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Mechanical and microbiological properties and drug release modeling of an etch-and-rinse adhesive containing copper nanoparticles

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ABSTRACT

Objectives. To evaluate the effect of addition of copper nanoparticles (CN) at different concentrations into a two-step etch-and-rinse (2-ER) adhesive on antimicrobial activity (AMA), copper release (CR), ultimate tensile strength (UTS), degree of conversion (DC), water sorption (WS), solubility (SO), as well as the immediate (IM) and 1-year resin–dentin bond strength (μTBS) and nanoleakage (NL).

Methods. Seven adhesives were formulated according to the addition of CN (0, 0.0075, 0.015, 0.06, 0.1, 0.5 and 1 wt%) in adhesive. The AMA was evaluated against *Streptococcus mutans* using agar diffusion assay. For CR, WS and SO, specimens were constructed and tested for 28 days. For UTS, specimens were tested after 24 h and 28 days. For DC, specimens were constructed and tested after 24 h by FTIR. After enamel removal, the ER was applied to dentin. After composite resin build-ups, specimens were sectioned to obtain resin–dentin sticks. For μTBS and NL, specimens were tested after 24 h and 1-year periods. All data were submitted to statistical analysis ($\alpha = 0.05$).

Results. The addition of CN provided AMA to the adhesives at all concentrations. Higher CR was observed in adhesives with higher concentration of CN. UTS, DC, WS and SO were not influenced. For μTBS an increase was observed in 0.1 and 0.5% copper group. For NL, a significant decrease was observed in all groups in comparison with control group. After 1-year, no significant reductions of μTBS and no significant increases of NL were observed for copper containing adhesives compared to the control group.

Significance. The addition of CN in concentrations up to 1 wt% in the 2-ER adhesive may be an alternative to provide AMA and preserve the bonding to dentin, without reducing adhesives' mechanical properties evaluated.

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1. Introduction

In recent years, due to an increasing demand for esthetic restorations, composite resins have gained a prominent role in restorative dentistry. Nowadays, they are the most widely used dental restorative material representing 65% of the restorations currently placed in the United States [1,2]. However, there are still concerns about the limited durability and clinical success of contemporary adhesive restorations [3,4]. It is reported that 50–70% of newly placed restorations are the result of failure of preexisting restorations, which results in millions of dental care dollars spent annually on replacement of these restorations [5–9].

Restorations performed with composites accumulate more biofilm and are subject to faster degradation than those made with other materials [10–15]. Furthermore, polymerization shrinkage can result in the formation of gaps between the adhesive resin and the dental structure [16]. This, in turn, can lead to microleakage and accumulation of biofilms at the restoration margins which acid by products cause secondary caries, which has been suggested as a primary reason for dental restoration failures [17–19].

For these reasons, further improvements could be achieved by developing dental materials with antibacterial properties to reduce biofilm formation at the tooth-restoration margins, without jeopardizing the mechanical properties of the adhesive formation [20–22]. Among the most promising agents with antibacterial properties are metallic nanoparticles, which exhibit increased chemical and biocide activity [23].

The antibacterial activity of copper and silver nanoparticles has been extensively investigated. Copper nanoparticles showed higher antibacterial activity compared with silver nanoparticles against *Escherichia coli, Bacillus subtilis and Staphylococcus aureus* [24,25]. In addition to superior antimicrobial activity, copper is cheaper as compared to silver, easily available and synthesis of copper nanoparticles is cost effective [26]. An additional advantage of copper nanoparticles is that they oxidize in air or aqueous media to produce copper oxide nanoparticles. While copper oxide demonstrates high enough bactericidal action, it is easily blend with polymers or macromolecules producing a formulation with a relatively stable chemical and physical properties [27–30].

The addition of silver nanoparticles in resin cement and dental adhesives produced materials with superior antimicrobial capacity, mechanical and adhesive properties [31,32]. Paradoxically, the use of copper nanoparticles in dental materials has not been much explored, despite its antimicrobial potential [33]. Two recent studies showed that an adhesive containing copper-nanoparticles presented an antimicrobial activity against *Streptococcus mutans* (*S. mutans*) [34,35], however, it is yet not clear which is the optimal concentration of copper that can be added to an adhesive system to express antimicrobial activity without compromising other mechanical properties of the adhesive formulation.

Therefore, this *in vitro* study aimed to investigate the antimicrobial activity, ultimate tensile strength, degree of conversion, water sorption, solubility, immediate and 1-year resin–dentin bond strength and nanoleakage, as well as, copper release kinetics of a commercial two-step etch-and-rinse adhesive system after adding different concentrations of copper nanoparticles.

2. Material and methods

2.1. Formulation of the experimental adhesives

We formulated experimental adhesives using the two-step etch-and-rinse adhesive system Ambar (FGM Prod. Odont. Ltda, Joinville, SC, Brazil). Seven experimental adhesives systems were formulated according to the addition of different concentrations of copper nanoparticles (99.9% pure, SkySpring Nanomaterials, Inc., Houston, TX, USA; www.ssnano.com) (wt%): 0% (control, commercial material), 0.0075%, 0.015%, 0.06%, 0.1%, 0.5% and 1.0%. The copper nanoparticles properties are shown in Table 1. The copper nanoparticles were characterized by field emission scanning electron microscope (FE-SEM), atomic force microscopy (AFM), Malvern zeta sizer and energy dispersive X-ray (EDX) analysis. Then, the participles were added to the adhesive and mechanically mixed by a motorized stirrer.

2.2. Antimicrobial activity

After isolating a metallic matrix (5.8 mm diameter, 1.0 mm thick) with petroleum jelly, we dispensed the adhesive until completely fill the mold. All visible air bubbles trapped in the adhesives specimens were carefully removed with a microbrush (Microbrush International, Grafton, WI, USA). Next, an air stream was applied for solvent evaporation for 40 s at a distance of 10 cm.

Under a plastic matrix strip, the adhesive was light-cured for 40 s with an LED light source at 1200 mW/cm^2 (Radii-cal, SDI, Baywater, Victoria, Australia), in close contact with each disc. After polymerization, the specimens were removed from the mold, and polished with 600-grit SiC paper to remove adhesive excess and the oxygen-inhibition layer.

After storage in a dark vial for 24 h, all specimens were disinfected by ultraviolet light with a 30 min exposure in each side of the disc. Each disc was then inserted placed on brain heart infusion (BHI) agar plates (Kasvi Ltda, Curitiba, PR, Brasil) containing a freshly prepared lawn of *S. mutans* (ATCC 25175). *S. mutans* was cultured microaerophilically in brain heart infusion broth (Difco Laboratories, Detroit, MI, USA) for 72 h in at 37 °C. Then, 100 μL of the bacterial suspension was swabbed

Table 1 – Copper nanoparticles specifications.

Type of particles	Specifications
Copper nanoparticles (Cu) 0820XH Skyspring nanomaterials	Purity: 99.9% trace metals basis Appearance: Black nanopowder APS: 40–60 nm SSA: ~12 m^2/g Morphology: spherical Bulk density: 0.19 g/cm^3 True density: 8.9 g/cm^3

APS, average particle size; SSA: specific surface area (http://ssnano.com/).