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Effect of nanolayering of calcium salts of phosphoric acid ester monomers on the durability of resin-dentin bonds

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ABSTRACT

To investigate the contribution of nanolayering on resin-dentin bond durability, two phosphoric acid ester resin monomers, 10-methacryloyloxy-decyl-dihydrogen-phosphate (10-MDP) or its analog, metha cryloyloxy-penta-propyleneglycol-dihydrogen-phosphate (MDA), were examined for their affinity for mineralized dentin powder in a column chromatography setup. Hydroxyapatite (HA) powder was dispersed in experimental primers consisting of 10-MDP or MDA solvated in ethanol/water and examined with FTIR, ^{31}P MAS-NMR and XPS. Light-curable 10-MDP or MDA primers were used for bonding to dentin, and examined after 24 h or one-year of water-aging by TEM for evidence of nanolayering, and for microtensile bond strength evaluation. Primer-bonded dentin was examined by thin-film XRD to identify short-range order peaks characteristic of nanolayering of resin monomer-Ca salts. Although 10-MDP had better affinity for mineralized dentin than MDA, both monomers completely eluted from the mineralized dentin powder column using ethanol-water as mobile phase, indicating that the adsorption processes were reversible. This finding was supported by chemoanalytic data. XRD of 10-MDP-bonded dentin showed three diffraction peaks hat were absent from MDA-bonded dentin. Nanolayering was identified by TEM in 10-MDP-bonded dentin, but not in MDA-bonded dentin. Significant drop in bond strength (in MPa) was observed for both groups after one-year of water-aging compared with 24-h: 10-MDP group from 48.3 ± 6.3 to 37.4 ± 4.6; MDA group from 50.7 ± 5.0 to 35.7 ± 3.8 ($P < 0.05$), with no significant difference between the two groups at the same time-point. Because both functional monomer-primed, resin-bonded dentin exhibited similar bond strength decline after water-aging, presence of nanolayering is unlikely to contribute to the overall resin-dentin bond durability.

Statement of Significance

The durability of resin-dentin bonds in 10-MDP containing self-etching adhesives has been anecdotally attributed to the presence of nanolayering of 10-MDP-calcium salts in the resin-dentin interface. Results of the present work indicate that such a claim cannot be justified. Complete elution of the phosphoric acid ester monomer from mineralized dentin powder in the column chromatography experiments using ethanol-water mobile phase to simulate the solvent mixture employed in most 10-MDP-containing dentin adhesives further challenges the previously proposed adhesion-decalcification concept that utilizes chemical bonding of phosphoric acid ester monomers to apatite as a bonding mechanism in 10-MDP containing dentin adhesives.

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1. Introduction

Dentin bonding interfaces degrade with time. Electron microscopy and other *in vitro* tests have provided ultrastructural evidence of degradation in hybrid layers, and decline in resin-dentin bond strengths that resulted from resin hydrolysis and collagen degradation [1,2]. Loss of micromechanical retention between adhesive and dentin eventually leads to clinical restoration failure [3]. Apart from micromechanical interlocking, chemical adhesion between specific functional resin monomers and tooth minerals has been reported as an alternative mechanism for adhesion of methacrylate resins to tooth structures. Chemoanalytic methods have identified prospective chemical reactions that occur in the resin-dentin interface, including adsorption of functional resin molecules on the apatite surface and the formation of resin monomer-calcium salts [4–6].

Due to challenges in quantifying chemical reactions in the resin-dentin interface, a direct link between chemical bonding and resin-dentin bond durability was difficult to be established [7,8]. The water solubility of 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP)-calcium salt was the lowest among salts produced by the reaction between phosphoric acid ester monomers and apatite [5,9,10]. This feature was used to account for the better *in vivo* and *in vitro* dentin bonding results achieved by 10-MDP-containing commercial adhesives [11,12]. In the absence of direct evidence, those long-term *in vivo* and *in vitro* bonding results were used anecdotally as indirect evidence for the contribution of chemical bonding to the overall bonding performance [13]. Because many confounding factors are involved in studies on dentin adhesives, it is taxing to attribute overall bonding performance to the presence of phosphoric acid ester monomer-calcium salts in the resin-dentin interface [14–16].

In 10-MDP primer-treated resin-tooth interfaces, 10-MDP-Ca salts self-assemble into nanolayers, with hypothetical structures consisting of the methacrylate groups of two 10-MDP molecules facing each other, and the functional hydrogen phosphate groups directed away from each other [17]. Ultrastructural manifestation of 10-MDP-Ca nanolayering was corroborated with the appearance of three characteristic peaks in the 2θ range of 2–8° in thin-film X-ray diffraction (XRD) scans of adhesive-coated dentin [18]. These three peaks represent short-range order of the precipitated salts [19]. Based on this finding, several claims have been made for the function of nanolayering in dentin bonding, including protecting collagen fibrils from water-induced degradation due to their hydrophobicity, increasing the resistance of residual apatite crystallites to acidic dissolution, and creating a more gradual transition between the inorganic bonding substrate and the biomaterial [17]. These claims, however, were not supported by experimental evidence.

According to the literature, the propensity of nanolayering formation in resin-dentin adhesives containing phosphoric acid ester monomers is affected by the presence of 2-hydroxyethyl methacrylate (HEMA), agitation, application time, monomer impurity and molecule structure of the phosphoric acid ester monomers [20–22]. These factors, alone or in combination, could have accounted for the paucity of nanolayering in resin-dentin interfaces created by commercial 10-MDP-containing adhesives [19]. Other functional resin monomers with similar structure as 10-MDP have also been investigated [10,23,24]. These monomers contain different hydrocarbon or fluorocarbon chains as spacer group between the methacrylate group and the phosphate group. The spacer group is known to influence monomer characteristics such as flexibility, solubility, hydrophobicity, viscosity, and wetting behavior. Long spacers are used to avoid steric hindrance during polymerization and to enhance mechanical properties [25]. These functional resin monomers were tested for their calcium salt solubility, chemical shifts after reacting with hydroxyapatite or powdered enamel and dentin with nuclear magnetic resonance (NMR) spectroscopy, bond strength and ultrastructural examination of the resin-dentin interface. Many of them produce nanolayering patterns with varying interlayer thickness. However, none of these studies was able to elucidate whether the presence of nanolayering was responsible for the durability of resin-dentin bonds.

To circumvent the problem of interference of other methacrylate resin monomers on nanolayering formation, 10-MDP and a new 10-MDP analog with a different spacer group were respectively used as the only resin monomer for creating experimental self-etching dentin adhesives. The analog member had been tested in a pilot study to confirm the absence of nanolayering when bonded to dentin, and served as the control analog for 10-MDP. This enabled the authors to evaluate the contribution of nanolayering to the longevity of resin-dentin bonds. The interactions between the two resin monomers and the dentin mineral phase were first investigated, followed by their influences on dentin bonding performance. The first null hypothesis tested was that there is no difference in the affinity of 10-MDP and the analog resin monomer for mineralized dentin. The second null hypothesis tested was that there is no difference in the capability of both phosphoric acid ester monomers to produce nanolayering on the dentin surface. The third null hypothesis tested was that nanolayering of phosphoric acid ester monomer-calcium salts is unstable after water aging and does not contribute to the resin-dentin bond durability.

2. Materials and methods

2.1. Materials

Sixty non-carious human third molars were used in the present study. The use of human teeth for research was approved by the Human Assurance Committee of the Augusta University, Georgia. The teeth were refrigerated at 4 °C in 0.9% NaCl that contained 0.02% sodium azide to prevent bacterial growth.

The two phosphoric acid ester monomers examined were: 10-MDP (molar mass: 322.35 g/mol) and methacryloyloxy-penta-propyleneglycol-dihydrogen-phosphate (10-MDP analog, designated as "MDA"; molar mass: 456.48 g/mol). The molecule structures of the two resin monomers are illustrated in Fig. 1. Both resin monomers were obtained from DM Healthcare Products, Inc. (San Diego, CA, USA). 2-hydroxyethyl methacrylate (HEMA; molar mass: 130.14 g/mol) and hydroxyapatite (HA; particle size ~200 nm) were obtained from MilliporeSigma (St Louis, MO, USA).

Two experimental primers were prepared by blending 10-MDP or MDA with ethanol and water in the ratio of 15:45:40 wt% [20]. For primers used in dentin bonding as part of the experimental adhesive systems, camphorquinone (CQ; 1 wt%) and ethyl-4-dimethylamino benzoate (EDMAB; 0.4 wt%), both from MilliporeSigma, were added to render the primers light-curable. The pH values of the experimental primer solutions (measured with a pH meter, Orion Star A211, ThermoScientific, Waltham, MA, USA) were 2.65 for the primer containing 10-MDP, and 2.38 for the primer containing 10-MDP analog.

2.2. Affinity of the two phosphoric acid ester monomers for mineralized dentin

A modified hydroxyapatite column chromatography procedure [26] was adopted in the present study to examine the elution characteristics of the two phosphoric acid ester monomers in the presence of mineralized dentin powder, as a measure of the affinity of