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# The effect of proanthocyanidin-containing 10% phosphoric acid on bonding properties and MMP inhibition

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ABSTRACT

*Objectives.* This study evaluated the effect of etching using 2% proanthocynidin-containing 10% phosphoric acid 2% PA/10% PhA vs. 35% phosphoric acid 35% PhA on immediate (IM) and 6-months (6 M) resin–enamel microshear bond strength (μSBS), resin–dentin microtensile bond strength (μTBS), nanoleakage (NL) and as well as *in situ* MMP inhibition potential.

*Methods.* The dentin surface of human were exposed and then etched using 35% phosphoric acid for 15 s or 2% PA/10% phosphoric acid for 30 s. After rinsing with water, the dentin was bonded with Single Bond Plus (3 M ESPE) and composite build-ups were constructed, followed by polymerization. The teeth were sectioned and the bonds were testing for microtensile bond strength (μTBS) and by SEM for NL analysis at IM and 6 M. For MMP activity, resin–dentin slices were prepared for *in situ* zymography, and analyzed under confocal microscopy. For μSBS, others teeth had flattened enamel surfaces etched according the experimental groups and prepared to microshear procedure. The specimens were tested IM and after 6 M by microshear bond strength. The data were submitted to two-way repeated measures ANOVA and Tukey's test ($\alpha = 0.05$).

*Results.* Acid-etching using the 2% PA/10% phosphoric acid did not lower the μTBS in IM ($p > 0.05$) compared to the control 35% phosphoric acid group. However, after 6 M, only the 2% PA/10% PhA etched dentin had remained stable the resin–dentin bond strength ($p < 0.05$). Bonds made with 35% PhA showed significant increase in NL% after 6 M ($p < 0.05$). Dentin bonds made with 2% PA/10% phosphoric acid showed no increase in NL% after 6 months. The MMP activity within the resin–dentin interface was almost completely reduced after 2% PA/10% PhA etching, while the 35% PhA exhibited intense MMP activity. For μSBS, the type of etchant and the storage period did not affect the resin–enamel bond strengths ($p > 0.05$).

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*Significance.* Ten percent phosphoric acid containing 2% PA can produce stable resin–dentin and enamel–resin interfaces, without requiring additional steps in the bonding procedure. Future studies for longer evaluation time are required.



## 1. Introduction

There is a general consensus that the resin–dentin bonds created with contemporary hydrophilic dentin bonding systems deteriorate over time [1,2]. For etch-and-rinse adhesives, there is a decreasing gradient of resin monomer diffusion within the hybrid layers [3,4]. This results in incomplete resin infiltration at the bottom the hybrid layer, leaving denuded collagen fibrils [3,5,6] that are susceptible to enzymatic degradation by host-derived collagen-bound matrix metalloproteinases (MMPs) and cysteine cathepsins [6–9]. Additionally, simplified etch-and-rinse systems are capable of activating these matrix metalloproteinases (MMPs) [6,10]. Consequently, procedures that enhance dentin collagen's resistance towards collagenolytic activity of host-derived enzymes have great potential to improving the longevity of dentin bonding.

To increase the collagen stability, one might employ MMP inhibitors and collagen cross-linking agents. Exogenous MMP inhibitors, such as chlorhexidine, are capable to reduce the protease activity and to prolong the durability of resin–dentin bonds [11–13] but they lack chemical bond with the collagen fibrils. Collagen cross-linkers establish chemical bonds with the collagen and may also increase the collagen resistance against the effect of host-derived proteases [14–16].

The delivery of these agents to demineralized dentin can be via application of primers where a therapeutic agent can be incorporated into one of the components of the bonding protocol [17]. The application of the agents as a primer is hampered by the fact that this procedure adds another step to the bonding protocol, which is against the clinician's preference for simplification. This fact has motivated some authors to combine MMPS inhibitors or cross-linking agents in the etchants [13,18,19].

Among the cross-linking agents, proanthocyanidin (PA)-rich grape seed extract (GSE) is a promising agent due to its effectiveness under shorter treatment times [20] and its absence of cytotoxicity [21]. Additionally, PA can stabilize the resin–dentin bond strength of the adhesive interface and decrease the dentin-bound MMPs activity [22].

In previous work, the incorporation of PA into a 10% phosphoric acid showed promising results, rendering the demineralized dentin collagen inert to bacterial collagenase digestion [18]. However, the authors did not evaluate the PA-etchant under clinically relevant bonding procedures, which prevent us from knowing whether or not the low concentrated PA-containing phosphoric acid is capable to promote an effective etching in enamel and dentin substrates, and if such treatment would produce stable resin–dentin bonds after water storage.

Therefore, the aim of this study was to evaluate the immediate and 6-month effectiveness of this modified phosphoric acid etchant in dentin and enamel through resin–dentin microtensile bond strength, resin–enamel microshear bond strength and nanoleakage studies. Additionally, the *in situ* MMP inhibition potential was also evaluated through *in situ* zymography [23].

## 2. Material and methods

### 2.1. Specimen preparation

A total of 26 extracted, caries-free, human third molars were used. The teeth were collected after obtaining the patients' informed consent under a protocol approved by the Ethics Committee Review Board from the State University of Ponta Grossa (Parana, Brazil). The teeth were disinfected in 0.5% chloramine, stored in distilled water, and used within 6 months after extraction.

In 16 teeth, a flat occlusal dentin surface was exposed after wet grinding the occlusal enamel with #180-grit silicon-carbide (SiC) paper for 60 s. The exposed dentin surfaces were further polished with wet #600-grit SiC paper for 60 s to standardize the smear layer. Ten teeth were used for evaluation of the resin–dentin microtensile bond strength (μTBS) and nanoleakage, while the remaining six was used for the evaluation of the MMP-activity.

In 10 teeth, the roots of all teeth were removed by sectioning at the enamel-cementum junction. The dental crowns were then sectioned parallel to the long axis of the teeth to produce four enamel specimens (buccal, lingual, and proximals). Forty enamel specimens were ground wet with # 180 and 600-grit SiC paper for 60 s each. The unsanded surfaces were potted in acrylic resin to stabilize the specimens that were used for the evaluation of resin–enamel microshear bond strength (μSBS).

### 2.2. Experimental groups and restorative procedure

The tooth specimens were randomly distributed into the control and experimental groups by a person not involved in the research protocol using computer-generated tables. In the control group, the dentin and enamel surfaces of all specimens were conditioned with a 35% phosphoric acid gel for 15 s (Scotchbond etchant, 3 M ESPE, St. Paul, USA, batch number N261433). In the experimental group, an experimental etchant made of 2% PA, containing 10% phosphoric acid was prepared by mixing GSE powder, ethanol, distilled water, and 85% phosphoric acid to final concentrations (weight percentage with respect to total mass) of 2% PA-rich GSE, 20% ethanol and 10% phosphoric acid [18]. The chemicals were purchased