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Effect of proanthocyanidin incorporation into dental adhesive on durability of resin–dentin bond

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ABSTRACT

Background: Proanthocyanidin has shown to have beneficial effects on dentin bonding via its collagen cross-linking and protease inhibitory effects.
Objective: This study evaluated the effect of incorporation of 1–3% PA into a dental adhesive on durability of resin–dentin bond.
Materials and methods: The experimental adhesive was first formulated by combining 50 wt% comonomer mixtures with 50 wt% ethanol. PA was then added to the ethanol-solvated adhesive to yield three groups of adhesives at concentrations of 1.0 wt%, 2.0 wt% and 3.0 wt%. The PA-free adhesive served as control. Flat dentin surfaces from forty extracted third molars were etched with 32% phosphoric acid and the specimens were randomly assigned to one of the four adhesive groups. Two layers of experimental adhesives were applied to etched dentin and light-cured for 20 s after solvent evaporation. Composite build-ups were performed using Filtek Z250 (3M ESPE). The bonded teeth were divided into three subgroups for different methods of storage: (1) 24 h indirect water exposure (IE), (2) 6 M IE and (3) 6 M direct water exposure (DE). After the designated period of water storage, the bonded teeth were sectioned into 0.9 mm × 0.9 mm beams for bond strength testing. Bond strength data were evaluated by two-way ANOVA and Tukey's tests ($\alpha=0.05$). Interfacial nanoleakage was examined using a field-emission scanning electron microscopy. Two-way ANOVA and Tukey's tests were used to examine the effects of PA concentration and water exposure on bond strength and percentage of nanoleakage ($\alpha=0.05$).
Results: Two-way ANOVA showed that the factors, water exposure and PA concentration had a significant effect on bond strength ($p<0.001$). Interaction between the two factors was also significant ($p<0.001$). Bond strength of all four adhesives decreased with PA concentrations and ageing. Type of water exposure had no effect on the bond strength of PA-incorporated adhesive; while direct water exposure significantly reduced the bond strength of PA-free adhesive. Conversely, the factors, water exposure and PA concentration showed a significant effect on nanoleakage percentage ($p<0.001$). Interaction between the two factors was not significant ($p>0.05$).
Conclusion: Incorporation of proanthocyanidin into a dental adhesive did not prevent resin–dentin bond degradation over time.

1. Introduction

The longevity of resin–dentin bond is challenged by degradation of collagen fibrils in the hybrid layer of the resin–dentin interface [1]. The hybrid layer is formed by infiltration of resin adhesive through the demineralized collagen scaffold, forming a resin-collagen inter-diffusion zone [2]. Even with the latest advancement in adhesive dentistry, complete coverage of the collagen scaffold produced by acid demineralization is challenging. There is a tendency of persistence of collagen fibrils at the base of the hybrid layer without resin protection [3,4]. These collagen fibrils degrade over time with exposure to exogenous or endogenous collagen-bound proteases, such as matrix metalloproteinases (MMPs) and cysteine cathepsins [5]. Furthermore, with repeated mechanical forces, the unprotected collagen fibrils may also undergo fatigue fracture [6].

In order to increase the longevity of resin–dentin bond, various strategies have been introduced, such as pre-conditioning the demineralized dentin with MMP inhibitors, use of collagen cross-linking

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agents to improve the mechanical properties of demineralized collagen matrix or use of deproteinization agents to remove the demineralized collagen matrix [7,8]. Matrix metalloproteinases inhibitors, such as chlorhexidine, have been used for preservation of collagen matrix by inhibiting MMPs and cysteine cathepsins [7,9,10]. Chlorhexidine has been regarded as the gold standard MMP inhibitor for preservation of resin–dentin bond. Although it can prevent degradation of the collagen matrix successfully, it has no effect on strengthening of the demineralized collagen fibrils.

Conversely, collagen cross-linkers, proanthocyanidin (PA), can perform the collagen cross-linking and protease inhibitory functions simultaneously. Due to its protein cross-linking effect, PA inhibits MMPs and cysteine cathepsins, as well as enhancing the mechanical properties of demineralized collagen [11–13]. Since PA is a plant flavonoid with a natural origin, it has lesser toxicity, which makes it more suitable for use in clinical practice.

To simplify the use of PA in clinical situations, PA has recently been incorporated directly into dental adhesives. This allows the release of PA from the cured resin, which remains in the hybrid layer for an extended period of time. In our previous study, it has been shown that incorporation of up to 2% PA into dental adhesive resin did not have a significant effect on resin–dentin bonding. However, the addition of 3% PA reduced the immediate resin–dentin bond strength significantly [14]. Further study is necessary to understand the effect of PA incorporation into dental adhesive on long-term resin–dentin bond integrity.

Water sorption by hydrophilic resin is another major cause of bonded degradation over time [15]. Being a hydrophilic material, incorporation of PA into an adhesive resin may increase the hydrophylicity of the resin. It has been reported that as long as the peripheral resin–enamel bond remains intact, it can protect the deeper resin–dentin bond from hydrolytic degradation for etch-and-rinse adhesives [16]. Hence, the objectives of this study were to evaluate the effect of incorporating different concentrations of PA into experimental dental adhesive on resin–dentin bond durability and the effect of surrounding bonded enamel on resin–dentin bonded interface after 6 months of water storage. The null hypotheses tested were: (i) the incorporation of different concentrations of PA into an experimental dental adhesive has no effect on bond strength and nanoleakage of the bonded interface after six months of storage in artificial saliva and (ii) the different water storage methods have no effect on the bond strength or nanoleakage of the bonded interface formed by tested adhesives.

2. Materials and methods

2.1. Tooth preparation

One hundred and eight caries-free human third molars that had been stored in a 0.5% chloramine T solution at 4 °C were used within one month after extraction. The teeth were collected after the patients' informed consent was obtained under a protocol reviewed and approved by the Institutional Review Board, the University of Hong Kong [UW 11-242]. The occlusal enamel was removed using slow-speed diamond impregnated disk (Isomet, Buhler Ltd., Lake Bluff, IL, USA) under water lubrication. The exposed dentin was polished wet with 180-grit silicon carbide paper for 15 s to create standardized smear layer on the mid-coronal dentin.

2.2. Preparation of experimental adhesives

A comonomer resin blend consisting of 40 wt% bisphenol A diglycidyl ether dimethacrylate (Bis-GMA), 30 wt% Bis[2-(methacryloyloxy)ethyl] phosphate (Bis-MP), 28.80 wt% 2-hydroxylethyl methacrylate (HEMA), 0.26 wt% camphorquinone (CQ) and 1 wt% ethyl N, N-dimenthyl-4-aminobenzoate (EDMAB) was used to formulate the four hydrophilic experimental adhesives. The experimental adhesive was first formulated by combining 50 wt% comonomer mixtures with 50 wt% ethanol. Proanthocyanidin (Grape Seed Extracts Oligomeric $>95\%$, International Laboratory of USA, USA) was then added directly to the ethanol-solvated experimental adhesives to yield four groups of adhesives with 0, 1, 2 and 3 wt% of PA in the adhesive formulations.

2.3. Bonding procedures

All dentin surfaces were acid-etched with 32% phosphoric acid gel (Uni-etch, Bisco Inc.) for 15 s and rinsed with water for 15 s before bonding. The etched dentin surfaces were blot-dried according to the wet bonding technique. The acid-etched teeth were then randomly assigned to the following 4 groups of adhesives ($n=18$ per group) for bonding:

Group 1 – PA-free adhesive
Group 2 – 1% PA- incorporated adhesive
Group 3 – 2% PA- incorporated adhesive
Group 4 – 3% PA- incorporated adhesive

The PA-free adhesive served as the control. The adhesives were applied to dentin surface for 15 s using a microbrush. This was followed by a second application of fresh adhesive, giving a total application time of 30 s. Excess solvent was removed with a gentle air stream for 10 s and followed by light curing the adhesive for 40 s using a light-curing unit (Optilux, Demtron-Kerr, Orange CA, USA) with an output at 600 mW/cm^2. Resin composite build-ups were performed with light-cured resin composite (Filtek Z250, 3M ESPE, St. Paul, MN, USA) in three increments of 1 mm each that were individually light-cured for 40 s with the same light intensity.

The bonded teeth were randomly divided into three subgroups ($n=6$ per group) for different methods of storage: Group 1: 24-h indirect exposure (IE), Group 2: 6-m IE and Group 3: 6-m direct exposure (DE) to artificial saliva. The bonded teeth from Group 1 were tested immediately after 24 h, while those from Group 2 were tested after direct exposure of the resin–dentin bonded interface to artificial saliva for 6 months and those from Group 3, the enamel surrounding the resin–dentin bonded interface was kept intact and the bonded teeth were sectioned into specimens for microtensile bond strength testing after exposure to artificial saliva for 6 months.

2.4. Microtensile bond strength testing

The bonded teeth were sectioned occluso-gingivally into 0.9 mm × 0.9 mm composite-dentin beams in accordance with the non-trimming technique of the microtensile bond test [17]. Five beams were retrieved from the two widest slabs of each tooth for microtensile bond strength testing. The mean microtensile bond strength of the beams originating from each tooth was used for statistical analysis. The cross-sectional area of each specimen was measured with a pair of digital calipers (Model CD-6BS; Mitutoyo, Tokyo, Japan). Each bonded beam was attached to the test apparatus with a cyanoacrylate adhesive (Zapit, Dental Ventures of North America, Corona, CA, USA) and stressed to failure under tension in a Bencor Multi-T device (Danville Engineering, San Ramon, CA) with the use of a universal testing machine Model 4440 (Instron, Inc., Canton, MA) at a crosshead speed of 1 mm/min.