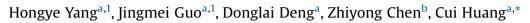
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# Effect of adjunctive application of epigallocatechin-3-gallate and ethanol-wet bonding on adhesive-dentin bonds



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#### ARTICLE INFO

ABSTRACT

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## Objectives: To determine the effect of the combined use of epigallocatechin-3-gallate (EGCG) and ethanol-wet bonding (EWB) on resin-dentin bonds.

*Methods:* Sixty molars were sectioned, polished, and randomly divided into six groups (n = 10) according to the following pretreatments: group 1, water-wet bonding (WWB); group 2, WWB with 0.02% (w/v) EGCG; group 3, WWB with 0.1% EGCG; group 4, EWB; group 5, EWB with 0.02% EGCG; and group 6, EWB with 0.1% EGCG. An etch-and-rinse adhesive was then used, followed by the resin composites building. The microtensile bond strength (MTBS), failure modes and interfacial nanoleakage were separately determined after 24 h water storage or 10,000 runs of thermocycling.

*Results:* Both pretreatment method (P < 0.05) and thermocycling (P < 0.05) significantly influenced bond strength and nanoleakage. Irrespective of thermocycling, the 0.02% EGCG/ethanol (group 5) pretreated adhesive-dentin interfaces showed higher MTBS than the control group (P < 0.05). Nanoleakage expression of all groups increased after thermocycling (P < 0.05) except group 5. Adhesive failure was the main fracture pattern in all groups.

Conclusion: This study showed that pretreatment with 0.02% EGCG/ethanol solutions can effectively improve immediate bond strength and bond stability of etch-and-rinse adhesives on dentin.

Clinical significance: The adjunctive application of EGCG and EWB provides a new strategy for dentists to obtain the desired bond effectiveness during adhesive restoration in clinical practice.

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## 1. Introduction

As the basis of esthetic restoration, contemporary dentin adhesive system has been developed and reached the eighth generation; however, the durability and stability of adhesivedentin bonds remain limited, particularly in clinical applications [1]. Poor bonding durability may weaken retention, produce marginal deterioration, and reduce service life of restorations [2]. Costs and resources have been consumed. Therefore, methods to improve dentin bonding durability have been extensively investigated in dentistry.

A decrease in bond strength is mainly attributed to the degradation of a hybrid layer at an adhesive-dentin interface [3,4]. In general, degradation is caused by incomplete infiltration of resin monomers, hydrolysis through water sorption, and

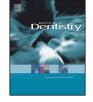
<sup>1</sup> These authors contributed equally to this work.

collagenolysis by endogenous matrix metalloproteinases (MMPs) and cysteine cathepsins [5,6]. Therefore, some effective methods, such as ethanol-wet bonding (EWB), MMP inhibitor, collagen cross-linker application, or biomimetic remineralization, have been developed to protect hybrid layer integrity [7,8].

Surface moisture is necessary to achieve effective dentin bonding [9]. Although water-wet bonding (WWB) is widely applied in dentin bonding, conventional hydrophilic adhesives likely increase water sorption and accelerate bonding interface degradation [10,11]. Therefore, the "EWB" technique, a method by which ethanol is used to replace water to support collagen fiber network of demineralized dentin, has been developed [12]. EWB can prevent collagen matrix collapse, promote infiltration of hydrophobic adhesive monomers into a collagen network, and avoid phase separation [13]. This approach has been successfully applied not only with experimental hydrophobic adhesives [14,15], but also with currently commercial adhesives, which are usually a combination of hydrophobic and hydrophilic monomers [16,17].

MMP inhibitors are used to prevent the degradation of incomplete resin-infiltrated collagen fibrils [8]. Among these







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inhibitors, chemical synthetics, such as chlorhexidine (CHX), are used to modify dentin adhesives; however, concerns on drug resistance have increased [18]. Epigallocatechin-3-gallate (EGCG), a green tea polyphenol, provides several beneficial functions, such as antioxidant, antimicrobial, antidiabetic, anti-inflammatory, and cancer-preventive properties [19]. EGCG induces low toxicity and inhibits MMP-2 and MMP-9 expression and activity [20]. Various concentrations of EGCG ranging from 0.0065% to 5% have been used in dentistry [21–24]. In this range, 0.02% and 0.1% EGCG/water solution can effectively facilitate dentin bonding [21].

To make dentin bonding more stable, the combined application of several well-developed methods is gaining people's attention. Ekambaram et al. [7] were the first to incorporate CHX to EWB, which enhanced the hydrophobic adhesive's ability to bond esthetic restorations with teeth. However, the possibility of drug resistance by CHX [18] and the complexity of self-made hydrophobic adhesive limited its clinical potential. Consequently, the combined use of EWB and EGCG, which possesses none of CHX's shortcomings, might provide clinicians with a better alternative. To the best of our knowledge, no report was available on this topic.

Therefore, the aim of this study was to explore the interactive effect of the adjunctive application of EGCG and EWB on adhesive–dentin bonds. The null hypothesis stated that the combined use of EWB and EGCG does not affect dentin bond strength, even after thermocycling is completed.

## 2. Materials and methods

#### 2.1. Specimen preparation and experimental groups

Sixty intact extracted human third molars were collected after the donors' informed consents were obtained in accordance with the protocols approved by the Ethics Committee for Human Studies of the School and Hospital of Stomatology, Wuhan University. The teeth were maintained in 1% chloramine T solution at 4°C for 1 month before use. A flat dentin surface was prepared by removing the occlusal crown with a low-speed water-cooled diamond saw (Isomet; Buehler, Evanston, IL, USA). The dentin surface was ground with water-irrigated 600-grit silicon-carbide paper for 60 s to create a standardized smear layer. Afterward, each dentin surface was etched with 35% phosphoric acid for 15 s, rinsed thoroughly with deionized water, and then blot-dried. The prepared teeth were randomly divided into six groups (n=10 each group) according to the following pretreatments: group 1 (WWB); group 2 (WWB+0.02% EGCG); group 3 (WWB+ 0.1% EGCG); group 4 (EWB); group 5 (EWB+0.02% EGCG); and group 6 (EWB+0.1% EGCG). Table 1 shows the composition and application methods of pretreatment solutions in each group and the dentin adhesive used in this study.

Briefly, the etched dentin surfaces in groups 1 were pretreated with a microbrush covered with distilled water for 60 s. In groups 2 and 3, 0.02% or 0.1% (w/v) EGCG solution was prepared in advance by dissolving 0.02 or 0.1 g EGCG (Sigma-Aldrich, St. Louis, MO, USA) in 100 ml of deionized water. The etched dentin surface was then pretreated with corresponding EGCG/water solution for 60 s. Excess liquid was removed from the specimens by gently blotting with filter papers to leave a visibly moist dentin surface. For groups 4-6, the similar operation was adopted but the solvent was replaced by 100% ethanol. The specimens in each group were bonded with Adper Single Bond 2 (3M ESPE, St. Paul, MN, USA). The procedures were performed by one dentist, and the manufacturer's instructions were strictly followed. The adhesive was polymerized using a LED light-curing unit (Bisco Inc., Schaumburg, IL, USA) at approximately 700 mW/cm<sup>2</sup> irradiance for 10 s. A resin composite (Charisma, Heraeus Kulzer, Hanau, Germany) was formed in four increments (thickness of 3-4mm), and each increment was polymerized for 20 s.

#### 2.2. Microtensile bond strength (MTBS) test

After the teeth were stored in deionized water at 37 °C for 24 h, the bonded teeth were longitudinally sectioned under watercooling to produce slabs with a thickness of 0.9 mm. Three slabs from each tooth were sectioned again to prepare beams with a dimension of 0.9 mm × 0.9 mm. After excluding unqualified beams which situated peripherally and showed presence of enamel, ten beams were obtained from each tooth. Five of them were immediately subjected to MTBS test, while the other five were thermocycled before being subjected to MTBS test. The beams were placed in a thermal cycling machine (Temperature Cycling Chambers; HUAN-S, Wuhan, China) from 5 °C to 55 °C for 10,000 cycles and dwell time of 15 s. The parameters (cycle times, temperature, and dwell time) were selected based on our previous study [25].

In MTBS test, each beam (50 beams for each subgroup) was attached to a jig with cyanoacrylate glue (Zapit, Dental Ventures of America, Corona, CA); the jig was placed in a universal testing machine (Microtensile Tester; Bisco, Schaumburg, IL, USA) and loaded in a tension at a crosshead speed of 1 mm/min until failure occurred. The maximum load was recorded, the fracture area of each beam was measured using a digital caliper; final MTBS values (MPa) were then calculated.

#### 2.3. Fracture mode analysis

After the MTBS test was conducted, the dentin side of the failed beams was observed under a stereomicroscope (Stemi 2000-C; Carl Zeiss Jena, Gottingen, Germany) at  $50 \times$  magnification and classified into four groups [26]: A, adhesive failure; CD, cohesive failure in dentin; CC, cohesive failure in composite; or M, mixed failure.

Table 1

Adhesive system, pretreatment solutions, comp	ositions and application modes in this study.
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Materials	Main components	Application modes
Adper Single Bond 2 (3M ESPE, St. Paul, MN, USA)	Bis-GMA, HEMA, dimethacrylates, polyalkenoic acid copolymer, initiators, water, and ethanol	Apply two coats of adhesive on pretreated dentin surface, gently air thin for 5 s, light-cure for 10 s
WWB (group 1)	Deionized water	1. Dentin surface was etched with 35% phosphoric acid 15 s,
WWB+0.02% EGCG (group 2)	Dissolving 0.02 g EGCG into 100 ml deionized water	rinse and blot dry
WWB+0.1% EGCG (group 3)	Dissolving 0.1 g EGCG into 100 ml deionized water	2. The etched dentin surface was pretreated with a
EWB (group 4)	100% ethanol	microbrush covering with the corresponding pretreatment solutions (groups 1-6) for 60 s, respectively
EWB+0.02% EGCG (group 5) EWB+0.1% EGCG (group 6)	Dissolving 0.02 g EGCG into 100 ml 100% ethanol Dissolving 0.1 g EGCG into 100 ml 100% ethanol	3. Excess liquid on dentin surface was gentle blotted with filter papers to leave a visibly moist dentin surface

Abbreviations: Bis-GMA, bisphenol-A-diglycidylether dimethacrylate; HEMA, 2-hydroxyethyl methacrylate; EGCG, epigallocatechin-3-gallate; WWB, water-wet bonding; EWB, ethanol-wet bonding.

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