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Effect of hesperidin incorporation into a self-etching primer on durability of dentin bond

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ABSTRACT

Objective. Collagen degradation at the resin–dentin interface deteriorates dentin bond durability. The use of natural cross-linkers might offer a positive approach to stabilize the resin–dentin interface. This study evaluated the effects of incorporation of natural cross-linkers into a self-etch adhesive primer on the immediate and long-term micro-tensile bond strengths (μTBS) to dentin.

Methods. Experimental primers were prepared by incorporating either 0.5%, 1%, 2%, 5% of hesperidin (HPN) or 0.5% of proanthocyanidins (PA) into Clearfil SE primer. Extracted human molar teeth were restored using the experimental primers or the pure SE primer (control). The mechanical properties of the bonded interfaces were measured using the nano-indentation tests. Beam-shaped bonded specimens were sub-divided for one-day and one-year μTBS test. Interfacial collagen morphology was observed using transmission electron microscopy.

Result. The immediate μTBS significantly increased in 0.5%, 1% and 2% HPN-incorporated groups when compared with the control. The mechanical properties of bonded interface were improved with 1% and 2% HPN-incorporated primers. For the long-term μTBS, the 2% and 5% HPN-incorporated group were significantly higher than the control. The morphology of the collagen fibrils were preserved by 5% HPN-incorporation after one-year storage. The PA group, however, failed to improve the μTBS and the mechanical properties of the bonded interfaces.

Significance. The incorporation of 2% HPN into the self-etching primer had a positive effect on the immediate μTBS and mechanical properties of the resin–dentin interfaces. The 5% HPN group preserved the morphology of the collagen in the hybrid layer after one-year storage in artificial saliva.

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1. Introduction

Adhesive restorations are widely distributed as the routine procedures in operative and restorative treatments. The use of self-etch adhesives became popular because it is less technique-sensitive, less aggressive to dentin, when compared with phosphoric-acid etching and shows less post-operative sensitivity [1]. However, deterioration of dentin bond occurs due to degradation in resin–dentin interface [2–5]. Unlike etch-and-rinse technique, self-etch technique does not completely expose the collagen matrix [6]; however, the stability of collagen fibrils within the hybrid layer is crucial for the maintenance of bond effectiveness over time [7,8]. Past studies have focused on two major factors that can degrade resin–dentin interface. The first factor is the hydrophilic characteristic of the monomer that can be hydrolyzed in aqueous solution [9–12]. The second factor is the uncured monomer that may remain at the bottom of the hybrid layer [5]. These two phenomena consequently expose the collagen matrix that can be degraded by proteolytic enzyme [13]. It has been reported that dentin collagenolytic and gelatinolytic activities can be suppressed by protease inhibitors [14]. Therefore, matrix metalloproteinase (MMPs) inhibitors such as chlorhexidine have been reported to be beneficial to preserve the hybrid layer and improve bond strength over time [15]. Recently, many researchers have reported that the use of collagen cross-linker to acid-etched dentin can prevent collagen degradation within the hybrid layer and maintain good dentin bond strength [8]. To simplify the use of cross-linker in clinical situations, cross-linkers should be incorporated directly into the dental primer or adhesives [16]. Since collagen matrices are partially denuded by acidity of the self-etching primer, the incorporation of cross-linker into the primer allows the cross-linking agents to interact with these denuded collagen substrate immediately upon removal of the mineral phase by the primer.

Hesperidin (HPN), hesperetin-7-O-rutinoside, is a flavonoid extracted from citrus fruits. The pharmacological properties and medicinal uses of HPN are associated with its wide range of benefits such as anti-inflammatory [17,18], analgesic [19], anti-microbial [20], and anti-oxidant effects [21]. HPN is also capable of carcinogenesis inhibition [22], bone loss prevention [23] and inhibition of MMPs' proteolytic activities [24]. We first attempted to apply HPN in root caries model in a pH cycle study, where HPN showed the potential to prevent collagen degradation against proteolytic enzyme [25]. Considering its cross-linking effect on dentin bonding, we have incorporated HPN into a primer of self-etch adhesive system, which effectively increased the immediate resin–dentin bond strength [26].

In the present study, we aimed to evaluate the effect of incorporation of different concentrations of HPN into the primer of a self-etch adhesive on micro-mechanical properties of resin–dentin interfaces, immediate and long-term resin–dentin bond strength.

The null hypotheses tested were that the incorporation of HPN has no effects (i) on the mechanical properties of resin–dentin interfaces and (ii) on the immediate and long-term resin–dentin bond strength.

2. Materials and methods

2.1. Micro tensile bond strength testing

The teeth used in this study were collected after obtaining the patients' informed consent. The Human Research Ethics Committee of Tokyo Medical and Dental University, Japan reviewed and approved this study under the protocol number 725. Forty-eight freshly extracted non-carious human molar teeth were used for bond strength testing. A flat dentin surface was created perpendicular to the tooth's longitudinal axis using a slow-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under water cooling to remove occlusal dentin. Smear layer was produced on each surface using #600 Silicon Carbide paper under water irrigation. Eight teeth per group were allocated for the following self-etching primers. HPN (hesperetin-7-O-rutinoside, Wako Pure Chemical Industries, Ltd., Tokyo, Japan) or grape seed derived proanthocyanidins (PA) (proanthocyanidins, Kikkoman Biochemifa, Chiba, Japan) was added to Clearfil SE primer (Kuraray Noritake Dental Inc. Tokyo, Japan) to formulate the experimental primer groups (0.5%, 1%, 2%, 5% HPN and 0.5% PA (Table 1). The pH value of each experimental primer was measured with a digital pH meter (Twin pH B-211, HORIBA, Ltd., Kyoto, Japan). The original SE primer served as control. The dentin surfaces were conditioned with the primers according to the manufacturer's instructions, then Clearfil SE bond (Kuraray Noritake Dental Inc. Tokyo, Japan) was applied and light-cured for 10 s (OPTILUX 501, Kerr corporation, CA, USA. light intensity 650 mW/cm^2). Composite resin (Clearfil AP-X, Kuraray Noritake Dental Inc. Tokyo, Japan) was placed on dentin surfaces incrementally up to 5 mm of thickness. Each increment was light-cured for 30 s. After storage in de-ionized water at 37 °C for 24 h, the bonded teeth were sectioned longitudinally into serial slabs, and further sectioned to obtain (0.9 mm × 0.9 mm) composite-dentin beams. The beams were divided into two test groups. Half of the specimens from each group were used for immediate micro-tensile bond strength (μTBS) testing and the remaining half were stored in artificial saliva for one year at 37 °C. The artificial saliva contained (mM): $CaCl_2$ (0.7), $MgCl_2{\cdot}6H_2O$ (0.2), KH_2PO_4 (4.0), KCl (30), NaN_3 (0.3), and HEPES buffer (20).

Table 1 – Study design and composition of SE primer and SE bond.

Group	Material tested	pH
1	Pure clearfil SE primer	2.0
2	Clearfil SE primer +0.5% HPN	2.0
3	Clearfil SE primer +1% HPN	2.0
4	Clearfil SE primer +2% HPN	2.0
5	Clearfil SE primer +5% HPN	2.0
6	Clearfil SE primer +0.5% PA	2.0

Clearfil SE Primer: MDP, HEMA, di-methyl-acrylate, monomer, water, catalyst.
Clearfil SE Bond: MDP, HEMA, di-methyl-acrylate, monomer, micro filler, catalyst.