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Original article

Effect of phytic acid etchant on resin–dentin bonding: Monomer penetration and stability of dentin collagen

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ABSTRACT

Purpose: Phytic acid (IP6) works well as an etchant in dentin bonding to remove the smear layer due to its acidity and chelating effect. This study compared the etching effect of IP6 with phosphoric acid (PA) and ethylenediaminetetraacetic acid (EDTA) on resin–dentin bond strength, micromorphology of the etched dentin surface and nanoleakage formation along resin–dentin interfaces and compared the protecting effect against collagen degradation.
Methods: Dentin disks and flat dentin surfaces were obtained from extracted human teeth. Specimens were etched with 35% PA (15 s), 0.5 M EDTA (30 s) or 1% IP6 (30 s). The surfaces and longitudinal sections of the etched dentin disks were observed using field emission scanning electron microscope (FE-SEM). An etch-and-rinse adhesive was used to create composite build up-specimens for microtensile bond strength (μTBS) testing and nanoleakage observation. To evaluate the effect on collagen degradation, demineralized bovine root dentin blocks were challenged with bacterial collagenase and then observed under light microscope.
Results: PA- and EDTA- treated groups showed significantly lower μTBS when compared to IP6-treated group. PA showed distinct nanoleakage and severe collagen degradation. Only slight nanoleakage was detected in IP6 group. IP6 showed better effect than EDTA in preventing collagen degradation induced by bacterial collagenase.
Conclusions: IP6 effectively removed the smear layer and etched dentin, providing high bond strength values and causing minimal nanoleakage and slight collagen degradation.
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1. Introduction

In 1955, Buonocore firstly introduced phosphoric acid (PA) for enamel etching [1]. While, acid etching remains to be the most effective technique for enamel bonding, etching of dentin is a more controversial issue. Dentin is composed of approximately 50% mineral phase, 30% organic component and 20% water [2]. Following the application of PA and water rinsing, mineral phase of dentin is removed and replaced by water. Ideally, this increased amount of water should be completely replaced by resin comonomers [2]. Otherwise, the etched dentin would be poorly impregnated, thus creating a weak zone of unfiltrated collagen at the bottom and within the hybrid layer which is prone to mechanical disruption when being stressed to failure [3]. Furthermore, these denuded collagen fibrils are susceptible to degradation by matrix metalloproteinase (MMPs) compromising the longevity of resin–dentin bonds [4].

The technique sensitivity of etch-and-rinse adhesive system resides in the difficulty of controlling the degree of moisture of etched dentin which is necessary to prevent collagen collapse that would hamper the infiltration of resin monomers [5]. In addition, increased depth of dentin demineralization which often happens with the use of PA could also result in imperfect resin impregnation [6,7]. In attempts to overcome these problems, previous studies have evaluated the use of PA at lower concentrations than that recommended by the manufacturers [8], as well as other etchants, such as maleic acid [8,9], citric acid [9] and ethylenediaminetetraacetic acid (EDTA) [10,11].

Because of its calcium (Ca) chelating effect [12], EDTA has the ability to remove the smear layer and mildly demineralize dentin, thus it is effective for etching dentin surface prior to adhesive application [10,11]. Etching dentin with EDTA was shown to improve the resistance of the resin–dentin interface to degradation by sodium hypochlorite [10,13]. The mechanical properties of dentin demineralized with EDTA was not affected after long-term storage [14]. EDTA was reported to exhibit MMP inhibition effect [15,16]. However, this effect was found to be time-limited [16].

Phtytic acid, inositol hexakisphosphate (IP6), has a strong metal chelating function. This organic acid is found in our daily diet including cereals, legumes, oil seeds and nuts [17]. It is also present in human body and animal in the form of extra or intracellular IP6 [18]. IP6 has been reported to have a role in cancer prevention or therapy and to inhibit calcium salt crystallization preventing the formation of renal stone [17]. It also has positive effect on blood glucose and cholesterol [17]. These pharmacological effects of IP6 are based on its ability to chelate multivalent cation such as Ca forming Ca-phytate salt that has very low solubility [19,20] and its ability to bind to some kinds of proteins [20–23]. Recently, IP6 was reported to be an effective root canal chelating agent [24] and dentin etchant that can provide good resin–dentin bonding [25,26]. It showed better biocompatibility to pulpal- and osteoblast-like cells when compared to PA or EDTA, respectively [24,25] and stabilized the morphology of collagen network possibly via cross-linking interaction [26].

Therefore, the aims of this study were to evaluate the effect of IP6 used as etchant on smear layer removal, resin–dentin bond strength, nanoleakage formation at the interface and on the degradation of dentin collagen comparing it to PA and EDTA. The tested null hypothesis was that there was no difference between the tested groups.

2. Materials and methods

2.1. Etched surface and longitudinal section observation

Caries free extracted human molars were collected with ethical approval following the guidance of the Ethical Committee at Tokyo Medical and Dental University under protocol number 725. The mid coronal dentin of 12 extracted non-carious human premolars were cut by a slow-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) to obtain 12 dentin disks which were approximately 1 mm thick. A diamond bur in a high-speed handpiece was used to drill a 0.5 mm deep groove in the middle of the pulpal surface of these dentin disks to facilitate separation of each disk into two halves, while the occlusal surface was polished with #600 silicon carbide (SiC) paper under water irrigation to standardize the smear layer. Then, the dentin disks were randomly assigned into four groups (n=3). The specimens of the control group received no treatment while those of experimental groups (PA, EDTA and IP6 groups) were etched for 15 s with 35% PA (Scotchbond™, 3M ESPE, St. Paul, USA, pH 0.22), 30 s with 0.5 M EDTA (Decalcifying Soln. B, Wako Pure Chemical Industries, Ltd., Osaka, Japan, pH 7.5) and 30 s with 1% IP6 (Phytic Acid Solution, Wako Pure Chemical Industries, pH 1.2), respectively. After etching, the dentin surfaces were rinsed with distilled water for 10 s. The specimens were fixed overnight at 4 °C with Karnovsky solution, pH 7.4. The specimens were thoroughly rinsed and dehydrated in ascending concentrations of alcohol (25%, 50%, 75% for 20 min each; then, 95% for 30 min and finally 100% for 60 min) and hexamethyldisilazane. Prior to sputter-coating with platinum/palladium, each specimen was fractured by hand along the pre-notched groove using bending force. The etched surfaces and the fractured surfaces were observed using a field emission scanning electron microscope (FE-SEM, S-4500, Hitachi Ltd., Japan).

2.2. Microtensile bond strength (μTBS) testing and failure mode analysis

Twelve extracted non-carious human molars were used for bond strength testing. A flat dentin surface was created perpendicular to the tooth's longitudinal axis using the slow-speed diamond saw. Smear layer was produced on that flat surface using #600 SiC paper. The specimens were allocated into three groups (n=4). Etching and rinsing were performed as described for the sample preparation for etched surface and longitudinal section observation. After rinsing, the specimens were blot dried. Then, a two-step etch-and-rinse adhesive (Adper™ Single Bond Plus, 3M ESPE, St. Paul, USA) was applied following the manufacturer's instruction and light-cured for 10 s (OPTILUX 501, Kerr Corp., CA, USA, light intensity 650 mW/cm^2). Composite resin (Z100™ Restorative, 3M ESPE, St. Paul, USA) was placed on the bonded surfaces incrementally up to a

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