EGYPTIAN DENTAL JOURNAL

VOL. 63, 1649:1658, APRIL, 2017

I.S.S.N 0070-9484

FIXED PROSTHODONTICS, DENTAL MATERIALS, CONSERVATIVE DENTISTRY AND ENDODONTICS

www.eda-egypt.org • Codex : 30/1704

MICROTENSILE BOND STRENGTH AND NANOLEAKAGE OF A RESIN COMPOSITE BONDED TO DENTIN TREATED WITH GALLA CHINENSIS EXTRACT

Emad Aboalazm* *and* Rania Badawy**

ABSTRACT

Aim: This in vitro study aimed to evaluate the effect of dentin surface-treatment with 4mg/ml Galla Chinensis Extract (GCE) on microtensile bond strength and nanoleakage of a resin composite restoration, subjected to two different water storage periods.

Materials and Methods: A total of thirty freshly extracted sound human molars were prepared and sliced coronally for application of a nanohybrid resin composite restorative material (Filtek Z350 XT). Exposed dentin surfaces, were acid-etched, to be either routinely covered by adhesive and resin composite, or treated with 4mg/ml Galla Chinensis Extract, followed by application of an adhesive and a resin composite. Treated teeth were divided into 3 subgroups according to time of assessment of their microtensile bond strength and nanoleakage after 24 hours, 3 months and 6 months of water storage.

Data were statistically analyzed with One-way ANOVA at 95% confidence interval. Kolmogorov Smirnov and Shapiro-Wilk tests were applied for normality testing. Independent t-test was used to compare between different groups for different water storage periods.

Results: Insignificantly different microtensile bond strength values between control and surface-treated teeth were recorded for the resin composite material immediately and after 3 months water storage periods. Lowest values were recorded after 6 months storage period, differing significantly ($p \leq 0.001$) between control and surface-treated dentin group. The immediately tested control group revealed significantly the highest mean microtensile bond strength values (32.1±7.84 MPa) compared to both other control groups at different storage periods. SEM micrographs showed extensive silver deposition throughout the entire hybrid layer thickness of silver-impregnated sections at resin/dentin interfaces after 6 months aging period. These depositions penetrated into dentinal tubules and around the resin tags in the control group.

Conclusions: Surface treatment of etched dentin with 4mg/ml Galla Chinensis Extract improved resistance of bonded resin composite restorations to hydrolysis in terms of improved microtensile bond strength and nanoleakage after 3and 6 months of water storage.

KEYWORDS: Galla chinensis, microtensile bond strength, nanoleakage, water storage, resin composite.

* Lecturer, Operative Dentistry, Suez-Canal University, Ismailia, Egypt
** Lecturer, Dental Materials, Suez-Canal University, Ismailia, Egypt

INTRODUCTION

Adhesion of resin materials to tooth structure has been a challenge in the history of adhesive dentistry. Currently, the issue of bond durability has attracted significant attention regarding resin–dentin bonding.[1,2] Despite improvements in dental adhesive technology and advances in bonding knowledge, resin–dentin bonding still shows limited durability for both etch-and-rinse and self-etch adhesive systems.[3,4]

Nevertheless, resin–dentin bonds created by infiltration of hydrophilic resin monomers into demineralized and mineralized dentin are imperfect and unstable.[5,6] Inadequate polymerization reduces the quality of the hybrid layer, leading to lower dentin bond strengths and increased nanoleakage.[7] Moreover, high permeability at the bonded interface and phase-separation during adhesive application contribute to hydrolytic degradation of the adhesive resin.[6]

Bonding to dentin is more complicated than bonding to enamel is, due to its high organic content and high humidity, having a tubular structure with an intrinsic wetness and a continuous outward flow of fluids.[8] The major component of dentin organic matrices is heterotrimeric fibrillar type I collagen representing 90%of the entire dentin content, which serves as oriented scaffold for deposition of mineral crystals and packing of non-collagenous matrices.[9–12] The covalent intramolecular and intermolecular cross-links formed by post-translational modifications, significantly stabilized type I collagen, forming the basis for its stability, fracture toughness, tensile strength and viscoelasticity.[13]

Biodegradation of the dentin collagen fibers, is a result of hydrolysis of the collagen fibers of demineralized dentin matrix by metalloproteinases (MMPs).[14] MMPs are a class of host-derived, zinc-activated, and calcium-dependent endopeptidases which become activated in low-pH environments; developed as a result of the acidic etchants or acidic resin monomers used during adhesive application. Human dentin contains MMPs-2, -8, and -9 which are capable of degrading type I collagen fibers.[14-16] However, the infiltration and encapsulation of demineralized collagen networks by the adhesive resins is thought to immobilize MMPs and thus provide protection against collagen degradation.[17] However, little attention has been paid to the contribution of collagen structure/stability to the bond strength. Studies have shown that with time, collagen in the hybrid layer is affected by enzymatic degradation; to finally cause bond failure.[14-16] This indicates that the improvement of dental materials alone is not sufficient enough to achieve a successful long-term bonding. Obviously, the stability of collagen fibrils should be maintained for a long period of time, the goal which may be achieved by bio-modification of dentin matrix.[17,18]

Galla Chinensis, the natural traditional Chinese medicine, of herbal origin, is known for being rich in hydrolysable polyphenols with a wide range of biological activities. Several studies have documented the capability of Galla Chinensis Extract (GCE) to inhibit cariogenic microbes and enamel demineralization, in a mode different from that of fluorides,[19] and positively preventing disintegration of uncovered dentin matrix.[19,20] The extract may even interact with dentin matrix to improve its biochemical and biomechanical properties, while maintaining the structural integrity and longevity of dentin,[21,22] also enhancing remineralization of carious lesions. Remineralization occurs in a natural repair process, transferring anions and cations to affected tooth areas, with an insignificant effect of pH.[23-25] Moreover, the GCE is ineffective on polymicrobial biofilms at alkaline pH.[26]

Due to the known promising effects of GCE on tooth structure and due to the importance of stability and quality of defect-treatments, this study aimed to test the hypothesis, that GCE will positively affect

the microtensile bond strength and sealing ability of resin composite to superficial dentin tested at two different time-intervals.

MATERIALS AND METHODS

GCE preparation

The extract of Galla Chinensis, was distilled as described by Cheng et al 2009.[19] The material produced in the Sichuan province of China was dried at 60°C for 3 days, powdered and double extracted with distilled water, and then ethanol for filtration and evaporation. Subsequently, GCE was further fractioned by adsorption chromatography and purified by successive column chromatography. The obtained powder was dissolved in ethanol to get a 4mg/ml concentration extract.

Samples preparation

A total of 30 freshly extracted human third molars (aged 20-30 years) were collected, cleaned with an ultrasonic cleaner and stored in distilled water at 4°C until tested. Each tooth was embedded in a cold-cured acrylic resin block, 1.5mm apical to the CEJ, having their long axis situated parallel to those of the molds. Occlusal enamel was cut perpendicular to the long axis of the tooth at the level of dentino-enamel junction (DEJ) using an automated diamond saw (IsoMet 4000, Buehler Ltd., Lake Bluff, IL, USA) under copious water coolant exposing the superficial dentin surface.(Figures 1a-c)

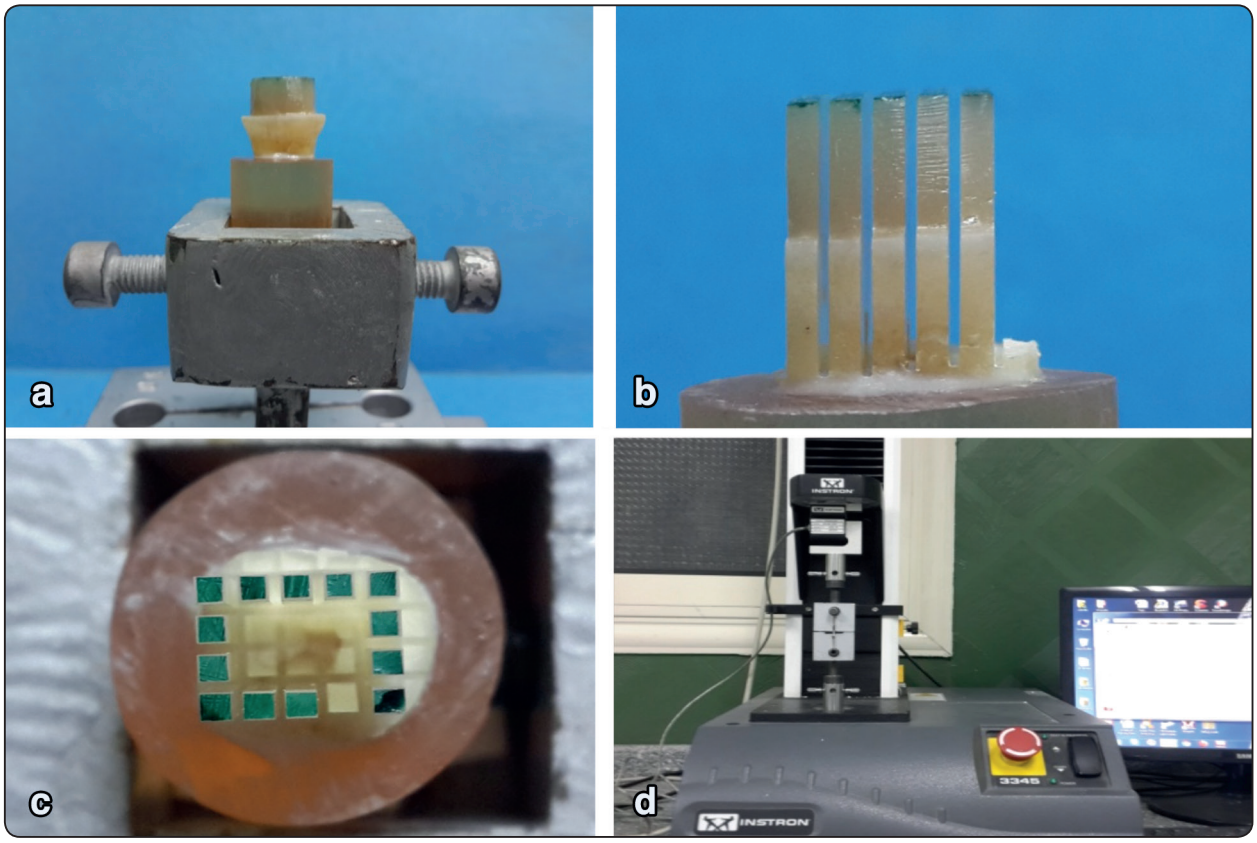

Fig. (1) a) Prepared dentin with composite build-up. b) Lateral view of prepared slabs. c) Occlusal view of prepared beams. d) Instron testing machine with mounted Jig

Specimen preparation for the micro tensile bond strength test(MTBS)

Prepared occlusal surfaces were acid-etched (37% phosphoric acid for 15 seconds, 3M ESPE), and after rinsing the acid, teeth were divided into 2 groups, A and B (15 specimens/grp).

Group A (Control group; 15 teeth): after acid etching, teeth were rinsed with water, dried and the adhesive system (Adper Single Bond 2, 3M ESPE) was applied on the polished dentin surface according to manufacturer's instructions. The adhesive was irradiated with a light-curing unit (Blue phase C5 LED, Ivoclar Vivadent AG, Shaan, Liechtenstein, Germany); 500mW/cm^2 light intensity at zero distance. Afterwards, resin composite FiltekZ350 XT was built-up incrementally (7mm length, 5 mm width, 4 mm height) to ensure sufficient bulk for the MTBS test (Figures 1a-d). Each increment was light-cured for 20 s.

Group B on the other hand was treated differently. After acid etching, the teeth were rinsed and dried, and a single coat of 4mg/ml GCE solution was applied to the dentin surface of the remaining 15 molars using a brush. The solution was kept on the dentin surface for 3 minutes prior to air-thinning. Afterwards the adhesive system and the resin composite were applied as mentioned previously for the control group.

Each group was further subdivided into 3 subgroups (5 teeth/grp) according to time of assessment. Subgroup 1; assessed after 24 hours of water aging, subgroup 2; assessed after 3 months of water aging and subgroup 3; assessed after 6 months of water aging. Teeth of each subgroup were stored separately in distilled water at room temperature in a sealed light-tight plastic container, which was labeled according to treatment and time of assessment.

Micro-tensile bond strength (MTBS) test

Each specimen was cut perpendicular to the bonded interface using a low speed diamond saw under water spray coolant for preparation of beams for microtensile bond strength testing, (0.9±0.1 mm thickness and 7±1 mm length). Beams sliced from each tooth were aligned in the central groove of a jig and their ends were glued using cyanoacrylate-based glue (Zapit, DVA Inc, USA). The jig was mounted on a universal testing machine (Instron, Model 3345, England) with a load cell of 500 N (Figure 1-d). Tensile load was applied, at a cross-head speed of 0.5 mm/min, until bond failure. Bond strength was recorded (MPa) using a computer software connected to the testing machine (Bluehill 3 Software, version 3.3 Instron, England). Prematurely-failed beams, either during preparation or testing, were considered of zero value.

Nanoleakage specimens' preparation

Additional 5 extracted human third molars/ subgroup were selected and stored as described for the MTBS test. The specimens were sectioned below the DEJ, ground flat with 180-grit silicon carbide abrasive paper under running water to provide uniform smear layers. The adhesive systems and composite build-ups for all specimens were applied on the dentin surface of each tooth according to the manufacturer's instructions. The specimens were sectioned parallel to the long axis of the tooth using a diamond saw under water coolant, all through the composite buildups and the bonded tooth, into approximately 1 mm thick slabs (Figure 1b). One central slab was chosen from each tooth, forming a total of 5 slabs per group. The bonded slabs were ground and polished in turn using wet paper discs up to 1200grit, and then they were coated with 2 layers of fast-drying nail varnish, applied up to 1 mm within the bonded interfaces. To keep the specimens hydrated, they were immersed in distilled water for 10 min prior to immersion in a tracer solution, ammoniacal silver nitrate, which was prepared according to the protocol previously

described by Tay et al.[27] The slabs were placed in the ammoniacal silver nitrate in total darkness for 24 h. Afterwards, the slabs were thoroughly rinsed in distilled water and immersed in photo-developing solution for 8 h under a fluorescent light, to reduce the penetrated silver ions into metallic silver grains. The silver-stained resin-bonded specimens were gently polished with diamond pastes, of 1µm particle size and placed in ultrasound cleaner for 5 min to remove the superficial silver adsorption. Finally, the prepared slabs were examined under scanning electron microscope (SEM) (Philips 505, Eindoven, Netherlands) for assessment of nanoleakage.

Statistical Analysis

Data were statistically analyzed at a 95% confidence interval, and the Kolmogorov-Smirnov, Shapiro-Wilk tests were used for normality testing. Independent t-test was used to compare between different groups under different storage periods. One Way ANOVA used to compare between time for each material (SPSS Inc., IBM Corporation, NY, USA- Statistics Version 24 for Windows).

RESULTS

Microtensile bond strength:

Means and standard deviation values of microtensile bond strength for tested resin composites subjected to 2 different surface treatments are presented in Table (1) and Figure (2).

TABLE (1): Means and standard deviations (SD) for different groups and storage periods.

		Groups				p-value
		Control		Group		
		Mean	SD	Mean	SD	
Microtensile Bond strength (MPa)	Immediate(24h)	32.10[a]	7.84	30.17[a]	9.38	0.395 NS
	3 Month	22.43[b]	7.79	25.48[b]	8.79	0.164 NS
	6 Months	16.80[c]	8.16	22.29[b]	6.85	0.008*
p-value		≤0.001*		0.003*		

Means with the same letter within each row indicates an insignificant difference at p≥ 0.05

NS= Non-Significant. *=Significant

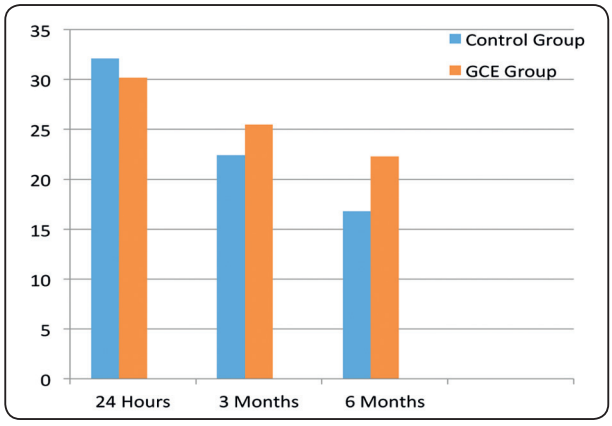

Fig. (2) Mean microtensile bond strength (MPa) values of control group and Galla Chinensis Extract-treated groups.

Effect of different surface treatments on microtensile bond strength

Insignificantly different microtensile bond strength values ($p \geq 0.05$) of the resin composite material were recorded between the control group and dentin surfaces treated with GCE prior to adhesive and resin composite application, for the immediate and 3 months' assessment (24 h: 32.1±7.84MPa; 3 months: 22.43±7.79MPa. respectively) Lowest microtensile bond strength values were recorded after 6 months' storage period in distilled water, differing significantly ($p \leq 0.001$) between control (16.8±8.16 MPa) and GCE-treated dentin group (22.29 ±6.85 MPa).

Effect of different storage periods on microtensile bond strength

The immediately tested control group revealed significantly the highest mean microtensile bond strength values (32.1±7.84 MPa) compared to the other control groups. A significant decrease in microtensile bond strength values occurred after 3 months (22.43±7.79 MPa), and a further significant decrease after 6 months (16.8±8.16 MPa) occurred for the 3 control groups at $p \leq 0.001$.

For differently-treated groups, immediate testing showed the highest significant mean microtensile bond-strength values (30.17±9.38 MPa), decreasing significantly (25.48±8.79 MPa, 22.29±6.85 MPa) after 3 and 6 months respectively.

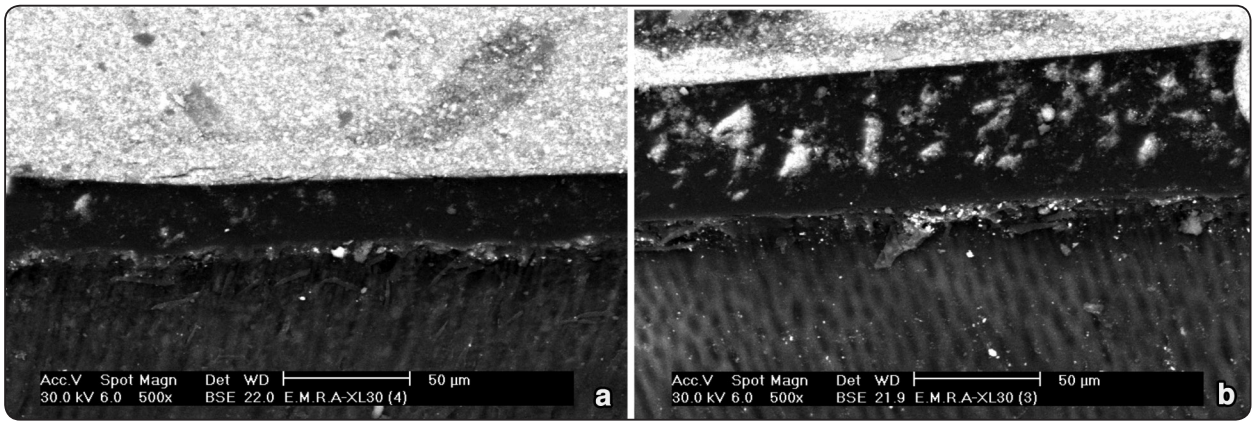

Fig. (3) a Microphotograph showing nanoleakage of control group after 24 hours. b) Microphotograph showing nanoleakage of Galla Chinensis-treated group after 24 hours

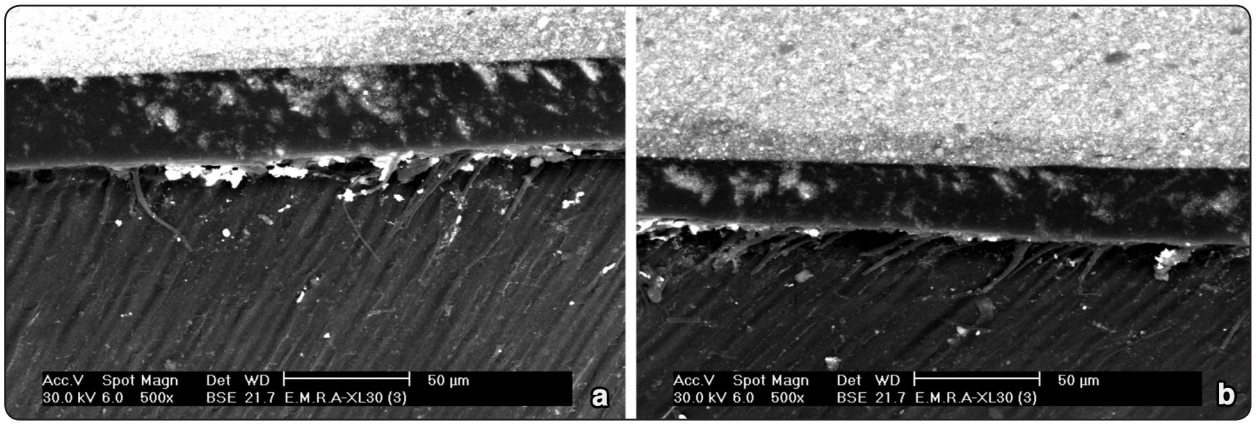

Fig. (4) a) Microphotograph showing nanoleakage of control group after 3 months b) Microphotograph showing nanoleakage of Galla Chinensis treated group after 3 months

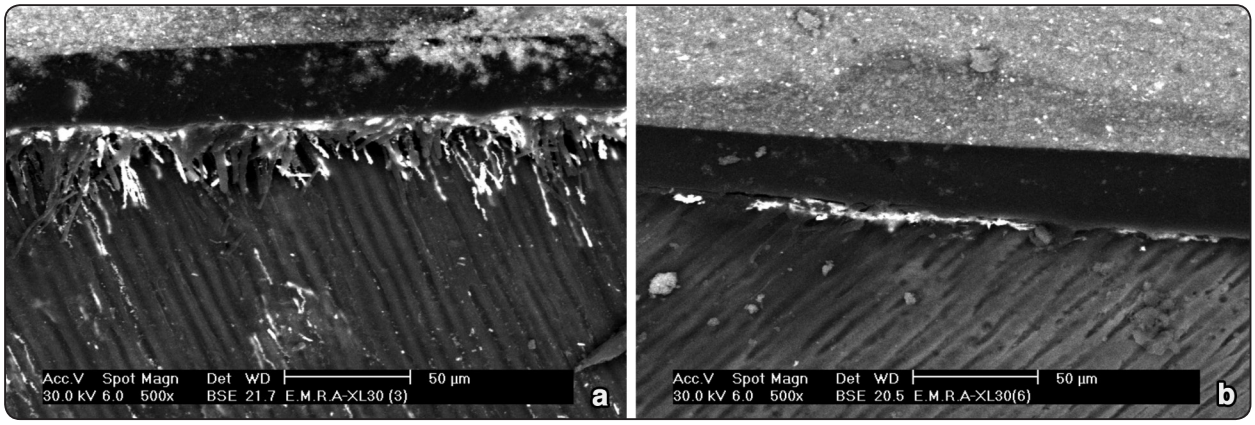

Fig. (5) a) Microphotograph showing nanoleakage of control group after 6 months. b) Microphotograph showing nanoleakage of Galla Chinensis-treated group after 6 months

Nanoleakage

Representative SEM micrographs obtained from silver-impregnated sections of resin/dentin interfaces of bonded specimens in 24 hours subgroups, control and GCE, are depicted in figures3-a and 3-b respectively. Both subgroups revealed nearly the same nanoleakage pattern in the form of very thin and interrupted-silver spotted deposition observed within the hybrid layer.

Further representative SEM micrographs obtained from silver-impregnated sections of resin/dentin interfaces of bonded specimens aged for 3 months forming subgroups, control and GCE-treated surfaces are depicted in figures 4-a and 4-b respectively. Both subgroups showed dense deposition of silver particles along the hybrid layer and all over the dentin surface, being higher than for the 24 hours subgroups with marked increase in control group compared to GCE-treated group.

Finally, the representative SEM micrographs obtained from silver-impregnated sections of resin/dentin interfaces of bonded specimens in 6 months aged subgroups, control and GCE treated surfaces, are represented in figures 5-a and 5-b respectively. The SEM showed extensive silver deposition throughout the entire hybrid layer thickness, with penetration into dentinal tubules and around the resin tags in the control group. On the contrary, the GCE-treated group showed dense silver-deposition along part of the hybrid layer and over the dentin surface, being markedly lower than that of the control group.

DISCUSSION

This in vitro study aimed to assess the interaction of GCE with the dentin matrix to improve the microtensile bond strength and thesealing abilityof sliced dentin surfaces treated with etch and rinse technique after 2 periods of water aging.

In the 2 step etch-and-rinse adhesive system, the adhesive resin is a hydrophilic mixture of the primer and bonding resin monomers, applied after rinsing the acid-etched dental substrate. The occurring monomer entanglement with the collagen fibrils at the resin/dentin interface creates a mixed structure known as hybrid layer.[6] It has been recommended to maintain the conditioned dentin surface in a moist state prior to application of the bonding resin, as the acid-etched collagen undergoes shrinkage and collapse on drying.[8]

Ideally, the organic primer should be able to completely displace the residual surface dentin

water and allow the adhesive resin to fully infiltrate and hybridize collagen after polymerization.[28] The presence of residual solvent and dentin transudation during the solvent-evaporation step, makes water replacement by resin far from ideal[29,30], thus collagen and resin hybridization mainly provides mechanical retention for resin-based restorations. Although, early in-vitro studies using this approach revealed satisfactory results with high bond strength values and sealing ability, still concerns were raised with regard to the long-term performance of the adhesive.[4,28]

The principal degradation mechanism of the hybrid layer isbased on both hydrolysis and enzymatic breakdown of the collagen fibrils and the polymerized resin matrix.[31-33] Hydrolysis breaks down the covalent bonds between the polymers by water incorporation into ester groups, which finally results in loss of the resin mass. Such incorporation of water molecules is believed to be one of the main reasons for resin degradation in the hybrid layer, decreasing bond strengths created by dentin adhesives over time.[31,34-37] Inadequate resin infiltration/encapsulation of collagen fibrils results in a hybrid layer that is more vulnerable to enzymatic degradation.

The results of this study showed higher significant microtensile bond strength values and lower nanoleakage scores at 6 months of water aging for the GCE-treated group compared to the control group, most probably due to one or more of the following reasons:

GCE contains significant amounts of monomeric and polymeric polyphenols (e.g, gallotannin, gallic acid). It has been reported that the polyphenol-protein interactions facilitate the arrangement and interaction of GCE with collagen fibrils, through decreasing repulsive electrostatic forces between protein molecules. Furthermore, because GCE contains a significant number of polyphenols, the hydroxyl and carboxyl groups of polyphenols could form multiple hydrogen bonds with basic amino acid side chains in the band region of collagens. It has been recognized that formation of H-bonds acts as a constraint to amide II and III bending vibrations, which results in shifting the frequency to higher values and downward shifting of H-bonded OH and N-H amide A bands. These shifts increased tensile strength, fracture toughness and viscoelasticity of collagen chains in addition to further hydrolytic stability, being in agreement with several studies[33-36].

Because GCE mainly contains gallotannins and gallic acid polysphenols, it has been used for several years due to its well-known antibacterial, anti-caries effect on tooth structure [17, 21,22], especially as it inhibits metabolism of oral bacteria and release of collaginase enzyme.[38,39] Along with its anti-inflammatory, antiviral and anti-diarrheal effect. The extract also proved its strong effect on tooth re-mineralization, regulating its demineralization/ remineralization balance. Moreover they revealed combined enhancing effects with fluoride on remineralization of dental caries.[40,41]

In this study, increased bond strength values due to the use of GCE was accompanied by lower leakage scores compared to control groups for different storage periods. These findings may be attributed to increased number of inter-/intrafibrillar cross-links which was accompanied by decrease in the bound water amount and finally decrease in water absorption.[33]

Moreover, due to the inhibitory effect of GCE on collagenase activity in dental caries, [42] dentin surfaces treated with GCE revealed reduced biodegradability of dentin matrix. This finding is most probably due to sterical masking of cleavage sites, which increases resistance of the dentin matrix to enzymatic cleavage. Furthermore, collagenase infiltration into dentin/adhesive interface is most probably impaired by the dense matrix network formed, improving its structural stability, hydrolytic resistance, biomechanical properties with reduced collagen degradation.[33,42]

CONCLUSIONS

1- Immediate microtensile bond strength values for both control and GCE-treated dentin revealed the significantly highest values compared to the other storage periods.

2- Immediate nanoleakage results revealed good sealing ability of both control and GCE treated groups with minimal precipitation of silver nitrate.

3- Prolonged water storage negatively affected microtensile bond strength and sealing abilityof control and GCE-treated dentin.

4- Surface treatment of etched dentin surface with GCE improved hydrolytic stability of bonded resin composite restoration in terms of microtensile bond strength and sealing abilityafter 3 and 6 months water storage periods.

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