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Incorporation of bactericidal poly-acrylic acid modified copper iodide particles into adhesive resins

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ABSTRACT

Objectives: This study aimed to investigate incorporation of polyacrylic acid (PAA) coated copper iodide (CuI) nanoparticles into dental adhesives, and to evaluate for the first time, their antibacterial properties, bond strength and cytotoxicity.

Methods: PAA-CuI nanoparticles were synthesized and incorporated into commercially available adhesives Optibond XTR (1.0 mg/ml) and XP Bond (0.5 and 1.0 mg/ml). The antibacterial properties of experimental and control specimens were evaluated ($n = 8$), after ageing for 18 h or 1 year, against *Streptococcus mutans* (1×10^8 cells/ml). Bond strength to human dentine of the control and experimental adhesives was evaluated by shear bond strength ($n = 10$). For cytotoxicity evaluation, HGF cells were cultured with gingival fibroblast media and exposed to control and experimental adhesive blends ($n = 3$). An MTT cell viability assay was used to assess cell metabolic function. A one-way analysis of variance followed by Tukey's test was used for data analysis.

Results: Significantly greater antibacterial properties were demonstrated for PAA-CuI containing adhesives after ageing for 18 h or 1 year relative to all control groups. A reduction in *Streptococcus mutans* viable cell count of 99.99%, 99.99% and 79.65% was shown for XP Bond – 0.5 mg/ml, XP Bond – 1.0 mg/ml and Optibond XTR – 1.0 mg/ml PAA-CuI after ageing for 18 h, and 99.99% for both XP Bond – 0.5 mg/ml and XP Bond – 1.0 mg/ml PAA-CuI after ageing for 1 year. No significant variations in shear bond strength or cytotoxicity were detected between the experimental resins and their corresponding controls.

Conclusions: PAA-CuI nanoparticles are an effective additive to adhesive blends as it renders them antibacterial without adversely affecting their bond strength or cytotoxicity.

Clinical significance: The incorporation of PAA-coated copper iodide particles into adhesive resins renders the adhesive antibacterial to *S. mutans* for at least 1 year *in vitro*. This may prevent or delay bacterial invasion and the consequent development of caries lesions if the adhesive interface becomes defective.

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1. Introduction

In the last few decades, resin-based composites (RBC) have gradually become the most widely used dental restorative material, representing 65% of the restorations currently placed in the United States.[1] Their wear resistance, life-like aesthetics and perceived ease of placement make them desirable for both patients and clinicians.[2] The use of RBC is anticipated to continue to increase due to patient demands for aesthetic restorations.[3–5] Despite its increased use and the significant developments taking place in the field of dental materials science, the durability of adhesive restorations remains a challenge.[5–7] It is well accepted that current RBC cannot prevent recurrent caries.[1–3,8–10] Its been reported that 50–70% of newly placed restorations are the result of failure of pre-existing restorations[8,11] with the cost to replace defective restorations in the United States surpassing 5 billion dollars annually.[12] While posterior high copper amalgam (HCA) restorations have an average lifespan of 11–12 years, the service life of posterior RBC restorations ranges between 6 and 7 years.[1,13] A study showed that after 8 years, the failure rate for posterior RBC restorations was 50% greater than that of HCA restorations.[14] The higher incidence of recurrent caries and consequently, the greater need for replacement, may explain their reduced lifespan.[6,7,9,10,15]

Silver and copper, present in amalgam, are known to have potent antimicrobial properties,[16] which may play an essential role in the clinical success of amalgam restorations. Modern amalgams contain approximately 60% silver and more than 12% copper by weight[17] making them potentially antimicrobial and delaying their failure from bacterial invasion.[18] The incidence of recurrent caries is 3.5 times higher in RBC than HCA restorations.[9] This notion is further supported by the observation that bacterial micro-leakage is the most frequent complication associated with RBC restorations, leading to recurrent caries as the primary cause of failure.[19–21] A study evaluating interproximal restorations in 650 radiographs found that failure due to recurrent caries was 43% for RBC and only 8% for HCA.[6] RBC micro-leakage and bacterial invasion was found to take place at the tooth-restoration interface. This adhesive interface, the so-called hybrid layer, is a biosynthetic layer composed of a mixture of type I collagen and adhesive resins,[22] and has been reported to be the weakest link of RBC restorations.[23] Continued developments in the field of dentine adhesion are still needed to improve the longevity of RBC restorations.

Recent research work has demonstrated that copper and silver nanoparticles incorporated into polymers have a broad spectrum of antimicrobial activity.[24–27] We speculate that this may help decrease the incidence of recurrent caries. Silver ion-containing resin composites have shown to have an antibacterial effect on oral *Streptococci*.[25] A study found that the incorporation of silver nanoparticles into experimental orthodontic resin adhesives decreased the attachment and slowed the growth of *Streptococcus mutans* and *Streptococcus sobrinus* relative to the control adhesive.[28] Although incorporation of silver nanoparticles did not have a negative effect on the bond strength to dentine,[29] it yielded a change in the colour of the resin to a degree that would limit its application to areas where aesthetics was not critical.[28] Likewise, copper particles are also black in colour and have a similar aesthetic concern. Alternative copper particles with enhanced aesthetic properties are available. Copper chalcogenide or copper halide (CuQ, where Q = chalcogens including oxygen or halogens) was found to have altered optical properties rendering them white (unpublished observations). Specifically, copper iodide particles are tooth coloured and were found to have minimal effect on the shade of the materials to which they were added (unpublished observations).

Copper particles are unique in that their antibacterial activity is derived from the localized leaching of copper ions, which generates an infinite electron sink that can alter the bacterial membrane charge causing lysis. These antimicrobial properties are further optimized by the high surface-to-mass ratio achieved from the structure of the nanosphere.[30] Copper particles are also significantly less expensive than silver, easily incorporated into polymers and relatively stable with regards to their chemical and physical properties.[24] Roselen et al. found that the addition of copper to sucrose significantly reduced the incidence of caries in rats when compared to sucrose diet alone.[31]

This study describes the development of an adhesive resin based on the incorporation of polyacrylic acid (PAA) coated copper iodide (CuI) nanoparticles that renders the adhesive resistant to the major processes of adhesive failure, bond degradation and destruction of the resin inter-diffusion zone from bacterial proliferation and invasion. The use of such antibacterial adhesives is anticipated to limit the recurrence of caries and failure of RBC restorations. The aims of this study were to develop an antibacterial adhesive resin incorporating PAA-CuI nanoparticles and to investigate for the first time, its antibacterial activity, bond strength and potential cytotoxicity. The null hypotheses evaluated were: (1) incorporation of PAA-coated CuI particles into adhesive resins would have no effect on their antibacterial properties; (2) incorporation of PAA-coated CuI particles into adhesive resins would have no effect on their shear bond strength to dentine; and (3) PAA-coated CuI particles would demonstrate no cytotoxicity to mammalian cells compared to control adhesives.

2. Materials and methods

2.1. *Synthesis of poly-acryic acid (PAA) coated copper iodide (CuI) particles and generation of PAA-CuI adhesives*

Copper (II) sulfate ($CuSO_4$, Puratonic, 99.999% metal bases, Alfa-Aesar, Ward Hill, MA), potassium iodide (PI, 99.995%, Acros, Waltham, MA) and poly (acrylic) acid (PAA, 50 wt% in water, MW = 5000, Acros) were used as received. All solutions were prepared using deionized 18.2 MΩ water (Milli-Q, Milipore, Billerica, MA). In a typical reaction, 78.8 ml of 0.2 M $CuSO_4$ was combined with 7.8 ml of 20 wt% PAA followed by the addition of 100 ml of 400 mM KI. Subsequently, more potassium iodide (50 ml of 400 mM) was added to drive the reaction to completion. The resulting white precipitate was washed with deionized water four times by centrifugation and dried under vacuum at 50 °C. The powder was characterized by field emission scanning electron microscope (FE-SEM) and