with collagen and 2-hydroxyethyl methacrylate (HEMA). The mechanical properties of demineralised dentin have been improved with the application of GDL.[6] Due to the cytotoxic concerns, the efficacy of naturally occurring crosslinkers has been studied on collagen-based tissues, including dentin.[7,8] Proanthocyanidin (PA) is a plant metabolite available in fruits, vegetables, nuts, seeds, flowers, leaves and bark. Grape seed is a rich source of PA where the seeds belonging to vitis vinafera grape have the highest yield. PA enhances resin-dentin bonds by improving the mechanical properties of dentin.[9,10] Grape seed PA has been found to be the most effective collagen biomodifier[11]

[1]Department of Science of Dental Materials, de'Montmorency College of Dentistry, Lahore, Pakistan; [2]Department of Prosthodontics, Faisalabad Medical University, Faisalabad, Pakistan; [3]Department of Restorative Dental Sciences, College of Dentistry Imam Abdulrahman Bin Faisal University Dammam, Saudi Arabia.
Correspondence: Saira Khalid. e-mail: dmsmdc@gmail.com

that increases the resistance at the dentin-adhesive interface to enzymatic degradation by matrix metalloproteinases and cathepsins, enhances the bond strength of endodontic sealers[12] and reduces caries.[13] PA renders demineralised dentin more receptive towards resin uptake and encapsulation around exposed collagen fibres after acid demineralisation.[14]

Genipin (GE) is another collagen crosslinker isolated from the fruit of jasmine. GE residues are not found to elicit cytotoxicity in tissues and are found to produce a low degree of inflammation compared to GDL, and also increase tissue regeneration.[15] GE increases the mechanical properties of different collagen-based biomaterials and provides better strength to gelatin compared to GDL.[16]

Much work has been put forth in order to make use of different collagen crosslinkers in a clinically feasible manner, but it needs to be further worked out. The current study was planned to investigate the effect of PA, GE and GDL on shear bond strength (SBS) at the composite resin-dentin interface.

Materials and Methods

The experimental study was conducted at the Postgraduate Medical Institute (PMI), Lahore, Pakistan, from June to September 2018. After getting approval from the institutional ethics review committee, the sample size was calculated using World Health Organisation (WHO) formula.[17]

While keeping the expected SBS for control group at 10.8±4.9 and for PA group at 17.7±9.4, power of study at 80% and level of significance equal to 5%.

$$n = \frac{Z_{1-\beta} + Z_{1-\frac{\alpha}{2}}^{2} \left(\sigma_1^2 + \sigma_2^2 \right)}{(\mu_1 - \mu_2)^2}$$

The sample was raised with extracted sound human premolar teeth collected from the Eexodontia Department, which were extracted due to orthodontic treatment. After cleaning by scrubbing, the extracted teeth were stored in normal saline and kept at 4^{O}C to avoid unwanted changes in the teeth that can happen after extraction. All the teeth were brought back to room temperature at 24^{O}C for sample preparation. The teeth were embedded in self-cure acrylic resin to make custom-made acrylic blocks. Tooth reduction was carried out using slow speed carbide saw (Jigsaw blade, type: U Length: 2-1/4, China) under constant water supply. After achieving a flat mid-coronal occlusal dentin surface, the occlusal and axial surfaces were reduced simultaneously until an occlusal diameter of 5mm and a height of 4mm for each tooth was achieved. A surface smear layer was created by grinding occlusal surfaces with 600 grit silicon-carbide abrasive papers. After the sample preparation, the dentin surfaces of sample teeth were conditioned with 10% phosphoric acid gel for 15s. Teeth were rinsed thoroughly with running tap water and dried using absorbent papers according to Kanca's wet bonding technique.[18] Teeth were randomly divided into control, PA, GE and GDL groups.

To prepare the experimental primers PA and GE, 6.5g of 95% grape seed extract powder (Italo Biological Technology Co., Limited. China) was dissolved in 100mL of the solvent (acetone-water 30:70) and 99% genipin powder in 100mL of the solvent respectively. Primer GDL was made by dissolving 6.5mL of 99% GDL in 100mL of the solvent. The potential of hydrogen (pH) of primers PA, GE and GDL were adjusted at 7 by dropwise addition of 1M sodium hydroxide (NaOH) (Sigma Aldrich, UK). The extracted samples were investigated with Fourier Transform Infrared Spectroscopy (FTIR) by using an attenuated total reflection (ATR) accessory (Thermo Nicolet 6700, USA). The resolution was 8cm-1 with 256 scan numbers and the range was 4000-525 cm-1.

Teeth in the control group did not receive any application of experimental primers, whereas the surface of teeth in groups PA, GE and GDL received 60s application of the respective primers by using micro-brush followed by thorough rinsing with running tap water after 60s and they were properly blot-dried using absorbent papers. Then all the teeth in the control and experimental groups entered the bonding phase which was carried out by applying two consecutive coats of bonding agent (Adper Single Bond 2, 3M-Exercise Science and Physical Education (ESPE) using a micro-brush with gentle application to improve penetration. The excess bonding agent was gently air-dried for 5s and photoactivated for 20s with a light-emitting diode (LED) curing unit (Woodpecker Medical Instruments, Co. Ltd..) as per the manufacturer's instructions. For the application of resin-based composite resin (Filtek Z250 XT 3M ESPE, USA; shade A1), a plastic ring with an internal diameter of 5mm and height of 2.5mm was used to build round cylinder of composite resin on the surfaces of prepared teeth as per BS EN 10477 specifications (Dentistry - Polymer-based crown and bridge materials).[19] The composite resin was placed in increments

and each increment was light-cured for 20s.

The restored samples were then stored in deionised water at 37°C for 24h. SBS was determined with Universal Testing Machine (Shimadzu Corporation, Tokyo, Japan). The restored surface was positioned perpendicular to the 0.9mm thick loading shear blade having a chisel-like configuration. The shear blade was placed at the junction of the tooth and composite resin interface following International Organization for Standardization (ISO) standards for bond strength test protocols-TR 11405.[20] The crosshead speed was kept at 0.5mm.min-1 until the debonding occurred.

Data was analysed using SPSS 22. Means and standard deviations were calculated for SBS, and expressed in MPa. One-way analysis of variance (ANOVA) post hoc Tukey's test was used to find significant difference in SBS in different groups. For pair-wise comparison, least significant difference (LSD) test was applied. Whereby, $p<0.05$ was taken as significant. After testing, specimens were examined under electronic zoom microscope (Olympus SZ x 7, Model SZ2-ILST Japan) and modes of failure were classified as adhesive (at the interface), cohesive (within the composite or tooth substrate) and mixed (combination of adhesive and cohesive failures). Representative samples of each group were prepared for optical microscopy analysis of the hybrid layer. Restored samples were kept under an electronic optical microscope (Optika B-600 MET, range 5X-1000X) at 100x magnification to observe the resin-restorative interface.

Results

Total of the 80 teeth, there were 20 (25%) in each of the 4 groups. Surface treatment in the three intervention groups significantly raised the shear bond strength at the composite resin-dentin interface compared to the control group ($p<0.05$) (Figure 1).

Significantly more fractures occurred at the interface for the control group (p=0.002), while cohesive failure within the tooth was highest in PA group (p=0.001). The number of mixed type of fractures were high in GE group (p=0.139)

Table: Type and percentages of fracture pattern.

Fracture Pattern	Study Group				Total Samples	p-value
	Control	PA	GE	GDL		
Adhesive Fracture	15 (75%)	4 (15%)	5 (25%)	7 (35%)	31 (38.8%)	0.001[a]
Cohesive Fracture	2 (10%)	16 (80%)	8 (40%)	11 (55%)	37 (46.3%)	< 0.001[a]
Mixed Fracture	3 (15%)	1 (5%)	7 (35%)	2 (10%)	13 (16.3%)	0.098[b]

[a]Chi-sqaure, [b]Fisher's exact test

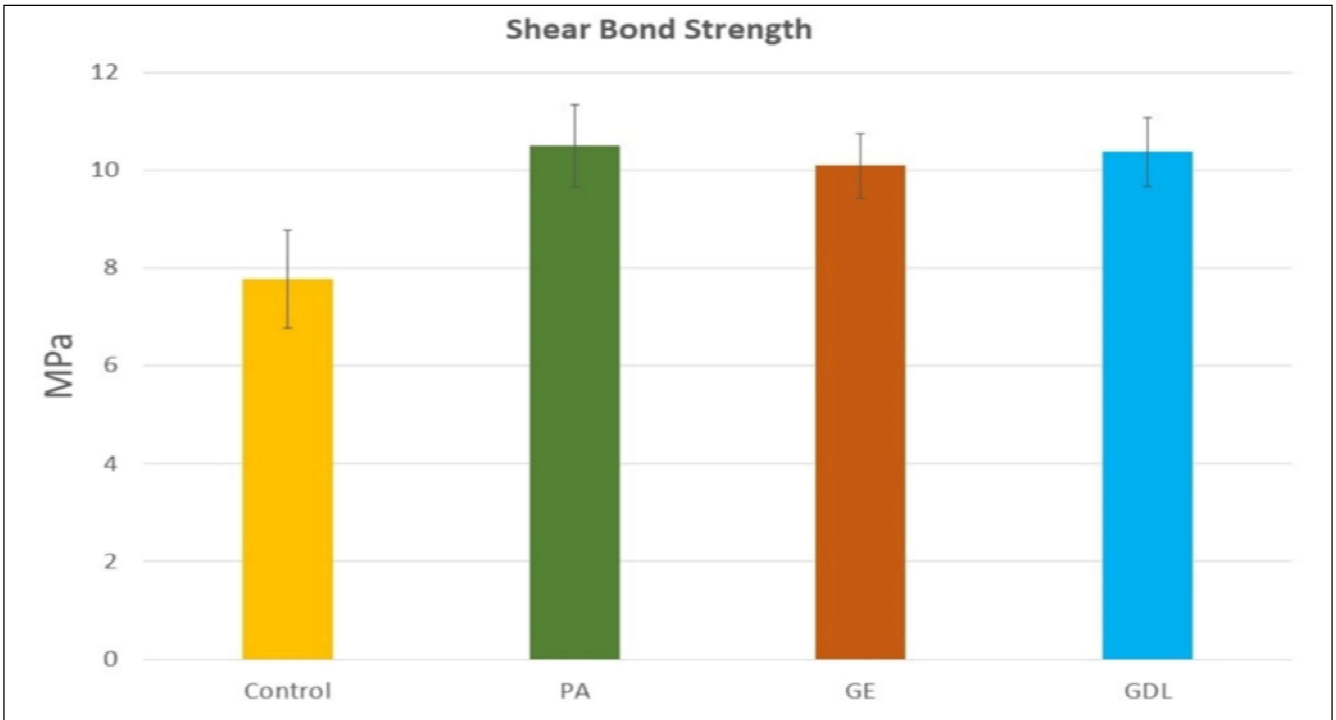

Figure-1: Comparative shear bond strength values (MPa) of control and experimental groups. PA: Proanthocyanidin; GE: Genipin; GDL: Glutaraldehyde.

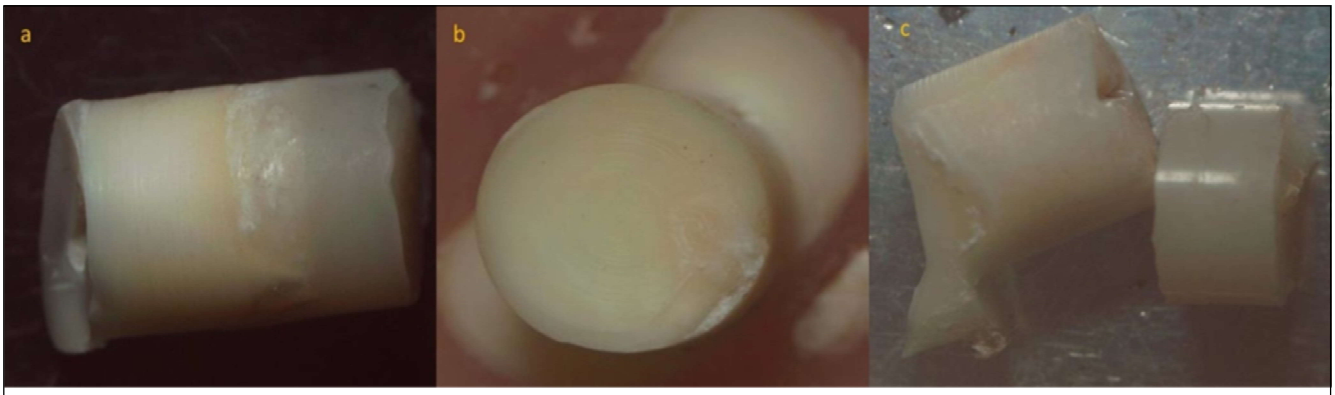

Figure-2: Fracture pattern behaviour of (a) control, (b) Proanthocyanadine (PA), and (c) Genepin (GE).

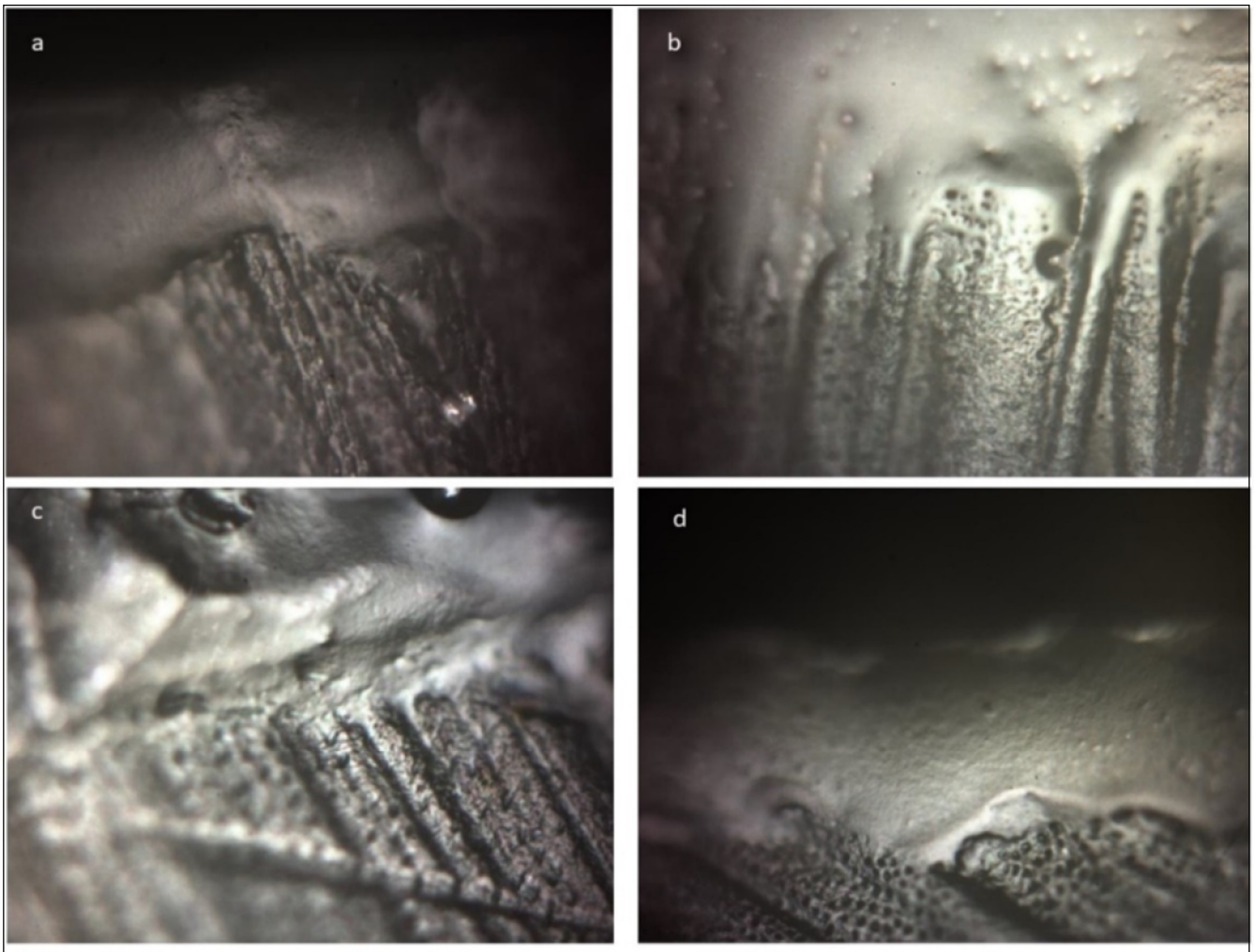

Figure-3: Microscopic images of dentinal infiltration of (a) control, (b) Proanthocyanadine (PA), (c) Genepin (GE), and (d) Glutraldehyde (GDL).

(Figure 2A-C).

Optical microscopy of the restored samples revealed that maximum resin penetration was observed for PA compared to the other groups, followed by GE and GDL groups (Figure 3).

FTIR spectra of the three experimental groups were also compared (Figure 4).

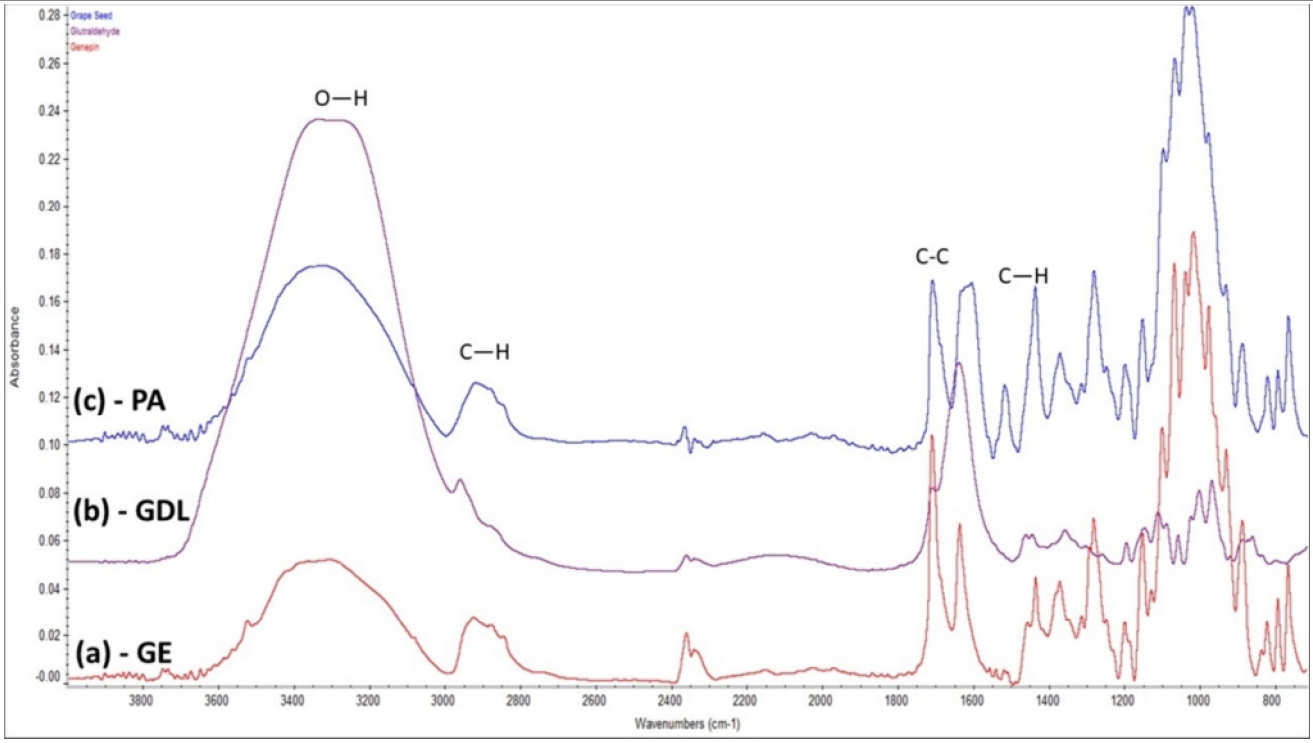

Figure-4: Comparative Fourier transform infrared spectroscopy (FTIR) spectra of (a) Genepin (GE), (b) Glutraldheyde (GDL), and (c) Proanthocyanadine (PA).

Descriptive statistics for shear bond strength (MPA)

n	Control 20	PA 20	GE 20	GDL 20
Mean	7.78	10.50	10.09	10.37
SD	1.00	0.84	0.65	0.71
Minimum	6.05	8.77	9.01	9.27
Maximum	9.79	11.51	11.17	11.87
p-value		0.000 (Significant Difference)		

GE: Genepin, GDL: Glutraldheyde, PA: Proanthocyanadine.

Multiple comparison test for shear bond strength between groups.

(I) Group	(J) Group	Mean Difference (I-J)	Sig.	95% Confidence Interval	
Control	PA	-2.71(*)	0.000	-3.23	-2.20
	GE	-2.30(*)	0.000	-2.82	-1.79
	GDL	-2.58(*)	0.000	-3.09	-2.07
PA	GE	0.40	0.118	-0.10	0.92
	GDL	0.13	0.609	-0.38	0.64
GE	GDL	-0.27	0.288	-0.78	0.23

GE: Genepin, GDL: Glutraldheyde, PA: Proanthocyanadine.

Discussion

In the present study, SBS was measured at the composite resin-dentin interface when PA, GE, and GDL experimental primers were applied on post-etch dentin surfaces. The alternate hypothesis was accepted that collagen crosslinkers (CCL) improved SBS at the composite resin-dentin interface when used as primer.

To etch the surface, a concentration of 10% phosphoric acid as conditioner was used to avoid unnecessary shrinking of the delicate collagen mesh of dentin. The stability and expanded network of dentin is important for a good bonding with adhesive resin.[21] The study followed wet bonding technique after post-etch rinsing. This was done to prevent exposed collagen network from collapsing that readily produces voids and reduce intertubular resin penetration. CCL are tissue fixatives and PA, GE, and GDL post-etch application for 60s improved SBS. PA, GE, and GDL are capable of forming hydrogen bonding with amino acids of collagen within 60s of surface application. For an effective hybrid layer formation, the adhesion must be gap-free between the resin and dentin. Previously, researchers found crosslinkers effective in modifying properties of demineralised dentin by soaking samples in a solution of crosslinking agents.[22,23] Our study observed that the benefits of crosslinker could be best availed when used as a primer which was later rinsed thoroughly. It is expected that leaving an accessory layer over the conditioned dentin surface might hinder resin penetration. Another reason for rinsing was due to the antioxidant behaviour of PA and GE. Antioxidants are scavengers of free radicals which are the main source of

the polymerisation reaction of the tooth-coloured adhesive restorative system. The quality of the hybrid layer and resin encapsulation depend upon the concentration of PA if it is added into the bottle of adhesive, as interference with the photoinitiator can result in a reduced degree of conversion and incomplete setting of the bonding agent.[24] Therefore, in the current study, the cross linkers were used as separate primers rather than adding in the bottle of bonding agent.

The mean SBS of one minute post-etch application of GDL in the current study was 10.37MPa which was statistically higher than the control group (7.78MPa). The effective rise in bond strength was due to the ability of GDL to readily bond with alpha-amino groups of amino acids, N-terminal amino groups of peptides and sulfadryl groups of cysteine amino acid. A number of different amino acids, like lysine and hydroxylysine, drop after treatment with GDL which supports that GDL reacts with amino acids and engage free amino groups in new crosslinks.[25] The rise in bond stability is also explained by GDL inhibition to flow dentinal fluid and an increase in bond durability of post-etch dentin.[26] The concentration of GDL in present study was kept 6.5% and showed effective results. It is reported that up to 10% aqueous solutions of GDL are not harmful to dentin. However, GDL when used in combination with HEMA, as a single preparation (GLUMA bond), reduce cell viability and cell metabolism.[27] However, higher concentrations can damage human fibroblast cells.[28]

The current study confirmed that the 6.5% PA was effective in raising bond strength from a value of 7.79MPa in the control group to 10.50MPa. PA strengthens collagen type I due to the presence of galloyl groups.[29] It has a strong potential to decrease activity of endogenous enzyme activity. It also reduce binding sites of degrading enzymes on collagen fibres irrespective of its application time.[30] Moreover, it removes unbound water from collagen molecules that hinder penetration of resin into intertubular space and result in microleakage around the restoration. It also tends to reduce hydrophilicity of dentin by crosslinking with collagen.[31]

Similarly, GE is an emerging crosslinking agent which is capable of forming bonds with amino groups of lysine, hydroxylysine and arginine. It is considered a safest alternative to synthetic crosslinking agents, unlike GDL, formaldehyde, and carbodiimide that have a tendency of leaving cytotoxic residues in the tissues.[32] The comparison of mean shear strength done between the three available CCLs showed that GE had slightly low mean SBS value (10.37MPa) compared to 10.50MPa in PA group and 10.09MPa in GDL group. This might be due to the slow rate of crosslinking of GE, but the difference was non-significant.

FTIR spectroscopy of grape seed PA in the present study revealed that crosslinking ability of PA was stronger compared to GE due to the presence of hydroxyl groups.[33] The hydroxyl groups readily form hydrogen bonds with carbonyl groups of amino acids of type 1 collagen. The acidity of hydroxyl groups results in the formation of negatively charged phenoxide ion which is capable of forming ionic and covalent bonds with amino acids.[34] Crosslinking of PA is superior in terms of multiple bond formation with collagen and reduction in hydrophilicity of dentin[35] whereas efficiency of GDL to form covalent bonds with amide groups in collagen is directly related to its concentration.[36] GE forms hydrogen bonds at the first instance. However, covalent bonds are also formed as a secondary mechanism that might explain the slow cross-linking behaviour of GE in which ester group of GE is substituted for amide group of collagen.[37]

Study of fracture pattern revealed that PA group had maximum number of cohesive failure in the dentin. This might indicate that the resin-dentin interface was stronger than the dentin substrate itself. Similarly, an adhesive failure that occurred at the interface whether above or below the hybrid layer was maximum (75%) for samples in the control group. The resin-dentin interface of individual groups revealed that PA group showed maximum resin penetration among the four groups that were clearly evident by the presence of long uniform and continuous resin tags. The GE group showed very thin tags, while GDL showed very definite but shallow tags. On the whole, penetration of experimental groups was better than the control group.

The above findings may be helpful in highlighting the role of different crosslinking agents for promoting efficient and stable bonding at the resin-dentin interface. There is much to explore and study regarding the role of GE in improving the quality of hybrid layer formed between demineralised dentin and dental composite system. Moreover, research must be carried out to develop an exact concentration of primers PA and GE that can be incorporated into resin restorative procedures to give maximum bond strength results with negligible issues of

biocompatibility. The effect of available crosslinkers on a variety of dentin types, for example, carious, fluorosed and sclerotic dentin, should also be established.

Conclusion

Chemical modification with collagen crosslinkers improved bond strength at the composite resin-dentin interface. Benefits of long-term durability can be achieved after an application time of as short as 60s following acid conditioning of tooth surface during composite resin restorative procedures.

Disclaimer: The text is part of an MPhil thesis.
Conflict of Interest: None.
Source of Funding: None.

References

1. Chandwani ND, Pawar MG, Tupkari JV, Yuwanati M. Histological Evaluation to Study the Effects of Dental Amalgam and Composite Restoration on Human Dental Pulp: An in vivo Study. Med Princ Pract 2014;23:40-4.
2. Betancourt DE, Baldion PA, Castellanos JE. Resin-Dentin Bonding Interface: Mechanisms of Degradation and Strategies for Stabilization of the Hybrid Layer" . Int J Biomater 2019 ; 11 pages.
3. Hitmi L, Bouter D and Degrange M: Influence of drying and HEMA treatment on dentin wettability. Dent Mater 2002;18:503-511.
4. Bedran-Russo AK, Pauli GF, Chen SN, McAlpine J, Castellan CS, Phansalkar RS et al. Dentin biomodification: strategies, renewable resources, and clinical applications. Dent Mater 2014;30:62-76.
5. Walter R, Miguez PA, Arnold RR, Pereira PNR, Duarte WR, Yamauchi M. Effects of natural cross-linkers on the stability of dentin collagen and the inhibition of root caries in vitro. Caries Res 2008;42:263-8.
6. Cilli R, Prakki A, de Araujo PA, Pereira JC. Influence of glutaraldehyde priming on bond strength of an experimental adhesive system applied to wet and dry dentin. J Dent 2009;37: 212-8.
7. Fang M, Liu R, Xiao Y, Li F, Wang D, Hou R et al. Biomodification to dentin by a natural crosslinker improved the resin-dentin bonds. J Dent 2012;40:458-66.
8. Tsai CC, Liang HC, Sung HW. In vivo evaluation of cellular and acellular bovine pericardia fixed with a naturally occurring crosslinking agent (genipin). Biomaterials 2002; 23:2447-57.
9. Liu RR, Fang M, Zhao SJ, Li F, Shen LJ, Chen JH. The potential effect of proanthocyanidins on the stability of resin-dentin bonds against thermal cycling. Chinese J Stomat 2012;47:268-272.
10. Liu R, Fang M, Xiao Y, Li F, Yu L, Zhao S, etal. The effect of transient proanthocyanidins preconditioning on the cross-linking and mechanical properties of demineralized dentin. J Mater Sci Mater Med 2011;22:2403-411.
11. Phansalkar R, Nam J, Chen S, Bisson J , Kulakowski D, McAlpine JB et al. Grape Seed Proanthocyanidins: A novel source of dental biomaterials and unique phytochemistry. Planta Medica 2016;82:OA50.
12. Trindade TF, Barbosa AFS, Castro-Raucci LMS, de Castro-Raucci LMS, Silva-Sousa YTC, Colucci V et al. Chlorhexidine and proanthocyanidin enhance the long-term bond strength of resin-based endodontic sealer. Braz Oral Res 2018;32:e44.
13. Epasinghe DJ, Yiu CKY, Burrow MF, Hiraishi N, Tay FR. The inhibitory effect of proanthocyanidin on soluble and collagen-bound proteases. J Dent 2013;4:832-9.
14. Zhou J, Chiba A, Scheffel DL, Hebling J, Agee K, Tagami J et al. Cross-linked dry bonding: A new etch-and-rinse technique. Dent Mater 2016; 32:1124-32.
15. Yao CH, Liu BS, Chang CJ, Hsu SH, Chen YS. Preparation of networks of gelatin and genipin as degradable biomaterials. Mater Chem & Phys 2004;83:204-8.
16. Chiou B, Avena-Bustillos RJ, Shey J, Yee E, Bechtel PJ, Imam SH et al. Rheological and mechanical properties of cross-linked fish gelatins. Polymer 2006;47:6379-86.
17. Lwanga SK, Lemeshow S, World Health Organization. Sample size determination in health studies: a practical manual / S. K. Lwanga and S. Lemeshow. Geneva: World Health Organization; 1991.
18. Kato G, Nakabayashi N. Effect of phosphoric acid concentration on wet-bonding to etched dentin. Dent Mater 1996;12:250-5.
19. International Organization for Standardization. Dentistry: polymer-based crown and bridge materials. ISO. 1992;10477:1992-E.
20. Kitasako Y, Burrow MF, Nikaido T, Harada N, Inokoshi S, Yamada T et al. Shear and tensile bond testing for resin cement evaluation. Dent Mater 1995;11:298-304.
21. Nezu T, Nishiyama N, Nemoto K, Terada Y. The effect of hydrophilic adhesive monomers on the stability of type I collagen. Biomaterials 2005;26:3801-8.
22. Dos Santos PH, Karol S, Bedran-Russo AK. Long-term nano-mechanical properties of biomodified dentin-resin interface components. J Biomech 2011;44:1691-4.
23. Bedran Russo AK, Pereira PN, Duarte WR, Drummond JL, Yamauchi M. Application of crosslinkers to dentin collagen enhances the ultimate tensile strength. J Biomed Mater Res B. Appl Biomater. 2007;80:268-72.
24. Epasinghe DJ, Yiu CK, Burrow MF, Tay FR, King NM. Effect of proanthocyanidin incorporation into dental adhesive resin on resin-dentin bond strength. J Dent 2012;40:173-80.
25. Macedo GV, Yamauchi M, Bedran-Russo AK. Effects of chemical cross-linkers on caries-affected dentin bonding. J Dent Res 2009;88:1096-1100.
26. Omae M, Inoue M, Itota T, Finger WJ, Inoue M, Tanaka K et al. Effect of a desensitizing agent containing glutaraldehyde and HEMA on bond strength to Er:YAG laser-irradiated dentin. J Dent 2007;35:398-402.
27. Scheffel D, Soares D, Basso F, de Souza Costa CA, Pashley DH, Hebling J. Transdentinal cytotoxicity of glutaraldehyde on odontoblast-like cells. J Dent 2015;43:997-1006.
28. Sun HW, Feigal RJ, Messer HH: Cytotoxicity of glutaraldehyde and formaldehyde in relation to time of exposure and concentration. Pediatr Dent 1990;12:303-7.
29. Nowshehri J, Bhat Z, Shah M. Blessings in disguise: Biofunctional benefits of grape seed extracts. Food Res Int 2015;77:333-48.
30. Seseogullari-Dirihan R, Apollonio F, Mazzoni A, Tjaderhane L, Pashley D Breschi L et al. Use of crosslinkers to inactivate dentin MMPs. Dent Mater 2016;32:423-432.
31. Ku CS, Mun SP. Characterization of proanthocyanidin in hot water extract isolated from Pinus radiata bark. Wood Sci and Technol 2007;41:235-47.
32. Nagaoka H, Nagaoka H, Walter R, Boushell L, Miguez P, Burton A et al. Characterization of Genipin-Modified Dentin Collagen. BioMed Res Int 2014;1-7.
33. He L, Mu C, Shi J, Zhanga Q, Shi B , Lin W. Modification of collagen with a natural cross-linker, procyanidin. Int J Biol Macromol 2011;48:354-9.
34. Gensel PG, Edwards D, editors. Plants invade the land: evolutionary and environmental perspectives. Columbia University, Press; 2001.
35. Matuda L, Marchi G, Aguiar T, Leme AA, Ambrosano GM, Bedran-Russo AK. Dental adhesives and strategies for displacement of water/solvents from collagen fibrils. Dent Mater 2016;32:723-31.

36. Migneault I, Dartiguenave C, Bertrand MJ, Waldron KC. Glutaraldehyde: behavior in aqueous solution, reaction with proteins, and application to enzyme crosslinking. Biotechniques 2004;37:790-806.

37. Al-Ammar A, Drummond JL, Bedran Russo AK. The use of collagen crosslinking agents to enhance dentin bond strength. J Biomed Mater Res B Appl Biomater 2009;91:419-24.