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Zn-doped etch-and-rinse model dentin adhesives: Dentin bond integrity, biocompatibility, and properties

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ABSTRACT

Objective. This study assessed a 6 month resin/dentin bond's durability and cytotoxic effect of Zn-doped model dentin adhesives. The mechanical and physicochemical properties were also tested.

Methods. A model etch-and-rinse single-bottle adhesive was formulated (55 wt.% Bis-GMA, 45 wt.% HEMA, 0.5 wt.% CQ, 0.5 wt.% DMAEMA) and Zinc methacrylate (Zn-Mt) or ZnO nanoparticles (ZnOn) were added to the model's adhesive, resulting in three groups: Group Control (control model adhesive); Group Zn-Mt (1 wt.% Zn-Mt incorporated to adhesive) and Group ZnOn (1 wt.% ZnOn incorporated to adhesive). The microtensile bond strength (mTBS) was assessed after 24 h or 6 months in water storage. Mechanical properties (diametral tensile strength/DTS, flexural strength/FS, flexural modulus/FM, resilience modulus/RM, and compressive strength/CS) and physicochemical properties (polymerization shrinkage/PS, contact angle/CA, water sorption/WS, and water solubility/WS) were also tested. Cytotoxicity was evaluated with SRB biochemical assay.

Results. No significant difference in the DTS, FS, FM, CS, CA, WS, and WS were found when 1% of ZnOn or Zn-Mt was added to the model dentin adhesive. Group Zn-Mt decreased the RM of adhesive. Groups Zn-Mt and ZnOn decreased the PS of adhesives. Group ZnOn reduced the cytotoxicity of adhesive. Group ZnOn preserved mTBS after 6 months storage without degradation areas as seen by SEM analysis.

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Significance. The 1 wt.% ZnOn may preserve the integrity of the hybrid layer and may reduce cytotoxicity and polymerization shrinkage of model dentin adhesive. The addition of Zn-Mt to the adhesive had no beneficial effects.

1. Introduction

The lack of durable dental adhesives is considered one of the main problems with the use of composite materials in dentistry. Failures of composite restorations are observed mainly at the dentin/adhesive interface. The bonding to dentin is obtained by the formation of a hybrid layer; however, the longevity of the hybrid layer has still been widely questioned as a consequence of hydrolytic degradation of the adhesive interface over time [1–3].

Among the various factors that are related to the degradation of the hybrid layer, two factors are highlighted: the hydrolytic degradation over time of the polymer present in the hybrid or adhesive layers [4], and a poorly infiltrated hybrid layer with unprotect collagen fibers [1,4]. This unprotected collagen can hydrolyze by host-derived matrix metalloproteinase (MMP) enzymes [5–7]. Therefore, new strategies are being exhaustively studied with the purpose of increasing the durability of the dentin/bond interface, one of which is the inhibition of MMP's proteolytic activity within the unprotected collagen fibers [5–8].

MMPs require Zn^{2+} ions to maintain their proper tertiary structure and functional active sites [9]. On the other hand, Zn can be used to protect collagen from MMPs activity [10–12]. Previous studies [5–8] observed the durability of the resin/dentin interface when Zn was added to commercial dental adhesives. Zn can promote subtle conformational in collagenase cleavage sites in collagen molecules that protect collagen from MMP's activity [5,7]. The incorporation of Zn in commercial adhesives has also resulted in the formation of apatite crystallites on the collagen fibrils, favoring dentin mineralization [5], reduce MMPs-mediated collagen degradation [5,12], may inhibit dentin demineralization [13], and may promote enamel remineralization [14].

However, any change in the chemical composition of adhesive systems involves potential mechanical and physicochemical failures and biohazards. Therefore, the insertion of Zn in adhesive systems as an alternative to preserve the longevity of the restoration, although having promising results, requires further reviews, due to the possibility of changing adhesive systems' biological, physico-chemical and mechanical properties, and, consequently, their clinical performance.

The aim of this study was to evaluate a 6 month resin/dentin bond's durability of Zn-doped etch-and-rinse model dentin adhesives. Mechanical properties (diametral tensile strength, flexural strength, flexural modulus, resilience modulus, and compressive strength) and physicochemical properties (polymerization shrinkage, contact angle, water sorption, and water solubility) were also tested. *In vitro* cytotoxicity of these adhesives was evaluated on human dental pulp fibroblasts.

This study tested three null hypotheses: (1) the model dentin adhesives tested can achieve similar bond durability to dentin; (2) the storage period does not affect the bonding effectiveness of model dentin adhesives; and (3) the model dentin adhesives tested can achieve similar results for mechanical and physicochemical properties and cytotoxicity.

2. Material and methods

2.1. Model dentin adhesives preparation

A model simplified etch-and-rinse adhesive was formulated through intensive mixing of bisphenol-A diglycidyl ether dimethacrylate (Bis-GMA) and 2-hydroxyethyl methacrylate (HEMA), with a mass ratio of 45:55 (HEMA:Bis-GMA). The photoinitiators used were 0.5 mol% of camphorquinone as a hydrophobic photosensitizer and 0.5 mol% of 2-(dimethylamino) ethyl methacrylate (DMAEMA) as a hydrophilic co-initiator. Model dentin adhesives were prepared in a brown glass vial in the absence of visible light. All materials were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA) [15].

Zinc methacrylate (Sigma Aldrich) and ZnO nanoparticles (Sigma Aldrich) were added to the control model dentin adhesive at 1, 2, 5, and 10 wt.% concentrations. The Zn-doped model dentin adhesives were shaken using a tube agitator in the dark for 10 min at 2000 rpm.

2.2. Degree of conversion (pilot study)

In order to obtain a maximal concentration that did not alter the conversion degree of the Zn-doped model dentin adhesives, the degree of conversion of polymerized Zn-doped adhesives was compared to the control model dentin adhesive. The degree of conversion was determined using Raman spectroscopy. Spectra were collected using a Micro-Raman spectrometer (model 2000, Renishaw Engineering, Wotton-under-Edge, UK) with an Argon laser as an excitation source. To determine the degree of conversion, spectra of a droplet of uncured adhesives and polymerized adhesives were acquired over a spectral range of 700–1800 cm^{-1}. The change of the band height ratios of the aliphatic carbon–carbon double bond (peak at 1640 cm^{-1}) and the aromatic C=C (peak at 1610 cm^{-1}) (phenyl) in both the cured and uncured states was monitored. The degree of conversion was calculated using the following formula based on the decrease