Original Research

Effect of Tannic Acid Application on Durability of Bond of Etch and Rinse Adhesive Resins

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Abstract

Aim: To determine the effect of different concentrations and application times of tannic acid on bond strength to dentin. **Materials and Methods:** Occlusal surfaces of 180 human molars were randomly divided into two groups of immediate shear bond strength evaluation and evaluation after 2 months of storage in collagenase solution. Each group was divided into 10 subgroups according to the solutions applied: no additional treatment (control), chlorhexidine 2% (Positive control), tannic acid 20% (30s, 1 min, 3min, 5min) and Tannic acid 30% (30s, 1 min, 3 min, 5 min). Failure mode were assessed using a stereo-microscope. The data were statistically analyzed by One-way ANONA and Tukey's post hoc test. **Results:** There was no significant difference in immediate bond strength among experimental groups. However, after 2 months storage in collagenase solution, a significant difference was found among the groups. Bond strengths of CHX and 20% TA applied for 3 minute were significantly higher than the control group. **Conclusion:** Use of tannic acid and chlorhexidine has no negative effect on immediate shear bond strength. However, their application significantly prevents compromised bond strength caused by storage in collagenase solution.

Keywords: bond durability, tannic acid, chlorhexidine, matrix metalloproteinases.

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Introduction

Use of adhesive resin systems is a mandatory step for restoring teeth with tooth-colored restorative materials. Although improvement in adhesive systems has established effective bond to tooth structure, the quality of bond becomes unstable and degrades over time, particularly in dentin (1). The main reasons for this reduction in bond include: hydrolytic degradation via water sorption, incomplete penetration of resin monomers, collagenolytic activity of matrix metalloproteinase (MMPs), Cystein and Cathepsin enzymes (2,3). MMPs are proteolytic enzymes with cellular origin consisting of 26 members. Dentin possesses endogenous MMPs which regulate its physiologic and pathologic metabolism (4,5). Since resin cannot penetrate to the whole depth of acid etched dentin, some collagen fibrils at the base of the hybrid layer remain exposed. These fibrils can be degraded by MMPs activity and hydrolysis process, so bond durability may decrease (6,7). Three main strategies have been suggested in order to inactivate MMPs or improve bond durability including application of MMP inhibitors (8,9), collagen cross linkers (10,11) and drying agents (12). Inhibitors decrease aging of bonding substrates and improve stability of collagen fibrils in the hybrid layer, thereby improving bond durability (7,13). They can act via binding to the active site of catalytic domain and changing the pro-enzyme molecular structure, inhibiting enzymes' catalytic activity or through Ca and Zn ions chelating (cation chelating mechanism), like tetracycline and doxycycline (14-16). One of the most important MMP inhibitors is

chlorhexidine which is well known as a disinfectant and serves as a great MMP inhibitor as well (13,17). Cross linking agents such as Glutaraldehyde, Proanthocyanidin and tannic acid improve mechanical properties and increase modulus of elasticity and tensile strength of demineralized dentin by inducing more exogenous cross links (10). They change collagen fibers' structure and stabilize them against degradation (2,18). However, some disadvantages have been mentioned for cross linkers. Synthetic cross linkers like Glutaraldehyde or Carbodiimide have high levels of cytotoxicity. Moreover, their long term stability is indistinct. On the other hand, natural cross linkers have fewer shortcomings (2). Tannic acid (TA) is a commercial form of Tannin, a polyphenol with weak acidity. TA was previously used as a vasoconstrictor to inhibit bleeding of gingival tissue and also as a dentin acid conditioner. However, it was only capable of removing smear layer and not demineralizing dentin. TA binds to collagen amid groups via its hydroxyl group, rendering fibrils more stable and resistant against MMPs (19,20).

The aim of this study was to evaluate the effect of different concentrations and application times of TA on bond strength and bond durability of a total etch adhesive resin. The null hypothesis of this study is that different concentrations and application times of tannic acid (TA) have no significant effect on immediate and two-month bond strengths.

Methods and Materials

180 human third molar teeth without caries and cracks were selected. After debridement, the teeth were stored in normal saline solution (Shafa, Iran) for 1-2 weeks in standard room temperature (25 ^{c}). The solution was renewed every week. Then the samples were vertically mounted in self-cure acrylic resin blocks (Acropars, Iran) from 1 mm below CEJ. The enamel of occlusal surfaces were removed and then dentin surfaces were gently ground flat with 400 and 600 grit silicon carbide sand papers under running water. The samples were randomly divided into 10 groups of 18. In the first group (control), dentin surfaces were etched by 15% phosphoric acid (Ultradent, USA) for 15 seconds and washed out for 15 seconds. Then, adhesive (PQ1, Ultradent, USA) was applied according to the manufacturer's instructions and polymerized using a LED curing unit (Blue Phase C8, IvoclarVivadent, Schaan, Lichtenstien) at 800mW/cm^2 for 20s. Consequently, one transparent plastic tube with 1.5 mm diameter and 4mm length was carefully placed on the dentin surface. Resin composite build-up was accomplished using one increment of 1mm and two 2-mm increments with Z250 (Shade A1, 3M ESPE; St Paul, MN, USA), each increment being light cured for 40s. Specimens were post-cured from each surface for 40s. In other experimental groups, all procedures were the same as the control group except application of the solution before using adhesives. In the second group (positive control), aqueous solution of 2% CHX (Consepsis, Ultradent, USA) was applied on the dentinal surface with micro brush (Pegasus, Astek Innovations, England) removing the remnants after 30 seconds with a clean micro-brush. (2, 18). In the third (TA20) and forth (TA30), 20% and 30% TA solutions were applied respectively. These groups were divided into four subgroups according to time of TA applications (30 s, 1 min, 3 min and 5 min). The remnants of TA were removed with a clear micro-brush.

In half of the samples of each experimental group, the shear bond strength test (SBS) was performed after 24 hour storage in 100% humidity by using Universal Testing Machine (Zwick/Z250, Type KAP-Z, Zwick Roell Group; Ulm, Germany) at crosshead speed of 1 mm/min. According to the time of evaluating bond strength samples were divided into 2 subgroups. Micro tensile bond strength test was done on half of the specimens after 24 hours from preparation. In other samples, SBS was evaluated after two month storage in collagenase solution (Cholestridium Histoliticum, 125 U/mg, Sigma Aldrich, USA). The solution was renewed every week. Failure modes were determined at 40X magnification using a stereomicroscope (LEO, 1450 UP, Zeiss; Oberkochen, Germany) and recorded as adhesive, cohesive in enamel, cohesive in composite or mixed. Data were processed using SPSS version 11.5 software (SPSS Inc. Chicago, IL). Normal distribution of the data was confirmed using Kolmogorov-Smirnov test, and data were analyzed using One-way ANOVA and Duncan tests with the level of significance set at 0.05.

Results

The mean values of SBS are presented in Table 1. There was no significant difference in immediate bond strength among experimental groups (P-value= 0.2). However, after 2 months storage in collagenase solution, a significant difference was found among groups. Bond strengths of CHX and 20% TA applied for 3 minute, were significantly higher than the control group. Failure assessment is summarized in Table 2. Adhesive failure was the most observed failure mode in all experimental groups.

Table 1. The mean value of SBS of experimental groups (MPa). Values with different uppercase letters denote statistically-significant difference at $P < 0.05$.

Groups		N	Mean ± Std. deviation
Immediate	Control	9	10.703 [a] ± 2.59
	CHX	9	11.650 [a] ±2.71
	TA 20%-30”	9	9.970 [a] ± 2.171
	TA 20%-1´	9	9.893 [a] ± 1.091
	TA 20%-3´	9	9.970 [a] ± 3.04
	TA 20%-5´	9	8.986 [a] ± 0.975
	TA 30%-30”	9	9.995 [a] ± 1.101
	TA 3%-1´	9	9.316 [a] ± 1.144
	TA 30%-3´	9	8.966 [a] ± 0.740
	TA 30%-5´	9	8.560 [a] ± 1.216
2 months storage	Control	9	5.285 [b] ± 1.020
	CHX	9	8.488 [d] ± 2.953
	TA 20%-30”	9	5.635 [b] ± 0.928
	TA 20%-1´	9	6.448 [bcd] ± 1.632
	TA 20%-3´	9	7.985 [cd] ± 1.754
	TA 20%-5´	9	6.125 [bc] ± 1.127
	TA 30%-30”	9	5.998 [bc] ± 1.705
	TA 30%-1´	9	6.458 [bcd] ± 1.790
	TA 30%-3´	9	6.561 [bcd] ± 1.387
	TA 30%-5´	9	5.830 [bc] ± 1.713

Table 2. Distribution of Failure modes in experimental groups

Groups		Adhesive	Cohesive of composite	Mixed	Cohesive of dentin
Immediate	Control	6 (66.6%)	0	3 (33.3%)	0
	CHX	5 (55.5%)	0	4 (44.4%)	0
	TA 20%-30”	8 (88.8%)	0	1 (11.1%)	0
	TA 20%-1´	9 (100%)	0	0	0
	TA 20%-3´	7 (77.7%)	0	2 (22.2%)	0
	TA 20%-5´	8 (88.8%)	1	0	0
	TA 30%-30”	9 (100%)	0	0	0
	TA 3%-1´	9 (100%)	0	0	0
	TA 30%-3´	8 (88.8%)	0	1 (11.1%)	0
	TA 30%-5´	9 (100%)	0	0	0
2 months storage	Control	9 (100%)	0	0	0
	CHX	7 (77.7%)	0	2 (22.2%)	0
	TA 20%-30”	9 (100%)	0	0	0
	TA 20%-1´	9 (100%)	0	0	0
	TA 20%-3´	8 (88.8%)	0	1 (11.1%)	0
	TA 20%-5´	9 (100%)	0	0	0
	TA 30%-30”	9 (100%)	0	0	0
	TA 3%-1´	8 (88.8%)	0	1 (11.1%)	0
	TA 30%-3´	8 (88.8%)	0	1 (11.1%)	0
	TA 30%-5´	9 (100%)	0	0	0

Discussion

The null hypothesis was partially rejected by the results of current study. Application of 20% tannic acid for 3 minutes could preserve bond strength. The results of current study are in consistence with previous studies which demonstrate that CHX can improve bond durability (21-23). CHX is the gold standard MMPs inhibitor which has been recommended by several studies. CHX decreases bond degradation and improves durability via inhibition of enzymes. It has been reported

by several authors that CHX doesn't interfere with immediate bond strength and has no negative effect on bond strength (4,24). More ever, it results in higher long term bond strength values and improves bond durability. (25, 26). This effect is attributed to substantivity and also in the prolonged retention of CHX to dentin surface that result in a more durable hybrid layer over time (6,25, 26). Breschi *et al.* (27) reported significantly less hybrid layer degradation and reduction in bond strength in CHX-treated specimens compared to control specimens (27). Carrilho et al. (8) in an *in-vitro* study on inhibitory effect of CHX, reported that CHX preserves hybrid layer against degradation and aging over time (8). Zhou et al. (7) in an *in-vitro* study added CHX to the primer of a two-step self -etch adhesive and confirmed the effect of CHX in preservation of bond to dentin(7).

Cross linking agents improve bond durability via concealing cleavage site of fibrils, decreasing enzymes activity and water sorption. They promote modulus of elasticity and tensile properties of dentin. Macedo *et al.* (28) claimed that using cross linkers such as glutaraldehyde and grapes seed juice significantly improved bond to carious dentin (28).

For the first time, Bedran-russo applied 10% and 20% TA as a cross linker for one hour. He reported that 20% TA cloud inhibit bond degradation in samples that were stored in collagenase for 24h (10). In the current study, only one adhesive system (PQ1) was tested. Our results were consistent with De Muancks et *al.* (29) study about etch and rinse adhesives. Results of the current study about immediate bond strength demonstrated that 20% and 30% TA didn't have significant differences. Different concentrations and application times of TA decreased the immediate bond strength in comparison to the control group, though not significantly. This could be attributed to the presence of water in TA solutions. It was in consistent with results of Pavan *et al.* (30); that used 25% TA as a conditioner for dentin. Some studies on cross linkers claim that they are time and concentration dependent, although our results didn't prove it (11, 19, 31). Zouet al. (32) reported that the cholesterol esterase enzyme in saliva can destruct monomers with aromatic rings such as Bis-GMA in 16 days (32). Other study also mentioned that methyl methacrylate polymers with ester links are susceptible to hydrolysis and MMPs (33). Armstrong *et al.* (34) demonstrated that samples stored in proximity of a cholesterol esterase (with origin of Clostridium hisblytion) showed lower bond strength. This issue is interpreted as enzymatic hydrolysis has a more destructive effect on resin-dentin bond compared to collagenase degradation since bond strength in collagenase group and artificial saliva group didn't differ significantly (34). Several authors reported that CHX doesn't decrease immediate bond significantly (27, 35). In the current study, the bond strength in CHX group, immediately and after 2 months (in collagenase), didn't differ significantly. This could be attributed to participation of CHX in promotion of bond durability as proved by other studies. Biocompatibility is one of the advantages of TA over other cross linkers such as glutaraldehyde. TA is supposed to have low cytotoxicity by several authors (36-38). Bitter (39) reported that 25% TA is well tolerated by pulp and has no adverse effects. According to our results, the best application time recommended for TA was 3 min, though it didn't differ significantly with 1 min and 30 sec application times. It seems that TA can establish hydrogen bond with collagen, which increases modulus of elasticity of dentin. Bedran-Russo *et al.* (11) and Castellan *et al.* (38) reported that TA slows down the rate of bond degradation through covering cleavage sites and reducing enzymes activity (11, 38).

Our study was an *in-vitro* study, thus conducting *in-vivo* and clinical studies with larger sample sizes are recommended. Furthermore, in the present study, a total etch adhesive was tested. Trials on self-etch adhesives are needed in future. Within limitations of this study, it's concluded that application of 20% TA for 3 minutes would improve bond strength of total etch adhesives to dentin.

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