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Designing dental composites with bioactive and bactericidal properties

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ABSTRACT

Objectives: The aim of this work was to fabricate and evaluate new antibacterial and bioactive composites capable of strictly controlling oral bacteria, enhancing apatite layer formation and retaining their mechanical properties.
Methods: A new Ag-doped bioactive glass (Ag-BG) was incorporated into flowable dental composite (COMP) in different concentrations (1, 5, and 15 wt.%) in order to fabricate new combined bioactive and antibacterial composite materials (Ag-BGCOMPs). The antibacterial properties, bioactivity, and total bond strength of the Ag-BGCOMPs were evaluated.
Results: The bioactivity of the Ag-BG was confirmed after its immersion in simulated body fluid (SBF). The total bond strength between the surrounding tooth tissue and the new composites or the control (dental composite alone) has not shown any statistically significant difference in the performed pilot study. Antibacterial activity was observed against *Escherichia coli* (*E. coli*) and *Streptococcus mutans* (*S. mutans*) for the Ag-BGCOMP 5 wt.% and 15 wt.% but not for the Ag-BGCOMP 1 wt.% or the control.
Conclusions: This work contributes to our long term aim which is the fabrication of dental materials capable of reducing bacteria invasion and enhancing remineralization of the surrounding dental tissues.
Significance: We anticipate that these new composites could ultimately prevent restoration failure by inhibiting the formation of secondary caries and by remineralizing the hard tissues surrounding tooth lesions.

1. Introduction

It has been observed that two-thirds of all restorative dentistry involves the replacement of failed restorations [1,2], while most teeth that have compromised restorations develop symptoms requiring dental pulp treatment [3], with 1.5 million US restorations requiring root canal therapy [4] and millions of teeth finally extracted. High rates of treatment failure suggest that the current restorative approaches are not yet optimized and have a potential for improvement. There is a need for developing innovative restorative materials exhibiting antibacterial function to prevent the recurrence of caries and to repair and/or regenerate the defected dental tissue. Up-to-date, appropriate materials capable of exhibiting these features are not yet available.

Towards this goal, several ion-releasing resin-based CaP cements have been proposed as potential cavity liner materials due to their ability to neutralize acid solution and to induce the remineralization of mineral-deficient carious dentine [5]. However, their relative weak antibacterial activity indicates the need for incorporation of other antibacterial agents in order to develop strong antibacterial composite materials for clinical use. Ag-containing resin-based materials have been also fabricated and used as adhesives in dental practice [6,7]. Although these materials presented antibacterial activity, their remineralization potential has not been confirmed.

There is also much merit in the studies examining how current dental materials can be modified to maintain long lasting bioactive and anti-bacterial properties [8–10]. Many of these studies provide evidence for the potential beneficial use of bioactive glasses (BGs) as additives to dental materials to enhance their biological effect. Bioactive glasses have been reported to induce mineralization of dentin disc surfaces [11,12]. These results suggest that dental materials with incorporated BGs could be instrumental in the remineralization of affected human dental tissues [13].

Indeed, new materials that can remineralize the dental hard tissues [14] will find multiple applications in the minimal invasive

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dentistry field. The principals of minimal therapeutic restoration may be enhanced by using bioactive materials capable of releasing specific ions within the bonding interface, to evoke a positive response from the biological environment and to induce protection and/or remineralization of the mineral-depleted dental tissues [15–17]. The application of such materials can lead to a perfect bonding between the restorative material and the surrounding tooth tissues. However, bioactive dental materials with combined antibacterial properties that can eliminate future micro-penetration and secondary caries have not been developed.

Due to their esthetics and direct-filling capability, resin composites have been the materials of choice in restorative dentistry [18,19]. One of the most common causes of replaced restorations is the secondary caries where active bacteria colonize the injury site and penetrate the dental pulp through material/dental tissues micro-gaps. Another major challenge with resin-based composites is bulk fracture [20]. Furthermore, it has been observed that composites allowed more accumulation of dental plaque on their surfaces than other restorative materials [21,22].

The aim of this work was to fabricate and evaluate new bioactive and antibacterial composite materials. Our hypothesis was that a silver containing bioactive composite could show enhanced antibacterial properties compared to a control composite material. A novel silver (Ag)-doped bioactive glass (Ag-BG) [23] was incorporated in different proportions in a flowable commercial composite to fabricate new bioactive and antibacterial composites (Ag-BGCOMPs). Moreover, we anticipate that Ca and P released ions from the incorporated BGs will enhance apatite layer formation, remineralizing the mineral-depleted dentin.

2. Materials and methods

2.1. Ag-BG fabrication

Ag-BG fabrication has been already presented in detail elsewhere [24]. Briefly, the fabrication of the new sol–gel derived Ag-doped bioactive glass is based on incorporating the sol–gel bioactive glass 58S (SiO_2 58-CaO 33-P_2O_5 9 wt.%) being in the solution stage, into the solution stage of a new sol–gel glass (in the system SiO_2 60-CaO 6-P_2O_5 3-Al_2O_3 14-Na_2O 5-K_2O 5-Ag_2O 7 wt.%). The resulting composite solution follows a specific heat treatment; initially aging process at 60 °C, then drying at 180 °C and finally stabilization at 700 °C. The final sol–gel derived Ag-doped bioactive glass (Ag-BG) is in the system SiO_2 58.6-CaO 24.9-P_2O_5 7.2-Al_2O_3 4.2-Na_2O 1.5-K_2O 1.5-Ag_2O 2.1 wt.% [24]. The novel Ag-BG is fabricated in powder form with particle size around ~25 μm.

2.2. Fabrication of Ag-BGCOMP specimens

Specimens with 0, 1, 5 and 15 wt.% of Ag-BG within the flowable composite (Ivoclar Vivadent, Tetric EvoFlow® Filling Material A1, United States and Canada) were formulated prior to the initiation of the test methods. Ag-BG in powder form with particle size of <35 μm was incorporated manually. Samples without Ag-BG incorporation were used as controls (controls: Ag-BG 0 wt.%).

Cured disc samples were prepared using Teflon molds (10 mm diameter, 2 mm thick, and weight 100mgr) applying the instructions of manufactures (two curing cycles of 10 s with halogen curing light (Valo, Ultradent (South Jordan, UT)) operating with a wavelength of 400–500 nm and an intensity of about 1000 mW/cm^2. Both top and bottom surfaces were exposed to light, while there was almost no distance between the light-tip and the sample surface) and uncured samples (weight 100 mgr) both with Ag-BG incorporated at 0, 1, 5 and 15 wt.% immersed in PBS for 8 days. Moreover the changes at the pH values were followed for immersion period of a month.

2.3. Antibacterial properties

The bactericidal properties of the extracts were tested against *S. mutans* ATCC 25175 basic cariogenic bacterium and *E. coli* ATCC 29522. A single colony of bacteria was inoculated in nutrient broth and grown overnight at 37 °C. After adjusting to an optical density equivalent to 10^8 cells per ml in PBS, sequential tenfold dilutions were added to tubes containing equal volumes of the extracts. The effect of the materials' extracts on bacterial growth was assayed by colony forming units (CFU) on nutrient agar plates after 24 h of growth.

Cured specimens were sterilized by ethylene oxide gas. Sterilized samples were placed onto BHI (brain heart infusion broth) agar plates inoculated with 350 μl of 1×10^8 CFU/ml of each bacterium suspension, and the plates were incubated at 37 °C for 48 h. The inhibitory effect against *E. coli* and *S. mutans* was assayed, as it is indicated by the literature [25]. In particular, after incubation, samples were then removed, and bacterial growth under the specimens was observed by scanning electron microscopy (SEM). For SEM observation the specimens were rinsed with phosphate buffered saline (PBS), and then immersed in 1% glutaraldehyde in PBS for 4 h at 4 °C. The specimens were rinsed with PBS and subjected to graded ethanol dehydrations. They were then rinsed twice with 100% hexamethyldisilazane. The specimens were then mounted on aluminium stubs with carbon cement and sputter coated with gold (Polaron E5100 Sputter Coater). Scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS) capability was used for the characterization of the samples (AMRAY 1910 Field Emission Gun-type Scanning Electron Microscope (FEG-SEM) and JEOL JSM-840A). The sample size was triplicated for each sample case and for each tested bacterium. Qualitative analysis of samples' surface was performed and representative images are shown.

2.4. Bioactive behavior

The apatite-forming ability of the bioactive glass was examined through immersion in simulated body fluid (SBF), as this technique is commonly used to evaluate the bioactivity [26]. Three specimens (dimensions 10 mm diameter and 2 mm thickness) were immersed in SBF (30 ml) at 37 °C up to 20 days (with time points after 3, 7, 10, 15 and 20 days). The SBF solution was replaced every three days since there is a decrease in cation concentration due to the changes in the chemistry of the samples. At the end of each selected time period, samples were removed from the SBF solution, rinsed with 70% ethanol and distilled water, dried and stored in airtight containers for further investigation. The SBF treated samples were examined by SEM to assess the possible formation of a hydroxyapatite (HAp) layer on the material surface, as a marker of bioactive behavior [27].

2.5. Mechanical properties

Microtensile test method was used in a pilot study to measure total bond strength (μTBS) of both dentin and uncut enamel. It is considered as an initial means of characterizing the mechanical properties of Ag-BGCOMP material [28]. In particular, third molars were potted along the long access of the tooth and cut mid-coronally to expose the dentin (or enamel alone in case of the uncut enamel tests). The teeth were sanded with 320 grit paper, etched for 15 s using 35% phosphoric acid etchant, blotted dry until they were slightly moist, and then bonded with commercial bonding agent (AdheSE®, Ivoclar Vivadent) as well as ultimately, layered with the fabricated composite formulations. Specimens of 1 mm × 1 mm in transverse cross-section were cut using a hard tissue microtome along the long axis of the tooth, obtaining matchstick-shaped beams and creating microtensile samples. The specimens were immediately tested for the μTBS test. This testing procedure was executed using customized microtensile fixtures on a testing set-up comprising a LAC-1 (high speed controller single axis with built-in amplifier) and LAL300 linear actuator that had a stroke length of 50 mm