ORIGINAL RESEARCH

Evaluation of the Dentinal Shear Bond Strength and Resin Interface after pre-treatment with various Dentin Biomodifiers: An In Vitro study

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ABSTRACT

Objectives: To compare and evaluate the effect of grape seed extract (6.5%), gluteraldehyde(5%), hesperidin(0.5%) and CPP-ACP(tooth mousse) on shear bond strength of Dentin and to evaluate the resin tags at resin tooth interface. **Study design:** Seventy fivesound human primary molars were collected and their occlusal surfaces were ground flat to expose dentin. Dentin surfaces were etched using a phosphoric acid and then teeth were randomly divided into 5 groups according to the dentin treatment: Group I (Control group (no treatment), Group II (5% gluteraldehyde),Group III(6.5% grape seed extract),Group IV(0.5% hesperidin) and Group V (CPP-ACP). 10 teeth from each group were tested for Shear Bond Strength and 5 for SEM analysis.The data obtained was statistically analysed by ANOVA and post hoc tests ($p<0.05$). **Result:** Grape seed extract group showed significantly increased shear bond strength than control group($p < 0.05$) and the mean length of resin tags in different dentine biomodifiers groups was also statistically significant ($p < 0.05$). **Conclusion:** The use of dentin biomodifierssuch as Grape Seed Extract,Gluteraldehyde, Hesperidin and CPP-ACP can potentially improve the long-term stability of the dentin bond, thereby enhancing the longevity of the tooth-restoration complex.

KEYWORDS: Dentin biomodifiers, Shear bond strength, SEM

INTRODUCTION

One of the most effective and gold standard way of improving the mechanical bonding and marginal seal between the restoration and dentin is by the use of acid-etching methods. Acid etching of enamel has provided a superb mechanism for mechanical bonding and an established procedure for placement of restorative resins.[1] But the challenge to adhesive researchers still remains the development of agents to dentin and cementum. Although research in this area has been in progress since 1950s, dentin still poses greater obstacles to adhesive bonding than does enamel especially in primary teeth.[2] Some studies have demonstrated lower bond strength values to primary dentin than that found in permanent teeth. Though the results in primary teeth are not well documented, previous reports have shown that the difference lies in adhesion to both types of dentin, attributed to their differences in structure and their chemical composition.[3]

However, irrespective of the challenges associated with the degradation of the dentin-adhesive interface over time, continuous research has been done to improve the dentin-resin interface. One such method is use of extrinsic collagen cross-linking agents to improve the intrinsic properties and modify the dentin.Well known synthetic agents, nature derived agents, and also physical methods have been shown to effectively interact with type I collagen.[4]

Studies have reported that Grape seed extract, composed mainly of proanthocyanidinsincrease the physical properties of dentin by inducing cross-linking with collagen.[5]

Gluteraldehydehas shown to induce cross –linking by improving the mechanical properties of dentin and thereby modifying the dentin.[6]

Hesperidin, extracted from citrus fruits has shown to inhibit degradation of collagen.[7]

Another novel approach is the use of calcium phosphate remineralization technology that has been developed based on casein phosphopeptide-amorphous calcium phosphate, CPP-ACP, has been claimed to be a synthetic cross-linker that can increase collagen cross linking in the mammalian tissue.[8]

Hence, this study was conducted to evaluate the effects of various dentin biomodifiers on shear bond strength of dentin and their effect on resin dentin interface.

MATERIALS AND METHODS

A total of 75 human primary non carious molars were collected for the study. Teeth with pre-shedding mobility, free of caries, non hypoplastic teeth, absence of enamel cracks, teeth without traumatic injuries were included in the study. Ethical clearance from institutional Ethical

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review board was taken prior to the study. All the teeth were cleaned with prophylactic instruments and stored in 10 % formalin until use. The teeth were randomly divided into five groups, one control group and four experimental groups (5% gluteraldehyde, 6.5% grape seed extract, 0.5% hesperidin and CPP-ACP).

In all the experimental groups, 60 teeth were pretreated with the respective dentin biomodifier, and in control group no pre-treatment was done. In all the groups out of 15 teeth, 10 teeth were tested for Shear Bond Strength and 5 for SEM analysis.

Sample preparation for Shear Bond Strength: The selected teeth were embedded perpendicular to their long axis into the acrylic resin mould with occlusal surface facing upwards. The occlusal surfaces were ground flat under running water to remove enamel and expose dentin. The flat surfaces were acid-etched for 15 sec with 37% phosphoric acid, and teeth were randomly divided according to the treatment and bonding system used.

In Group I (control group)no pre-treatment was done on the exposed dentin surface.Two successive coats of the adhesive Adper Single bond 2 (3EM ESPE) were applied on the prepared dentin surface of the specimens according to the manufacturer's instructions, air dried and light curing was done for 20sec. Composite build up was done by placement of two increments of 2-mm thick with each increment being light cured for 20 sec.

In experimental groups (II, III, IV and V) the exposed dentin surface was treated with 6.5% Grape Seed Extract solution, 5% Gluteraldehyde, 0.5% Hesperidin solution and CPP-ACP (Tooth Mousse), respectively using an applicator brush for 1 min and then rinsed with water and blot dried, followed by thebonding/composite buildup procedure as described above. The specimens were stored in distilled water until subjected to testing procedure.

Shear Bond Strength Test: All the completed samples were subjected to shear bond strength test using universal testing machine (Instron machine, ADMET, Enkay Enterprises, New Delhi) at a cross-head speed of 0.5 mm/min using a straight knife-edge chisel applied at the tooth restoration interface. Load was applied until restoration failure occurred. The load at failure was measured in Newtons. The results were tabulated and statistically analysed. The shear bond strength data were analysed by ANOVA and post hoc test at confidence level of 95%.

Fracture Mode Analysis: After shear bond evaluation the samples were emersed in methylene blue for 24 hrs. Following this the teeth were rinsed for 15minutes under running water and were left to dry.

Failure modes were observed under stereomicroscope at 40 X magnification and classified as cohesive, adhesive or mixed.

Specimen Preparation For SEM Study: Five samples from each group were prepared for SEM analysis. After the occlusal preparation the samples were sectioned vertically through the resin build up and dentin with the help of diamond disc under running water into two halves (mesial and distal) to expose the resin–dentin interface.

Specimens were placed in 4% NaOCl for 20 min, followed by 20% HCL acid for 30 sec and rinsed with distilled water. All samples were then sequentially dehydrated in ascending grades of ethanol i.e 60%, 70%, 80%, 90% alcohol for 20 min each and in 100% alcohol for 1 hr.

Tested samples were dried, mounted on aluminium stubs which were then placed in vaccum chamber and sputter coated with Alluminium layer and were observed under a scanning microscope.

The bonding interfaces were observed with Scanning Electron Microscope in order to illustrate the resin/dentin-bonding interface.

Microscopic Evaluation: Series of photographs were taken field by field at a magnification of 2000x, 5000x and 10000x for viewing the dentin resin interface.

Measurement of the Length of the Resin Tags: Length of the resin tags were measured on the photographs with a ruler according to the scale given on the photograph.

Scoring criteria for visual evaluation: A four step (0-3 scale) method according to Ferrari et al[9,10] was used for evaluation of resin dentin interface.

The evaluation for length of resin tags was then subjected to statistical analysis.

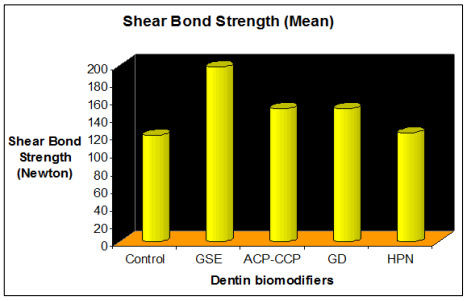
RESULTS

Shear Bond Strength: There was no statistically significant difference in the shear bond strength of control and experimental groups as $p > 0.05$ (Table 1).

	N	Mean ±Std. Deviation	Std. Error	p value
Control	10	120.21 ±77.64990	24.55506	.225**
Grape Seed	10	197.31 ±12.85616	35.68825	
CPP-ACP	10	150.52 ±92.61446	29.28726	
Gluteraldehyde	10	150.44 ±59.52182	18.82245	
Hesperidin	10	122.92 ±61.97322	19.59765	

Table 1: Distribution of Mean ± Standard deviation of Shear Bond Strength in different groups ** Not significant (p>0.05)

One way ANOVA showed no significant difference in the mean of Shear Bond Strength in different dentine biomodifiers groups (Graph 1).



Graph 1: Analysis of variance for mean shear bond strength in different groups

However, multiple comparisons of shear bond strength in different groups, on post hoc Least Significant difference (LSD) test showed that the mean difference of 77.100 N of Shear bond strength between control and Grape seed is significant ($p<0.05$) (Table 2).

Group	Groups	Mean Difference	Std. Error	p value
Control	Grape Seed	77.10000*	37.27035	.044*
	CPPACP	30.31000	37.27035	.420
	Gluteraldehyde	30.23000	37.27035	.422
	Hesperidin	2.71000	37.27035	.942
Grape Seed	Control	77.10000*	37.27035	.044*
	CPPACP	46.79000	37.27035	.216
	Gluteraldehyde	46.87000	37.27035	.215
	Hesperidin	74.39000	37.27035	.052
CPPACP	Control	30.31000	37.27035	.420
	Grape Seed	46.79000	37.27035	.216
	Gluteraldehyde	.08000	37.27035	.998
	Hesperidin	27.60000	37.27035	.463
Gluteraldehyde	Control	30.23000	37.27035	.422
	Grape Seed	46.87000	37.27035	.215
	CPPACP	.08000	37.27035	.998
	Hesperidin	27.52000	37.27035	.464
Hesperidin	Control	2.71000	37.27035	.942
	Grape Seed	74.39000	37.27035	.052
	CPPACP	27.60000	37.27035	.463
	Gluteraldehyde	27.52000	37.27035	.464

Table 2: Multiple Comparisons of Shear Bond Strength in different groups* Significant ($p<0.05$)

The order of mean shear bond strength in different groups according to the mean values was:

Grape Seed >CPP-ACP ≅ Gluteraldehyde>Hesperin>Control

Fracture Mode: Table 3 shows failure mode of all the specimens of all the groups under stereomicroscope.

	Control	Grape Seed	Gluteraldehyde	CPP-ACP	Hesperidin
	FM	**FM**	**FM**	**FM**	**FM**
1	ADH	ADH	ADH	COH	COH
2	ADH	ADH	ADH	ADH	ADH
3	MIX	ADH	ADH	ADH	ADH
4	ADH	ADH	ADH	ADH	COH
5	ADH	ADH	COH	ADH	ADH
6	ADH	ADH	COH	ADH	ADH
7	ADH	COH	ADH	COH	COH
8	ADH	ADH	MIX	COH	ADH
9	COH	ADH	ADH	COH	ADH
10	ADH	ADH	ADH	ADH	ADH

Table 3: Mode of failure observed in all the groups. (FM: Fracture mode, ADH: Adhesive failure, MIX: Mixed failure, COH: Cohesive failure)

Overall failures modes in all groups include:

- Adhesive failures: (74%)
- Mixed failures: (4%)
- Cohesive failures: (22%)

The specimens predominately showed adhesive failure with highest bond strength values. Figure 1 (A-C) shows the debonded dentin surfaces.

Statistical Analysis of Resin/Dentin Interface: A total of 25 specimens (control and experimental) were taken and statistical assessment was done by two methods:

- Visual inspection
- Measurement of resin tags

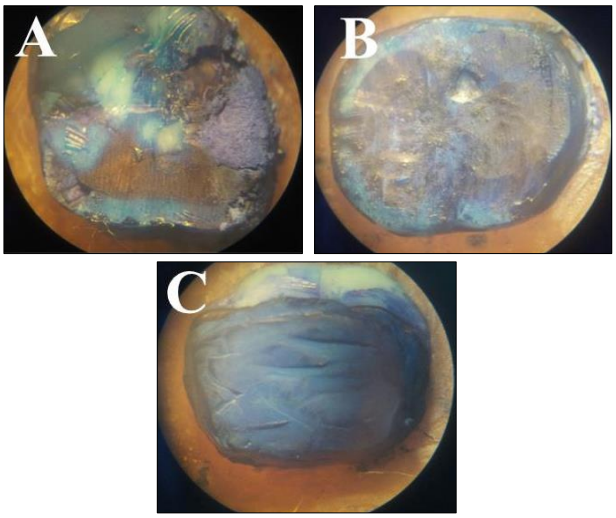


Figure 1: Failure modes as seen under stereomicroscope A) Mixed failure (B) Adhesive failure (C) Cohesive failure

Visual Inspection: Visual inspection of photographs was done. The resin tags in the photographs were graded using a four-step (0-3) scale method proposed by Ferrari *et al.*[9,10]

Figure 2 (A-D) and table 4 shows the grading scores obtained for different dentin cross-linkers. As depicted from table 4, grape seed extract shows the highest grading score of 3.

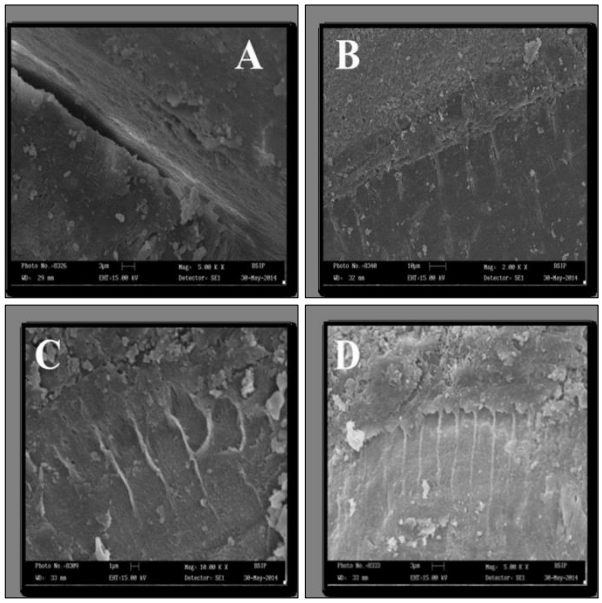


Figure 2: Grading Scores of different cross-linkers as seen under Scanning Electron Microscope (A) Grading score 0 (B) Grading score 1 (C) Grading Score 2 (D) Grading Score

Evaluation Of Resin Tag Length: Table 5 shows the distribution of mean length of resin tags in each group which was statistically significant ($p<0.05$). Multiple comparison (Table 6) between and within the groups using post hoc Least Significant difference (LSD) showed that the mean difference of Length of resins tags between control and Grape seed (85.20µm), Control & CPP-ACP (33.60µm), Control & Gluteraldehyde (37.20µm) and Control & Hesperidin (16.20µm) is

S.no		Control group	CPPACP group	Gluteral-dehyde Group	Grape seed group	Hesperidin Group
1	Scr	1	2	2	3	1
2	Scr	1	2	2	2	1
3	Scr	1	1	2	3	2
4	Scr	1	2	1	3	2
5	Scr	1	2	2	3	2

Table 4: grading scores for each dentin biomodifier

	N	Mean ±Std. Deviation	Std. Error	P value
Control	5	48.20 ±6.099	2.728	0.000*
Grape Seed	5	133.40 ±28.068	12.552	
CPPACP	5	81.80 ±18.295	8.182	
Gluteraldehyde	5	85.40 ±21.220	9.490	
Hesperidin	5	64.40 ±9.555	4.273	

Table 5: Distribution of Mean ± Standard deviation of Length of resin tags in different groups. *Significant (p<0.05)

Group	Sub-Group	Mean Difference	Std. Error	P value
Control	Grape Seed	85.200*	11.666	.000*
	CPPACP	33.600*	11.666	.009*
	Gluteraldehyde	37.200*	11.666	.005*
	Hesperidin	16.200	11.666	.180**
Grape Seed	Control	85.200*	11.666	.000*
	CPPACP	51.600*	11.666	.000*
	Gluteraldehyde	48.000*	11.666	.001*
	Hesperidin	69.000*	11.666	.000*
CPPACP	Control	33.600*	11.666	.009*
	Grape Seed	51.600*	11.666	.000*
	Gluteraldehyde	3.600	11.666	.761**
	Hesperidin	17.400	11.666	.151**
Gluteraldehyde	Control	37.200*	11.666	.005*
	Grape Seed	48.000*	11.666	.001*
	CPPACP	3.600	11.666	.761**
	Hesperidin	21.000	11.666	.087**
Hesperidin	Control	16.200	11.666	.180**
	Grape Seed	69.000*	11.666	.000*
	CPPACP	17.400	11.666	.151**
	Gluteraldehyde	21.000	11.666	.087**
* The mean difference is significant at 0.05 level. ** The mean difference is not significant				

Table 6: Multiple Comparisons using Post Hoc Tests

significant (p<0.05). The length of resin tags in experimental groups is significantly greater than the control group.

Thus from SEM observation following order of tag length was seen:

Grape Seed > Gluteraldehyde > CPP-ACP >Hesperidin > Control

DISCUSSION

In dentin, collagen is composed of inter and intra molecular cross links that can increase tensile strength, elasticity. Use of extrinsic collagen cross-linking agents can induce additional formation of such cross –linking and improve the biomechanical properties of dentin.[5]

In dentistry, naturally occurring compounds have received a great attention in the past decade. The commonly used cross linkers in dentistry are proanthocyanidins, gluteraldehyde, genipin, carbodi-imide.[11]

Grape seed extract primarily composed of Proantho-cyanidins, are naturally occurring plant metabolites available widely in fruits and vegetables. Belonging to a category known as condensed tannins, Proanthocyanidins are highly hydroxylated structures capable of forming an insoluble complex with carbohydrates and proteins. They are suggested to interact with proteins via covalent, ionic, hydrogen bonding or hydrophobic interactions.[12] Grape seed extract has been reported to induce cross-linking and increases resistance to degradation.[13] Authors have shown grape seed as a non –toxic cross-linking agent, the rate of cross linking and degree of penetration of which can be controlled by various factors like pH, temperature, forms of proanthocyanidins, (PA) varying PA concentration.[14]

Our study showed increased shear bond strength in grape seed extract group with the control group, hence improving dentin-resin bonding. The significant increase in bond strength may be attributed to the improved dentin collagen stability (Han et al 2002, Bedran-Russo et al, 2007). Grape seed and control group showed a bond strength of 197.31 ±12.85616 N and 120.21 ±77.64990 N, respectively. On the contrary, CPP-ACP, Gluteraldehyde and Hesperidin showed a shear bond strength of 150.52 ± 92.61446, 150.44 ± 59.52182 and 122.92 ± 61.97322, respectively. On post hoc Least Significant difference (LSD) test the mean difference of Shear bond strength between control and 6.5% Grape seed, was significant (p<0.05) therefore, improve dentin-resin bonding.

The bonding agent used for all the groups is Adper single bond 2 which is an ethanol based adhesive, using 37% phosphoric acid to etch dentin. This removes smear layer completely. In addition, Hagerman et al reported thatethanol may be a preferable solvent for proanthocyanidin–based preconditioners as ethanol stimulates proanthocyanidin and collagen interactions rather than acetone by decreasing the dielectric constant of the media.[15]

Our results demonstrate that dentin biomodification did not compromise the bond strength longevity and also effectiveness and stability of cross-linking treatment. The present study observed the effect of cross-linking agents after 5 min treatment, which is not a time consuming treatment and hence much more clinically feasible.At the resin-dentin interface, treatment with GSE showed very long tags extending almost through the entire resin dentin inter-diffusion zone. Few studies in the past stated the presence of formation of thicker hybrid layers in primary teeth with shorter resin tags. According to authors, the actual resin tag length is of minor importanceusually, the top 5–10 mm of the tags are believed to contribute the most to retention and sealing effectiveness of most dental adhesives.[16] The penetration of resin tags into the dentinal tubules is believed to contribute little to the final bond strength. But the adaptation to the inner tubule walls probably contributes significantly much more to bonding efficacy. Maximum of adhesive failures in grape seed may indicate that the hybrid layer was strengthened by

the cross linker.

Gluteraldehydewhich is a known synthetic cross–linking agentwhich has been proposed to reduce the lysine and hydroxylysine residues and decreases these residues in the reducible cross-links.The amino acids and cross-link composition is altered by this compound, increasing the cross-links and making the dentin stiffer.[17,18] A disadvantage of gluteraldehyde is its high cytotoxicity, which limits clinical applicability.[19,20]

Our results have shown long resin tags at the resin-dentin interface after treatment with gluteraldehyde than the control group, even though shear bond strength was not statistically significant. In vitro studies demonstrate that GD is effective in reducing dentin permeability, and that resin bonding to surfaces previously treated with GD based primers is not adversely affected. In fact, the application of GD on acid-etched dentin has been shown to improve the efficacy of dentin bonding systems in vitro. The observed enhanced bond strengths could be related to the covalent crosslinking between collagen and gluteraldehyde.[21]

Authors have shown a significant reduction of free lysine and hydrolysine residues on dentin collagen of permanent teeth after treatment with gluteraldehyde-HEMA aqueous solution.[22] Similar studies on primary teeth are scanty.

Hesperidin (HPN) is a flavonoid extracted from citrus fruits.The hydrophobic property of HPN is probably more pronounced which may enhance its association to collagens, resulting in increased mechanical properties and resistance to biodegradation of collagen matrix. However, the possibility of other modes of crosslinks remains unclear.[23] Statistically, there was no significant difference in the mean of Shear Bond Strength in Hesperidin and control group ($p > 0.05$). But the length of resin tags were longer than control.

Islam *et al*. applied HPN to tooth structures in an *in vitro* caries model and concluded that its cross-linking properties might inhibit degradation of collagen and demineralization of bovine root dentin. Moreover, HPN prevents demineralization of dentin by acid and has the capacity to help remineralize dentin.[24]

Hiraishi N et al investigated the effect of various plant-derived agents (hesperidin, proanthocyanidin, epigallo-catechingallate and genipin) on the stability of dentin collagen matrix to resist collagenase degradation. The Ultimate Tensile Strength and swelling ratio measurements revealed that the mechanical property of dentin was improved by the use of these natural agents. The greatest reduction in collagen degradation was shown following the use of hesperidin, proanthocyanidin, and epigallocatechingallate at 0.5%.[23]

HPN and proanthocyanidins are phenolic flavonoids, with a chroman ring. As a result, the chemical effect of HPN on collagen fibrils can be accounted for, similar to the cross-linking effect of proanthocyanidins.[25,26] Similar studies on primary teeth are rare.

CPP-ACP, a bovine milk protein derivative has been claimed to be a synthetic cross linker that can increase collagen cross-linking in the mammalian tissue.[8] Mechanism of action of CPP-ACP as a cross linker is yet to be proved and which is to be studied at a molecular level.The Distribution of Mean of Shear Bond Strength in CPP-ACP group was similar to gluteraldehyde group (150.44 ± 59.52182 N). Our results showed no statistically significant difference in the mean of Shear Bond Strength in control and CPP-ACP group but the length of the resin tags in CPP-ACP were longer than the control group indicating deeper penetration of the resin into the dentinal tubules.

As one of the component of CPP-ACP is colloidal silica.[27] Besinis A et al have investigated, the ability of colloidal silica and hydroxyapatite (HA) nanoparticles to infiltrate the collagen structure of demineralized dentin. Authors found that Silica nanoparticles have the ability to penetrate dentin and remain embedded within the collagen matrix and may provide a suitable scaffold for the remineralization of dentin, whereby the infiltrated particles act as seeds within the collage matrix and given the appropriate remineralizing environment, mineral growth may occur.[28]

Borges BC et al evaluated the push-out bond strength of dimethacrylate (Clearfil SE Bond/Filtek Z250; and Adper SE Plus/Filtek Z250) and silorane-based (Filtek P90 adhesive system/Filtek P90 composite resin) restorative systems following selective dentin pre-treatment with a CPP-ACP-containing paste (MI Paste). Bond strength values for the Adper SE Plus/Filtek Z250 restorative systemincreased after applying the CPP-ACP containing paste.[29]

In this study bond strength values of all the experimental groups was higher than the control group.The Karl Pearson's correlation coefficient showed a strong co-relation between Shear bond strength & Length of resin tags in all experimental groups and control and was statistically significant as $p < 0.05$. Hence, as the resin tags increases, shear bond strength also increases.

CONCLUSION

The present study has demonstrated that increased bond strength can be obtained by the use of biochemical cross-linkers in sound dentin. It has been proposed that the deterioration of dentin collagen fibrils contributes to the mechanism responsible for bond degradation.The use of dentin biomodifiers such as Grape Seed Extract and Gluteraldehyde, Hesperidin and CPP-ACP could potentially improve the long-term stability of the dentin bond, thereby enhancing the longevity of the tooth-restoration complex. Since, permanency of restorations in primary dentition is very low and the predicted life spanof re-restorations is even shorter.The results of this study may deliverunderstandings into evolving novel approaches for efficient and stable dentin bonding, with the use of naturally occurring and synthetic cross-linking

agents in primary teeth. Also, the natural cross linkers may be less toxic when compared with synthetic chemicals, the use of plant–derived agents should be considered as promising agents to improve the bond to tooth structure in the rigors of the oral environment.

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