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A zinc chloride-doped adhesive facilitates sealing at the dentin interface: A confocal laser microscopy study

Manuel Toledano*, Raquel Osorio, Estrella Osorio, Inmaculada Cabello, Manuel Toledano-Osorio, Fátima S. Aguilera

University of Granada, Faculty of Dentistry, Dental Materials Section Colegio Máximo de Cartuja s/n, 18071 Granada, Spain

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ABSTRACT

The aim of this study was to ascertain the effect of Zn-doping of dental adhesives and mechanical load cycling on the micromorphology of the resin-dentin interdiffusion zone (of sound and caries affected dentin). The investigation considered two different Zn-doped adhesive approaches and evaluated the interface using a doubled dye fluorescent technique and a calcium chelator fluorophore under a confocal laser scanning microscopy. Sound and carious dentin-resin interfaces of unloaded specimens were deficiently resin-hybridized, in general. These samples showed a rhodamine B-labeled hybrid layer and adhesive layer completely affected by fluorescein penetration (nanoleakage) through the porous resin-dentin interface. It was thicker after phosphoric acid-etching and more extended in carious dentin. Zn-doping promoted an improved sealing of the resin-dentin interface, a decrease of the hybrid layer porosity, and an increment of dentin mineralization. Load cycling augmented the sealing of the Zn-doped resin-dentin interfaces, as porosity and nanoleakage diminished, and even disappeared in caries-affected dentin substrata conditioned with EDTA. Sound and carious dentin specimens analyzed with the xylenol orange technique produced a clearly outlined fluorescence when resins were Zn-doped, due to a consistent Ca-mineral deposition within the bonding interface and inside the dentinal tubules. It was more evident when load cycling was applied on specimens treated with self-etching adhesives that were Zn-doped. Micropermeability at the resin-dentin interface diminished after combining EDTA pretreatment, $ZnCl_2$-doping and mechanical loading stimuli on restorations. It is clearly preferable to include the zinc compounds into the bonding constituents of the self-etching adhesives, instead of into the primer ingredients. The promoted new mineral segments contributed to reduce or avoid both porosity and nanoleakage from the load cycled Zn-doped resin dentin interfaces. EDTA + SB-$ZnCl_2$ or SEB·Bd-Zn doping are preferred to treat caries-affected dentin surfaces. ZnO-doping encouraged for etch-and-rinse adhesives.

1. Introduction

Dentin represents the most common dental substrate to be used in multiple adhesive techniques for restoration (De Munck et al., 2005). Sound dentin is composed of type I collagen fibrils with associated non-collagenous proteins. It forms a three-dimensional matrix that is reinforced by mineral (apatite) (Bertassoni et al., 2009). Dentists usually must bond adhesives to irregular dentin substrates such as carious dentin. Caries-affected dentin is partially demineralized with a predominantly intact collagen matrix. It should be preserved during clinical treatment because it is remineralizable and serves as a suitable substrate for dentin adhesion. Caries-affected dentin exhibits a higher degree of porosity which is commonly associated with a partial lack of minerals around and within the collagen fibrils.

To promote adhesion to sound or carious dentin, a part of the mineral phase from the substrate has to be removed. The voids left by the mineral removal should be filled with the adhesive resin that undergoes complete *in situ* polymerization to form the hybrid layer (Spencer et al., 2010). Two main strategies are used to create dentin bonding. The first approach involves the use of etch-and-rinse adhesives, which require the pre-treatment of dentin with phosphoric acid (PA) or EDTA. These acids remove the smear layer, demineralize the underlying dentin, and expose a dense filigree of organic-matrix fibrils, essentially represented by type I collagen (Breschi et al., 2010). This is followed by the application of a primer/bonding adhesive. This procedure causes a decreasing gradient of resin monomer diffusion within the acid-etched dentin which results in a phase of demineralized collagen matrix at the base of the hybrid layer (Pashley et al., 2011).

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* Corresponding author.
E-mail address: toledano@ugr.es (M. Toledano).

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The second strategy uses self-etching adhesives. They are based on the polymerizable acidic monomers that simultaneously condition/prime and bond to the dentin, to form the hybrid complex (Tay and Pashley, 2003). With the use of this technique, it is accepted that there is less discrepancy between the depth of demineralization and the depth of resin infiltration into the dentin (Pashley et al., 2011).

Dentin adhesives should not only be long-lasting. They should protect and remineralize the resin-dentin interfaces, triggering the bioactive nature of dentin matrix, by releasing bound bioactive molecules (Burwell et al., 2012). Zinc ions inhibit matrix metalloproteases (MMPs) and reduce collagen degradation in demineralized dentin. It is known that the activity of host-derived dentin proteases is responsible for the enzymatic degradation of improperly impregnated dentin collagen at the resin/dentin interface (Seseogullari-Dirihan et al., 2016). Therefore, a MMPs inhibitor as zinc may be of help to avoid hybrid layer degradation. Zinc may also stimulate dental remineralization (Osorio et al., 2014a). Zinc-doped adhesives can be obtained by using 20 wt% ZnO or 2 wt% $ZnCl_2$ without altering adhesive physic, chemical and mechanical properties (Toledano et al., 2013a; Pomacóndor-Hernández et al., 2015).

Restored teeth are constantly subjected to cyclic stresses during physiologic chewing and swallowing. Occlusal stress may cause mechanical degradation and accelerate chemical degradation within resin-dentin interfaces (Nikaido et al., 2002; Osorio et al., 2005). Mechanical loading enhances collagen's resistance to enzymatic degradation in demineralized dentin (Toledano et al., 2013b). It could be speculated that a relationship between this finding and partial dentin remineralization might occur. Nevertheless, degradation and remineralization studies assessing interfacial porosity and micropermeability of the bonded layer, in order to determine nanoleakage, require additional research. Fluorescence microscopy has been used to assess the interfacial morphology of the resin-dentin inter-diffusion zone and the distribution of dentin bonding agents. Fluorescent dyes are incorporated into the adhesives, highlighting the morphology of the hybrid layer created in thin optical sections, with minimal preparation of the sample. Micropermeability studies have revealed important information regarding bonded layer interfacial porosity, especially in samples apparently free of interfacial gaps. The extent of the hybrid layer permeability is dependent on the penetration of adhesive components into the etched dentin and on the development of porosities or gaps. Confocal laser scanning microscopy (CLSM) allows to point out the ability of fluids to penetrate the bonded interface, thus verifying the presence of micropermeability in a bonded interface (D'Alpino et al., 2006; Sauro et al., 2012). Micropermeability correlates with the presence of porosities, and so with nanoleakage. Recently, a new method to assess dentin remineralization, based on the use of a calcium-chelator fluorophore, has been proposed (Profeta et al., 2013).

The aim of this study was to analyze the effect of zinc-doping and mechanical loading on morphology and micropermeability of the dentin adhesive interfaces using two different adhesive approaches. The null hypothesis is that nanoleakage reduction and mineral precipitation are not produced at the Zn-doped resin-dentin interface after using different protocols of dentin conditioning and mechanical loading application.

2. Material and methods

2.1. Samples preparation

Eighty eight third molars without opposing occlusion were employed for the study. Extracted non-carious (44 specimens) and with occlusal caries (44 specimens) teeth were collected after written patients' informed consent (20–40 yr of age), under a protocol approved by the Institution Review Board (891/2014). Samples were stored in 0.01% (w/v) thymol at 4 °C for less than 1 month. A flat mid-coronal dentin surface was exposed using a hard tissue microtome (Accutom-50; Struers, Copenhagem, Denmark) equipped with a slow-speed, water-cooled diamond wafering saw (330-CA RS-70300, Struers, Copenhagen, Denmark). The inclusion criteria for carious dentin were that the caries lesion, surrounded by sound dentin, should be limited to the occlusal surface that it extended at least half the distance from the enamel-dentin junction to the pulp chamber. To obtain caries-affected dentin, grinding was performed by applying the combined criteria of visual examination, surface hardness employing a dental explorer, and staining by a caries detector solution (CDS, Kuraray Co., Ltd., Osaka, Japan). Using this procedure helps to remove all soft, stainable, carious dentin. It was left the relative hard, caries-affected non staining dentin, on the experimental side (Erhardt et al., 2008). A 180-grit silicon carbide (SiC) abrasive paper mounted on a water-cooled polishing machine (LaboPol-4, Struers, Copenhagem, Denmark) was used to produce a clinically relevant smear layer (Koibuchi et al., 2001).

An etch-and-rinse adhesive system, Single Bond Plus (3M ESPE, St Paul, MN, USA) (SB), was first tested. It was zinc doped by mixing the bonding resin with 20 wt% ZnO microparticles (< 1 µm particle size, Panreac Química, Barcelona, Spain) (SB-ZnO) or with 2 wt% $ZnCl_2$ (Sigma Aldrich, St Louis, MO, USA) (SB-$ZnCl_2$). A two-step self-etching system, Clearfil SE Bond (Kuraray, Tokyo, Japan) (Clearfil SE) was also tested. It was zinc doped by mixing the primer of SEB (SEB·P) with 20 wt% ZnO (Panreac Química, Barcelona, Spain) (SEB·P-ZnO) or with the bonding (SEB·Bd) resin (SEB·Bd-ZnO), or by mixing the primer of SEB with 2 wt% $ZnCl_2$ (Sigma Aldrich, St Louis, MO, USA) (SEB·P-$ZnCl_2$) or with the bonding resin (SEB·Bd-$ZnCl_2$). To achieve complete dissolution of $ZnCl_2$ and dispersion of ZnO nanoparticles, adhesive mixtures were vigorously shaken for 1 min in a tube agitator (Vortex Wizard, Ref. 51075; Velp Scientifica, Milan, Italy). The complete process was performed in the dark. Employed chemicals and adhesives description are provided in Table 1. Selection of employed zinc-doped modes and percentages was the result of a previous study on changes of physicochemical properties after zinc-doping dental adhesives (Osorio et al., 2014b; Pomacóndor-Hernández et al., 2015).

The specimens were divided into the following main groups ($n = 4$) based on the tested adhesive systems and dentin-etching procedure: (i) SB was applied on 37% phosphoric acid (PA) treated dentin, 15 s (PA + SB); (ii) SB was applied on EDTA-treated dentin, 0.5 M, 60 s (EDTA + SB); (iii) SB-ZnO was applied on 37% PA treated dentin (PA + SB-ZnO); (iv) SB-ZnO was applied on EDTA-treated dentin, 0.5 M, 60 s (EDTA + SB-ZnO); (v) SB-$ZnCl_2$ applied on 37% (PA + SB-$ZnCl_2$); (vi) SB-$ZnCl_2$ applied on EDTA-treated dentin, 0.5 M, 60 s (EDTA + SB-$ZnCl_2$). Concerning the self-etching adhesives, the specimens were divided into the following main groups ($n = 4$): (i): Clearfil SEB (SEB); (ii): SEB·P-ZnO was applied followed by the resin bonding, SEB·Bd, (SEB·P-ZnO); (iii): SEB·P-$ZnCl_2$ was applied followed by the resin bonding, SEB·Bd, (SEB·P-$ZnCl_2$); (iv): SEB·Bd-ZnO was applied after the primer, SEB·P, placement (SEB·Bd-ZnO), and (v): SEB·Bd-$ZnCl_2$ was applied after the primer, SEB·P, placement (SEB·Bd-$ZnCl_2$). Each experimental group had 4 sound and 4 caries-affected specimens.

The bonding procedures were performed in sound or caries-affected dentin following the manufacturer´s instructions. A flowable resin composite (X-Flow^{TM}, Dentsply, Caulk, UK) was placed incrementally in five 1 mm layer and light-cured with a Translux EC halogen unit (Kulzer GmbH, Bereich Dental, Wehrheim, Germany) for 40 s.

2.2. Confocal microscopy evaluation

Previous to adhesive application, bond resins were doped with 0.05 wt% Rhodamine-B (RhB: Sigma-Aldrich Chemie Gmbh, Riedstr, Germany). In 32 specimens, the pulpal chamber was filled with 1 wt% aqueous/ethanol fluorescein (Sigma-Aldrich Chemie Gmbh, Riedstr, Germany) for 3 h (Sauro et al., 2012; Toledano et al., 2013a). The rest of the molars were immersed in 0.5 wt% xylenol orange solution (Xo: Sigma-Aldrich Chemie Gmbh, Riedstr, Germany), excited at 514-nm for 24 h at 37 °C (pH 7.2). The latter is a calcium-chelator fluorophore