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The antibacterial and physicochemical properties of a one-step dental adhesive modified with potential antimicrobial agents

Alexandra R. Cocco [a,b], Tamires T. Maske [a,b], Rafael G. Lund [a,b], Rafael R. Moraes [b,*]

[a] Laboratory of Oral Microbiology, Federal University of Pelotas, Brazil
[b] Graduate Program in Dentistry, Federal University of Pelotas, Brazil

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ABSTRACT

This study investigated the effect of antimicrobial agents on the antibacterial potential of a one-step dental adhesive. Zinc silicate microparticles (ZnSi), silver microparticles (Ag), or essential oil of tea tree (terpinen-4-ol, Tr) were added at 0.5 wt% or 1 wt%. Additional analysis included pH, degree of C=C conversion (DC), translucency parameter (TP), water sorption/solubility ($W_{SR/SL}$), morphology of bonded interfaces, and dentin microtensile bond strengths (μTBS) after 24 h or 6 months. Antibacterial potential was assessed using a microcosm biofilm model. Data were statistically analyzed at α=0.05. DC, $W_{SR/SL}$, and bonding morphology were not affected by antimicrobial incorporation. ZnSi and Ag increased pH and improved immediate μTBS, generating more stable dentin bonds after 6 months. Tr showed the poorest results for μTBS. Ag 1% was the adhesive with lower TP. In general the best antibiofouling results were observed for Ag 0.5 wt%, although all antibacterial agents showed some antibiofouling effect.

1. Introduction

The main reasons for failures of dental composite resin restorations reported in clinical studies are fractures, occurring with time due to mechanical fatigue, and development of caries lesions adjacent to restorations [1,2]. Presence of residual microorganisms along the cavity margins or interfaces, among other factors, is inarguably a component playing a role on the development of secondary caries. Current research efforts aim to provide dental adhesives and restoratives materials with antibacterial potential. These materials might aid in reducing demineralization around restorations, improving maintenance of composite surface properties overtime, as well as reducing staining along cavity margins [3].

Amongst the agents used to render dental adhesives antibacterial properties, components such as chlorhexidine, fluoride, essential natural oils, ion-releasing particles, and antibacterial monomers have been tested [4–11]. Besides differences in antibacterial potential, these strategies may also differentiate from each other by the possibility of the agents of being or not released into the oral environment. Leachable agents may potentially have a long-distance effect over microorganisms, whereas agents that are physically or chemically attached to the polymer chains may have antibacterial properties by contact. For the former, the main concern is the difficulties in controlling the release kinetics of the agents, which might be associated with decreased antibacterial potential overtime [12,13]. The main concern for non-leachable agents regards their effect restricted to microorganisms adhered to the restorative surfaces.

Among antibacterial agents, silver has been commonly investigated [10,14]. Silver particles have demonstrated antibacterial and antifungal potential, but its grey aspect might affect other physical–chemical properties of dental adhesives. Other components such as particles containing Zn or natural essential oils could also provide antibacterial activity to dental adhesives [15,16], perhaps without dramatically influencing other crucial material properties. Tea tree oil, for instance, a volatile component derived from *Melaleuca alternifolia,* has been used in many topical formulations to treat cutaneous infections [17]. The antibacterial activity of tea tree oil is due to its main component, terpinen-4-ol [17], which is a monocyclic terpene alcohol that has demonstrated antibacterial, antifungal, and anti-inflammatory activities [18,19].

The purpose of this study was to investigate the effect of antimicrobial agents (essential oil of tea tree, zinc silicate microparticles, or silver microparticles) added to a one-step, self-etch dental adhesive on its antibacterial potential. The hypothesis tested was that incorporation of these agents would render the adhesives antibacterial effect without being detrimental to other physical-mechanical properties.

* Correspondence to: Graduate Program in Dentistry, Federal University of Pelotas, Rua Gonçalves Chaves 457, room 505 96015-560 Pelotas-RS, Brazil. Fax: +55 53 3225.6741x135.
E-mail address: moraesrr@gmail.com (R.R. Moraes).

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2. Materials and methods

2.1. Materials tested

A one-step self-etch adhesive from Septodont Confi-Dental Division (Louisville, CO, USA) was tested. The bonding agent reflects a typical formulation of one-step dental adhesives: 2-hydroxyethylmethacrylate, phosphate dimethacrylate, water, camphorquinone as photosensitizer, and N,N'-dimethyl-p-toluidine as coinitiator. The material was modified by the manufacturer by addition of 0.5 wt% or 1 wt% of three different antimicrobial agents, which were selected and purchased by Septodont Confi-Dental Division. All modified and non-modified materials were provided by the company. In total, seven versions of the adhesive were tested, defined by the antimicrobial agents used and their concentration: control (no antimicrobial agent), silver microparticles (Ag), zinc silicate microparticles (ZnSi), and terpinen-4-ol (Tr). Table 1 presents the characteristics of the antimicrobial agents tested. The pH of the adhesive solutions ($n=3$) was measured using a digital pHmeter (Analion PM 608 plus; Ribeirão Preto, SP, Brazil).

2.2. Degree of C=C conversion (DC)

DC was evaluated using real-time Fourier transform infrared spectroscopy (Prestige21; Shimadzu, Tokyo, Japan) with an attenuated total reflectance device incorporating a horizontal diamond crystal (Pike Technologies, Madison, WI, USA). Each adhesive was placed in the total reflectance cell and a preliminary reading for the uncured material (monomer) was taken using 24 coadded scans and 4 cm^{-1} resolution. The adhesive was photoactivated for 10 s using a LED light-curing unit (Radii; SDI, Bayswater, Victoria, Australia) with 1400 mw/cm^2 irradiance. Readings were carried out again (polymer) and %DC was calculated as previously described [20]. Five specimens were tested for each material.

2.3. Translucency parameter (TP)

The TP of cylindrical specimens of each adhesive (diameter 7 mm, thickness 1 mm) was measured. The adhesives were placed into silicone molds, polyester strips were placed on top and bottom surfaces, and photoactivation was carried out for 60 s on top and bottom surfaces. The specimens were stored in distilled water at 37 °C. The CIEL*a*b* color coordinates were measured after 24 h using a spectrophotometer (SP60; X-Rite, Grand Rapids, MI, USA). Color readings were taken over white ($L^*=93.07$, $a^*=1.28$, $b^*=5.25$) and black ($L^*=27.94$, $a^*=0.01$, $b^*=0.03$) Munsell-like neutral value scale sheet backgrounds (AG-5330; BYK-Chemie, Wesel, Germany). The TP for each specimen was calculated using the formula: $TP=[(L^*_W-L^*_B)^2+(a^*_W-a^*_B)^2+(b^*_W-b^*_B)^2]^{1/2}$, where W and B refer to the color coordinates measured on the white and black backgrounds [21,22]. Five specimens were tested for each material.

2.4. Water sorption and solubility

Dentin disks (diameter 7 mm, thickness 1 mm) were obtained from the buccal faces of bovine incisors, placed in Eppendorf tubes, and dry-stored in an oven at 37 °C until a constant mass was achieved. The adhesives were actively applied to dentin for 30 s using microbrush, followed by 10 s air-drying and photoactivation for 10 s. The specimens were dry-stored again at 37 °C until a new constant mass (m_1) was obtained. Distilled water was added to the tubes and the specimens stored at 37 °C. After 7 days, the specimens were reweighed (m_2) and dry-stored again at 37 °C until a new constant mass was obtained (m_3). Water sorption ($\%W_{SR}$) and solubility ($\%W_{SL}$) were calculated based on mass gain or loss during sorption and desorption cycles. Five specimens were tested in each group.

2.5. Dentin microtensile bond strength (μTBS) and failure analysis

Seventy bovine incisors were stored in 0.5% chloramine-T aqueous solution for 7 days. The buccal surfaces were flattened to expose medium dentin and the teeth were randomly assigned into seven groups according to the adhesive system tested. The adhesives were applied to dentin as described before and composite resin restorations were built up incrementally on the surfaces (N'Durance; Septodont). Restorations were stored in distilled water at 37 °C for 24 h then sectioned into beam-shaped composite-dentin specimens with 1 mm^2 cross-sectional area. At least four beam-shaped specimens were obtained per tooth and randomly divided into two storage periods in distilled water at 37 °C (24 h and 6 months, $n=20$ per group), for the evaluation of dentin μTBS. The μTBS test was carried out on a mechanical testing machine (DL500; EMIC, São José dos Pinhais, PR, Brazil) at 1 mm/min crosshead speed until failure. After testing, the specimens were individually analyzed using a stereomicroscope at 40 × magnification for determining their mode of failure, which was classified as adhesive failure (interfacial failure), cohesive within dentin or composite resin (failure within one of the bonded substrates), or mixed failure (failure partially adhesive and cohesive). Premature failures (spontaneous debonding) were also recorded.

2.6. SEM analysis of the bonded interfaces

The morphology of the bonded interfaces was analyzed using scanning electron microscopy (SEM) at 15 kV (JSM 6610, JEOL, Tokyo, Japan). The adhesives were applied to dentin disks, which were bonded in pairs using a thin layer of photoactivated composite resin, generating a dentin-composite-dentin sandwich specimen. The specimens were embedded cross-sectionally in epoxy resin leaving the dentin-composite interface visible. After 24 h, the specimens were wet-polished with 600, 1200, 1500, and 2000-grit SiC abrasive papers and polished with 3, 1, and 0.5-μm diamond suspensions. The polished surfaces were etched with 50% phosphoric acid aqueous solution for 5 s and deproteinized by immersion in 2.5% NaOCl solution for 10 min. The specimens were

Table 1
Antimicrobial agents tested in the study.

Antimicrobial agent	Supplier	Characteristics[a]
Silver microparticles (Ag)	BioEpiderm, Nürnberg, Germany	Silver-gray powder, 99.9% porous pure metallic silver particles, 10 μm average particle size (not including nanoparticles), up to 5 m^2/g surface area
Zinc silicate microparticles (ZnSi)	Sukgyung AT Co., Gyeonggi-do, Korea	White ZnO–SiO_2 complex powder, 3.2 μm average particle size (including nanoparticles), up to 57 m^2/g surface area, treated with 10 wt% silane, refractive index 1.60
Terpinen-4-ol (Tr)	Sigma-Aldrich, Milwaukee, WI, USA	Light yellow liquid, ≥95% sum of enantiomers, molecular weight 154.25 g/mol, refractive index 1.479

[a] Data obtained from the suppliers.