EGYPTIAN
DENTAL JOURNAL

Vol. 65, 2801:2811, July, 2019

I.S.S.N 0070-9484

Fixed Prosthodontics, Dental materials, Conservative Dentistry and Endodontics

www.eda-egypt.org • Codex : 152/1907

IMPACT OF NATURAL CROSS-LINKING AGENTS ON DENTIN BONDING

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ABSTRACT

Collagen is the most important structure of the hybrid layer, and chemical alteration to its structure could be gainful during bonding procedures. Aim: To evaluate the influence of two natural cross-linking agents (pomegranate peel extract [PP], and Apple peel extract [AP]) compared to chlorohexidine 2% [CHX on the shear bond strength (SBS) of sound and demineralized dentin before and after aging.

Materials and Methods: 160 sound human premolars were collected and their occlusal surfaces were ground flat to expose dentin. Eighty teeth were submitted to cariogenic challenge by pH cycling to induce demineralized dentin [DD] after those teeth were randomly divided according to the dentin treatment protocol: chlorohexidine 2 % solution (CHX solution, Consepsis, Ultradent USA), pomegranate peel extract 10% solution (PP, Naturalin Bio-Resources Co., Ltd, Lu-Valley Enterprise Square. Changsha, City. Hunan Province. China), apples peel extract 10% solution (AP, Naturalin Bio-Resources Co., Ltd, Lu-Valley Enterprise Square. Changsha, City. Hunan Province. China) and the control group without any treatment. Then each group was further subdivided into two subgroups the first one was evaluated after 24 hours water storage and the second one after six months of water storage. Data were statistically analyzed by repeated measures Analysis of Variance (ANOVA) to study the effect of material, substrate, storage and their interaction on shear bond strength. Bonferroni's post-hoc test was used for pair-wise comparisons when the ANOVA test is significant. The significance level was set at $P \leq 0.05$. The interaction between the tested variables had a statistically significant effect on mean shear bond strength. PP scored the statistically highest mean shear bond strength. There was no statistically significant difference between CHX and AP; both showed lower mean values. While the control showed the lowest.

Conclusion: Chemical alteration to the dentin matrix endorsed by PP and AP lead to enhance in the shear bond strength. The application of natural collagen cross-linkers during bonding procedures may be an innovative approach to enhance dentin bond strength properties

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INTRODUCTION

During cavity preparation, the durability of restoration can be affected by bacterial remnants in the prepared cavity. It has been documented that bacteria remaining after restorative procedure may survive and multiply, especially in the presence of leakage, which may lead to pulpal irritation,[1,2] risk of recurrent caries,[3] and/or postoperative sensitivity,[4] and therefore failure of the dental restoration.[5,6]Attempts at the complete removal of deep carious dentin, by solely mechanical means, may result in pulpal violation and/or gross destruction of the tooth structure.[7,8] Moreover, the complete mechanical caries removal approach has failed to generate a completely bacteria-free cavity.[9,10] Thereafter, cleaning the cavity preparation with antibacterial agents, to aid in bacterial elimination, began to gain wide acceptance among dental practitioners.[11,12]

Multiple disinfectants have been used in clinical dentistry, in an effort to reduce or eliminate bacteria during cavity preparation and prior to the placement of dental restorations. Some of these agents have been reported to cause pulpal irritation, due to their inherent chemicals, and therefore have fallen into abandonment.[13, 14]In addition to their effectiveness in sterile cavity preparation; the effects of these different agents and techniques on restorations, especially bond strength, and tooth structure have been a major concern for researchers.

Constantly the bonding of restorative materials to dentin has been more demanding than enamel bonding. It is considered more challenging and less predictable with time, for the reason that dentin is a heterogeneous tissue composed of high water, organic content and dentinal tubules.[15, 16]

The stability of the bond between dentin and an adhesive system rely on the structural integrity and mechanical properties of acid-de-mineralized collagen fibers.[17]The mechanical properties of collagen and its resistance to enzymatic degradation can be enhanced by the formation of intra-and inter-molecular and inter-micro-fibrillar cross-links. These techniques can be accomplished using a variety of collagen cross-linkers, both synthetic and natural, on the dentin substrate preceding to the bonding .[18-22]

Chlorhexidine digluconate (CHX) is a biguanide biocide that inhibits the formation and progression of dental plaque and has been used as an oral antimicrobial agent since the 1970s.[23] Presently, CHX is one of the most widely used antimicrobial agents in oral health[24] and is considered the "gold standard" of oral antiseptics.[25,26] Different concentrations and forms of CHX are available: 0.12 to 0.2% mouth rinses, 2% cavity-cleaning solutions, and 0.5 to 1% gels. It has been reported that the 2% solution is the most widely used CHX form in clinical dentistry and dental research.

Although chemical agents are well recognized for their efficiency, antagonistic properties can be observed with longstanding use as teeth staining, calculus formation, and taste alterations. Though, oral mucosal desquamation and parotid swelling may also be reported.[27]

Newly there is a recent tactic for natural remedies as the division of medical and dental therapeutics, which has been termed as "phytotherapeutics" or "ethnopharmacology".[28] About half of the pharmaceuticals in use today are derived from natural products.[29]

The awareness of proanthocyanidins (PA), and their presences in foods has increased. Various clinical and laboratory studies revealed their anti-infectious, anti-inflammatory, anti-carcinogenic and cardio-protective properties. These actions have been ascribed to their act as potent antioxidants and free radical scavenger; inhibit lipid peroxidation; aiming various protein targets within the cell, altering their activity.[30]

Pomegranate (*Punicagranatum*) is one such natural source of proanthocyanidins that are currently finding important applications in the field of dental health.[31] The healing property of pomegranate was discussed in one of the oldest medical texts, the Eber's Papyrus from ancient Egypt (1500 BC). In ayurvedic medicine, pomegranate is considered "a pharmacy unto itself" and as a remedy for diabetes in Unani medicine. Various components of this plant such as the leaves, flowers, roots, barks and fruit extracts have been used for a variety of ailments. There is ample evidence regarding the antimicrobial efficacy of Pomegranate peel in various In Vitro studies.[32]

"Eat an apple on going to bed, and you'll keep the doctor from earning his bread." Science seems to be patronaged this old idea. Japanese nutritionist pointed out that the contents of nutrients in apple peel and its adjacent peel are four times that in the pulp. These nutrients contain polyphenols which have many effects such as reducing blood lipid, anti-aging, anti-cancer. In addition to polyphenols, apple peel is rich in dietary fiber, iron, potassium and vitamins A, C, E, K, etc. Among them, the dietary fiber in apple peel is half of the total fiber content of apple.[33,34]

Five common nutrients in apple peel; proanthocyanidins in apple peel have the strong antioxidant capacity and have the effects of reducing blood lipid, resisting cancer, eliminating halitosis, preventing tooth decay, whitening skin. Epicatechin is also a high antioxidant active ingredient, which can reduce cholesterol concentration in blood, prevent dental caries and periodontal disease, and inhibit harmful bacteria in the intestinal tract and Helicobacter pylori in the gastric inside. Anthocyanin in apples is mainly found in the peel. Anthocyanin can improve vision, prevent eye fatigue, and has an effect in preventing eye diseases such as cataract and glaucoma. In addition, anthocyanin can also promote metabolism and smooth blood circulation. The rich water-soluble dietary fiber between apple peel and pulp is called "apple pectin". This apple pectin has more than twice the detoxification capacity of other pectin and can inhibit harmful bacteria. In addition, it also has the functions of lowering cholesterol level, inhibiting blood sugar level rise, preventing constipation, and promoting visceral fat decomposition, which is also helpful for cancer prevention. Potassium is responsible for the excretion of excess salt in the human body, has diuretic and detumescence effects, and helps prevent hypertension and arteriosclerosis.[35-39]

Though of pomegranate peel and apple peel extracts have been used in dentistry for their antibacterial properties, no study till date has been conducted using them as natural crosslinkers. Thus, the present study was carried out to evaluate and compare the roles of pomegranate peel and apple peel extracts as potential cross linkers agents on the shear bond strength of the self-etch adhesive system to demineralized and sound dentin before and after six months water storage. The null hypothesis tested was that the use of natural agents in vitro would positively influence the shear bond strength when compared to a control (no treatment).

MATERIALS AND METHODS

Ethical clearance was obtained by the institutional ethical committee of Imam Abdulrahman Bin Faisal University (EA:2019025). 160 sound human extracted premolars were collected, cleaned from debris and kept in distilled water with 0.5% thymol crystals solution at _4 8C.

Using Isomet low-speed saw (Buehler, Lake Bluff, I ll, USA) the occlusal surfaces of the selected teeth were ground flat at 2.5 mm deep from the cuspal tip and perpendicular to their long axis. After fixation of teeth in acrylic resin blocks, to have a standardized smear layer further grinding was done with 600-grit silicon carbide paper for 30 s.

Eighty teeth were subjected to pH cycling to induce demineralized dentin as described by ten Cate et al. (1995).[40] In brief, nail polish was painted parting an area of exposed dentin 3 mm diameter, followed by pH cycling. Pre and post, pH cycling the teeth microhardness values were recorded by the Vickers microhardness test (Microdurometer HMV-2000 Shimadzu Corporation, Kyoto, Japan), using a load of 25 kgf, for 10 s. For confirming the demineralization, after pH cycling, the mean values of microhardness were 33.2 to 42.7 VHN.[40]

The sound (SD) and demineralized dentin (DD) (n = 80) were randomly and equally allocated into four groups as follows: The dentin in experimental groups were treated with one of the following: chlorohexidine 2 % solution (CHX solution ,Consepsis, Ultradent USA), pomegranate peel extract 10% solution (PP, Naturalin Bio-Resources Co., Ltd, Lu-Valley Enterprise Square. Changsha, City. Hunan Province. China), apples peel extract 10% solution (AP, Naturalin Bio-Resources Co., Ltd, Lu-Valley Enterprise Square. Changsha, City. Hunan Province. China) and the control group without any treatment. The pH of all solutions had accustomed to 7.4 using NaOH preceding to treatment.[41]

Then each group was further subdivided into two subgroups (n=10) the first one was evaluated after 24 hours of water storage and the second one after six months of water storage.

The solutions were applied with micro brush rubbing motion for 2 min then the excess was removed with cotton pellets. The surfaces were air dried for 10 seconds.[42]

AdheSE Primer (AdheSE, Ivoclar Vivadent, Schaan, Liechtenstein) was applied to the dentin for15 s and air dried by a light air-jet for 10 s. Next, AdheSE Bond was applied, dried, and light activated for 20 s, following the manufacturer's instructions.

Composite resin Tetric Evo Ceram universal shade A3 [Ivoclar Vivadent, Schaan, Liechtenstein] was applied on the treated dentin surface with the use of a cylindrical split Teflon mold [2 mm height and 3 mm diameter] and light activated in accord the manufacturer's instructions. Post-polymerization and elution of the unreacted components, specimens were kept in distilled water 37˚C for 24 hours before testing.

Aging conditions

The assigned specimens were submerged in distilled water 37˚C for six months in dark container before testing.

SBS testing

Specimens were subjected for SBS test; the specimen was secured in the universal testing machine (Instron Model 8871, Instron Corp., Canton, MA, USA). Following, the adhesive interface in shear mode (knife-edge chisel) was adjusted at a crosshead speed of 0.5 mm/min until failure. The SBS values (MPa) were calculated from the peak load at failure divided by the cross-sectional area of the bonded specimen

Statistical analysis:

Normality distribution of the numerical data were clarified by (Kolmogorov-Smirnov and Shapiro-Wilk tests). Parametric distribution was observed in all data. Data were reported as mean and standard deviation (SD) values. Repeated measures Analysis of Variance (ANOVA) test was applied to reveal the effect of material, substrate, storage and their interaction on shear bond strength. Bonferroni's post-hoc test was used for pair-wise comparisons when ANOVA test is significant. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

RESULTS

Repeated measures ANOVA results showed that material type, substrate, and storage had a statistically significant effect on mean shear bond strength. The interaction between the three variables had a statistically significant effect on the shear bond strength.

Influence of material regardless of other variables

TABLE (1) Repeated measures ANOVA test results for the effect of different variables on mean shear bond strength

Source of variation	Type III Sum of Squares	df	Mean Square	*F*-value	*P*-value	Effect size *(Partial eta squared)*
Material	322.333	3	107.444	674.326	<0.001*	0.966
Substrate	53.319	1	53.319	294.345	<0.001*	0.803
Storage	564.842	1	564.842	3544.976	<0.001*	0.980
Material x Substrate x Storage interaction	9.052	3	3.017	16.657	<0.001*	0.410

*df: degrees of freedom = (n-1), *: Significant at $P \leq 0.05$*

TABLE (2) The mean, standard deviation (SD) values and results of repeated measures ANOVA test for comparison between shear bond strength of different materials regardless of other variables

Material	Mean	SD	*P*-value	Effect size *(Partial eta squared)*
Control	12.3^{C}	3.6	<0.001*	0.966
CHX	13.3^{B}	3.7		
PP	14.4^{A}	1.5		
AP	13.4^{B}	1.1		

: Significant at $P \leq 0.05$, Different superscripts in the same column are statistically significantly different

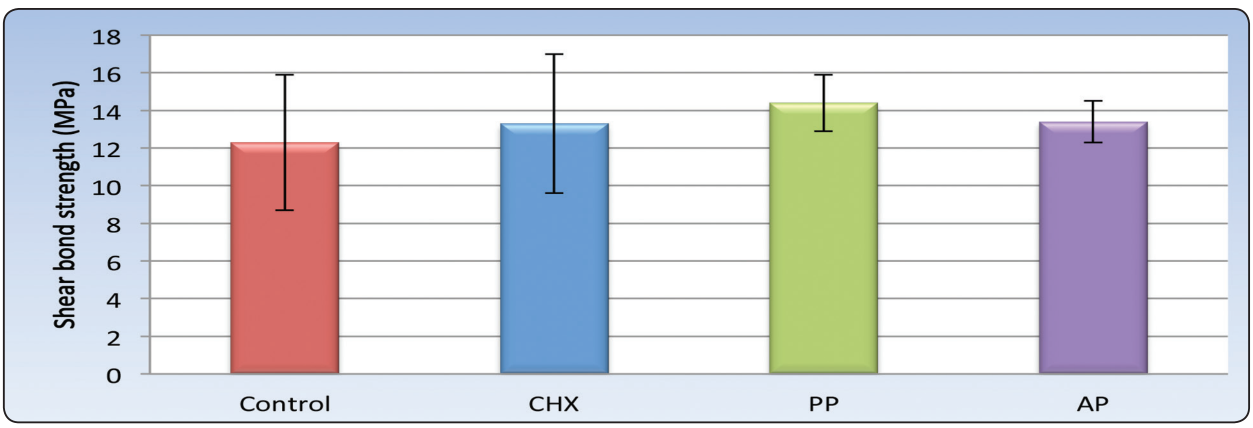

Fig. (1) Bar chart representing mean and standard deviation values for shear bond strength of different materials regardless of other variables

PP scored the statistically significantly highest mean shear bond strength. There was no statistically significant difference between CHX and AP; both showed lower mean values. Control group secured the lowest.

Impact of substrate regardless of other variables

Sound dentin had higher mean shear bond strength than demineralized dentin.

TABLE (3) The mean, standard deviation (SD) values and results of repeated measures ANOVA test for comparison between shear bond strength of the two substrates regardless of other variables

Sound dentin		Demineralized dentin		*P*-value	Effect size *(Partial eta squared)*
Mean	SD	Mean	SD		
13.5	2.5	12.3	2.8	<0.001*	0.803

: Significant at P ≤ 0.05

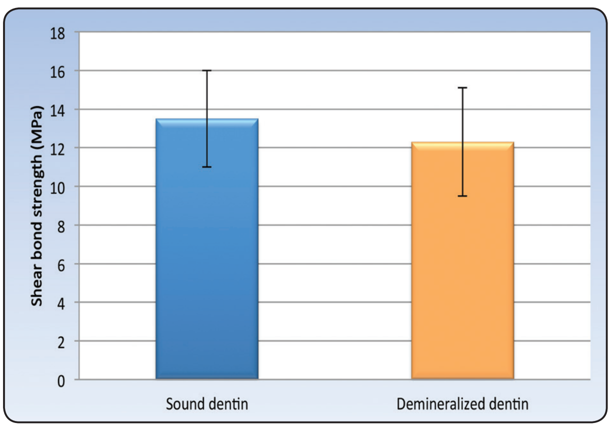

Fig. (2) Bar chart representing mean and standard deviation values for shear bond strength of the two substrates regardless of other variables

Effect of storage regardless of other variables, a statistically significant decrease in shear bond strength after storage was observed.

TABLE (4) The mean, standard deviation (SD) values and results of repeated measures ANOVA test for comparison between shear bond strength before and after storage regardless of other variables

Before storage		After storage		*P*-value	Effect size *(Partial eta squared)*
Mean	SD	Mean	SD		
14.8	1.9	11	1.9	<0.001*	0.980

: Significant at P ≤ 0.05

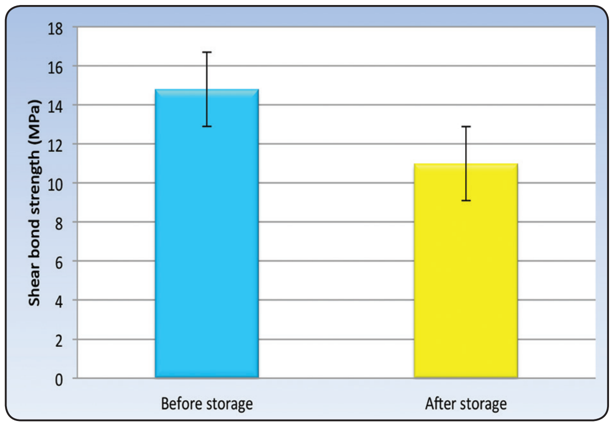

Fig. (3) Bar chart representing mean and standard deviation values for shear bond strength before and after storage regardless of other variables

Effect of different interactions on shear bond strength

Comparison between materials

Before storage

With sound dentin; CHX recorded the statistically significantly highest mean shear bond strength. PP scored lower mean shear bond strength followed by AP. The control secured a statistically significantly lowest mean shear bond strength. With demineralized dentin**;** PP scored the highest mean shear bond strength. CHX recorded lower mean shear bond strength followed by AP. The control secured the lowest mean shear bond strength.

After storage

With sound dentin**;** PP scored the statistically significantly highest mean shear bond strength. AP had lower mean shear bond strength. No statistically significant difference between control and CHX was observed; both showed the lowest mean shear bond strength values. With demineralized dentin; PP recorded the highest mean shear bond strength. AP had statistically significantly lower mean shear bond strength followed by CHX. The control secured the lowest mean shear bond strength.

Effect of storage:

As regards all materials whether regarding sound or demineralized dentin; there was a statistically significant decrease in shear bond strength after storage in water.

TABLE (5) The mean, standard deviation (SD) values and results of repeated measures ANOVA test for comparison between shear bond strength values of different interactions of variables

Storage	Material	Sound dentin		Demineralized dentin		*P*-value (Between substrates)	Effect size *(Partial eta squared)*
		Mean	SD	Mean	SD		
Before storage	Control	13.4^{D}	0.5	11.1^{D}	0.4	<0.001*	0.652
	CHX	18^{A}	0.4	15.4^{B}	0.2	<0.001*	0.722
	PP	15.4^{B}	0.3	16.1^{A}	0.4	<0.001*	0.160
	AP	14.7^{C}	0.2	14.2^{C}	0.4	0.012*	0.084
	P-value (Between materials)	<0.001*		<0.001*			
	Effect size *(Partial eta squared)*	0.904		0.924			
After storage	Control	10.1^{C}	0.6	7.6^{D}	0.4	<0.001*	0.703
	CHX	10.5^{C}	0.5	9.2^{C}	0.6	<0.001*	0.373
	PP	13.3^{A}	0.3	12.7^{A}	0.5	0.003*	0.118
	AP	12.5^{B}	0.3	12.2^{B}	0.3	0.102	0.037
	P-value (Between materials)	<0.001*		<0.001*			
	Effect size *(Partial eta squared)*	0.852		0.936			
		Sound dentin		Demineralized dentin			
Control [*P*-value for Effect of storage, (Effect size)]		<0.001* (0.805)		<0.001* (0.835)			
CHX [*P*-value for Effect of storage, (Effect size)]		<0.001* (0.958)		<0.001* (0.941)			
PP [*P*-value for Effect of storage, (Effect size)]		<0.001* (0.634)		<0.001* (0.825)			
AP [*P*-value for Effect of storage, (Effect size)]		<0.001* (0.653)		<0.001* (0.622)			

: Significant at $P \le 0.05$

Different superscripts in each column indicate a statistically significant difference between materials

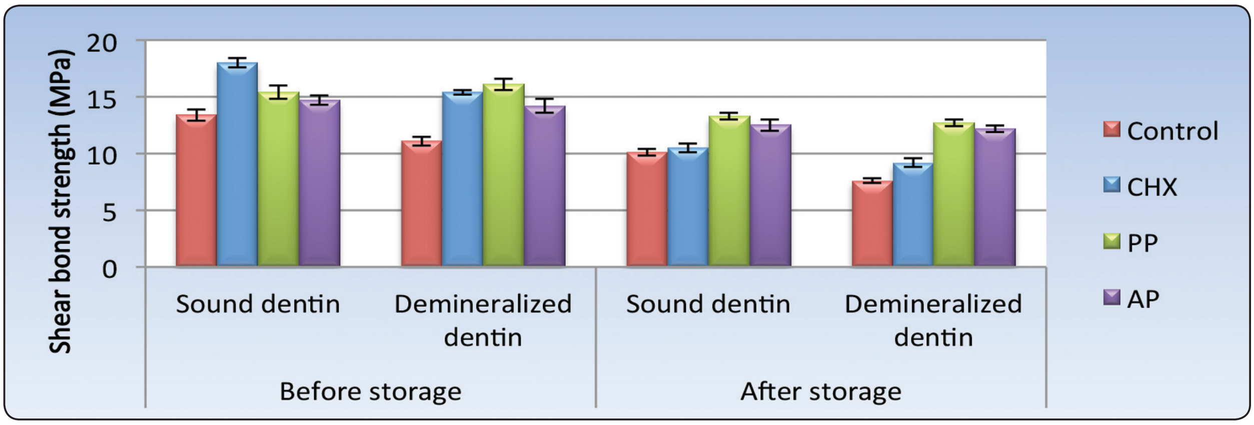

Fig. (4) Bar chart representing mean and standard deviation values for shear bond strength of different materials with different interactions of variables

DISCUSSION

Currently, the researches approved the self-etching adhesives concept, but still with enormous worry for their bonding durability.[43]This study investigated the impact of the tested cavity disinfectant and crosslinkers on the shear bond strength of self-etch adhesives to sound and demineralized dentin after selected aging condition: six months of water storage.

The use of cavity disinfectants may reduce or completely remove the bacteria from tubules, and this may reduce recurrent caries, damage to pulp or restoration failure. These problems could be avoided with the use of cavity disinfectants.

In the present study, the SBS test was applied for its ease, simplicity of specimen preparation, and the accuracy in qualitatively comparing various materials consistent with their bond strength value.[44]

Six months of water storage was selected as an aging condition. Collagen hydrolysis has been established to happen over time at the resin-dentin bonded interface in a previous laboratory and clinical studies.[45, 46]

In the present study, PP scored a statistically significantly highest mean shear bond strength. No statistically significant difference between CHX and AP was recorded; Control secured the lowest mean. These findings agreed with other studies that showed that modifying of the dentin matrix using cross-linking agents might positively affect both the strength and stability of resin-dentin bonds.

Crosslinking is defined as "the process of forming tridimensional networks", where polymer chains may be linked by covalent or non-covalent bonds.

Recently, the Dentinal matrix metalloproteinases MMP has focused attention regarding their role in bonding degradation. MMP is wrapped in dentin during the tooth development process. Once the dentin is treated with different agents of adhesive resin systems, the MMPs are released and activated in the bonding procedure.[47, 48] Activated MMPs are not fully penetrated with resin. They can gradually degrade the collagen fibrils at the resin-dentin interface.[49] Thus; the use of such cavity disinfectants which are MMP inhibitors is a strategy to avoid degradation of dentin bonds and to amplify the durability of bonded restorations.

Chlorhexidine is widely used in the dental field an antibacterial agent. For the fact that CHX is an MMP inhibitor, the surface pretreatment with CHX is considerate to enhance the durability of bonded restorations. CHX molecule binds the catalytic site (Ca2+ or Zn2+) of MMPs by a chelating action. It inhibits MMPs at very low concentrations.[50]

Conversely, in the present study unfavorable effects were recorded in the bond strength after water storage. No statistically significant difference between control and CHX was reported; both showed the statistically significantly lowest mean shear bond strength values. While PP scored the highest with both SD and DD. Several studies were in agreements and stated that after pretreatment with CHX, bonding properties diminished with DD and after storage in water.[51] It has been speculated that pretreatment with CHX inhibits polymerization of the bonding resin by increasing water sorption which accelerates the hydrolysis of the bond structure, consequently, the physical properties of the resin, decreased with time.[52]

Proanthocyanidins (PA) were used as collagen crosslinkers and MMP inhibitors to increase degradation resistance of bonding interface of the adhesive and dentin. Crosslinkers stiffen the collagen so that it cannot unwind.[20] PA has a high affinity for proline-rich proteins like collagen forming a proline-PA complex which is more resistant to degradation and does not hinder the resin infiltration.[53]There is controversy on the interaction mechanism of PA with collagen. Four different mechanisms are proposed: covalent interaction, ionic interaction, hydrogen bonding, or hydrophobic interactions.[21]

PA can be found in many natural products some of them was tested in the current study their ability to enhance the bond strength was recorded.

Sound dentin had higher mean shear bond strength than demineralized dentin. When analyzing the effect of dentin type (SD and DD) on bond strength, it was reported that the condition of dentin had a significant effect on bond strength: even if SD and DD had similar intertubular structure, bond strength to DD would still be significantly lower than to SD. The change in chemical and morphological characteristics of DD would be also reasons for the lower bond strength. The hybrid layers formed to DD thicker than those of SD, because DD is more vulnerable to the acid due to partially demineralization, consequential the formation of a deeper demineralized zone.[54, 55]

One of the limitations of this study is in the method of obtaining DD, mostly with reference to the laboratory demineralization-remineralization model, which does not consider the organic degradation nature of caries that clinically occurs.

CONCLUSION

The major advantages of using natural alternatives are easy availability, cost-effectiveness, increased shelf life, low toxicity and lack of microbial resistance reported so far. The present results appear promising for the application of natural collagen cross-linkers during adhesive restorative procedures as a new approach to improve dentin bond strength properties. However, several clinical trials are recommended.

When compared to the control group, the use of PP and AP, as cavity disinfectant and collagen cross-linkers, increased the shear bond strength to sound and demineralized dentin.

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