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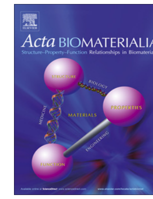

# Fabrication and characterization of bioactive and antibacterial composites for dental applications

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A B S T R A C T

There is an increasing clinical need to design novel dental materials that combine regenerative and antibacterial properties. In this work the characterization of a recently developed sol–gel-derived bioactive glass ceramic containing silver ions (Ag-BG) is presented. The microstructural characteristics, ion release profile, zeta potential value and changes in weight loss and pH value as a function of the immersion time of Ag-BG in Tris buffer are evaluated. Ag-BG is also incorporated into natural extracellular matrix (ECM) hydrogel to further enhance its regenerative properties. Then, the micro and macro architectures of these new composites (ECM/Ag-BG) are characterized. In addition, the antibacterial properties of these new composites are tested against *Escherichia coli* and *Enterococcus faecalis*, a bacterium commonly implicated in the pathogenesis of dental pulp infections. Cell–material interaction is also monitored in a primary culture of dental pulp cells. Our study highlights the benefits of the successful incorporation of Ag in the bioactive glass, resulting in a stable antibacterial material with long-lasting bactericidal activity. Furthermore, this work presents for the first time the fabrication of new Ag-doped composite materials, with inductive pulp-cell proliferation and antibacterial properties (ECM/Ag-BG). This advanced composite made of Ag-BG incorporated into natural ECM possesses improved properties that may facilitate potential applications in tooth regeneration approaches.


## 1. Introduction

The growing importance of biomaterials that prevent microbial growth is leading to the development of new bioactive materials exhibiting antibacterial action, which create a bacteria-free environment while healing and regenerating the defect area [1]. A large number of biomaterials doped with different ions, particles, therapeutic agents and various natural and inorganic substances expected to exhibit antibacterial activity are routinely used for clinical applications [2]. In addition, the increase in the number of antibiotic-resistant bacterial strains has generated increasing interest in the controlled delivery of alternative antibacterial agents with low impact on resistance and extended activity, such as heavy metals like silver, copper or zinc, which could be an alternative strategy to inhibit bacteria growth [3]. Silver at low concentrations is a metal well known for its bactericidal action against a wide range of Gram-positive and Gram-negative bacteria, fungi, protozoa and certain viruses [4]. Silver is also efficient against common bacterial strains involved in implant-associated infections and antibiotic-resistant strains [5,6]. Importantly, there is no observed cytotoxicity effect for mammalian cells when silver ion concentrations released are lower than 1.6 ppm [7–10]. Silver ions are able of inactivating the bacterial proteins by affecting DNA molecules, which are not able to replicate and interact with thiol groups in the protein [10]. Ag is indeed a well-known antiseptic agent which has been effectively used in a big variety of materials such as glass, titanium and polymers [11,12].

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Elemental silver or $Ag^{+}$ (silver salts or silver complexes) is usually incorporated into organic or inorganic matrices in the most Ag-doped antibacterial biomaterials, including silicate bioactive glasses (BGs) [13,14]. The successful development of a silicate Ag-doped bioactive and bactericidal glass has been reported previously [15,16] but there is still a lack of Ag-doped bioactive glasses exhibiting stable structure, low sensitivity to light and with the silver ion less bound into the silica matrix, enabling long-lasting antibacterial activity [17,18]. On the contrary, the rapid release of silver and the unstable behavior of silver ions, which is met on the already developed Ag-doped bioactive glasses, have brought out an increasing interest in silver nanoparticles as a bactericidal source due to their low solubility in aqueous media. Many methods have been developed for the fabrication of silver-doped glasses and ceramics, which are candidates for antibacterial materials [19]. In particular, the sol–gel technique is a favorable method for the fabrication of glasses and glass ceramics, with many advantages such as high purity, low processing temperatures, ultrahomogeneity and, most important, the ability to be applied in different forms to make glasses and glass ceramics of novel compositions [20–22].

Although the dental materials currently used in dental restorations have good clinical success, failure often occurs due to recurrent decay, which can lead to subsequent loss of the restorations [23,24]. Several attempts have been made to overcome this problem by adding to the dental material antimicrobial components such as fluoride and chlorohexidine in order to prevent recurrent decay. However, these restorative materials show limitations due to diminishing fluoride release and uptake with time [25,26]. Moreover, quaternary ammonium or metal particles were also used as additives in dental materials but their incorporation was affecting the physical properties and the bond strength of the material [27]. The use of an Ag-doped BG that supports cell proliferation for tissue regeneration in a bacteria-free environment and enhances the bond strength at the defect area could prolong the lifetime of the restoration.

On the other hand, three-dimensional scaffolds are considered as one common approach aiming to restore tissue function, allowing cell growth on designed scaffolds that create a microenvironment for cell support. Extracellular matrix (ECM) scaffolds have been used to reconstruct and remodel many different tissues in both preclinical animal studies and in human clinical applications. Many ECM materials have been commercialized and used in therapeutic applications to reconstruct a variety of tissues such as the integument [28,29], body wall [30], urinary bladder [31], rotator cuff [32], intestine [33], urethra [34], ureter [35] and diaphragm [36].

In dental applications, scaffolds have been fabricated with a variety of natural and synthetic biomaterials [37,38]; however, the clinical applications in the field of regenerative dentistry demand further development of suitable injectable scaffolds. The use of a naturally derived hydrogel is proposed for the first time in this work as a useful injectable scaffold that can enhance dental pulp cell attachment, proliferation and differentiation. Naturally derived hydrogels present superior biocompatibility and bioactivity compared to their synthetic counterparts [39]. However, limited antibacterial properties have been observed [40,41]; thus the incorporation of an Ag-doped BG could advance their properties, creating a new injectable material capable of healing and regenerating the defect areas.

In this work, we examined the properties of an Ag-doped BG and evaluated the combined properties of novel composites fabricated by incorporating a new Ag-doped BG into natural ECM scaffold obtained from porcine urinary bladder matrix (UBM). The new developed bioactive and bactericidal composites could be potentially used in envisaged dental applications for the healing and regeneration of the defect areas.

## 2. Materials and methods

### 2.1. Bg

The fabrication of the Ag-free BG and Ag-doped BG in the systems: $SiO_2$ 58.6–CaO 24.9–$P_2O_5$ 7.2–$Al_2O_3$ 4.2–$Na_2O$ 2.1–$K_2O$ 3 (wt.%) and $SiO_2$ 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.%), respectively, has been already described [42]. Briefly, the fabrication protocol is based on the sol–gel process by mixing the solution stage of the 58S sol–gel BG (in the system $SiO_2$ 58–CaO 33–$P_2O_5$ 9 (wt.%)), with the respective solution stage of a new sol–gel porcelain A (in the system $SiO_2$ 60–CaO 6–$P_2O_5$ 3–$Al_2O_3$ 14–$Na_2O$ 7–$K_2O$ 10 (wt.%)), as has been presented in detail in our previous work [43,44]. After extended stirring, the final homogeneous solution follows a specific heat treatment under aging at 60 °C, drying at 180 °C and stabilization up to 700 °C. The fabricated material is the Ag-free composition, which undergoes a specific modification of the fabrication protocol and components in order to fabricate the Ag-doped BG. In detail, the Ag-doped BG is fabricated by decreasing the concentrations of $Na_2O$ and $K_2O$ in the composition of the Ag-free BG and adding simultaneously $Ag_2O$. The fabricated materials were in powder form with particle size <35 μm.

The characterization of the developed materials was implemented by using different techniques. UV/vis spectroscopy (Lambda18 Perkin-Elmer with an Integrating Sphere) was used to confirm the colorless character of the systems. $^{27}$Al magic angle spinning nuclear magnetic resonance (MAS-NMR) spectroscopy was applied to study Al coordination in the structure. The spectra were recorded using a Varian-400 plus NMR spectrometer (Palo Alto, CA) operated at 104.21 MHz. A delay time of 10 s and a pulse length of 4.0 ms were used.

The thermal behavior of the fabricated materials was studied by heating up to 1000 °C with a heating rate of 10 °C $min^{-1}$ in air using a ThermoPlus instrument, TG 8120. The microstructural characterization of the heat-treated materials at 970 °C (the temperature range of the exothermic process) was performed by X-ray diffraction (XRD) analysis using a Phillips PW1700 Series automated powder diffractometer. Cu $K_\alpha$ radiation of 40 kV and 40 mA was obtained using a secondary crystal monochromator. Specimens were aligned perpendicular to the incident beam and the analysis parameters were: step size 0.017° (2θ), 10 s per step and a diffraction range of 5–70° (2θ). The microstructural and elementary characterization of the fabricated materials was studied by scanning electron microscopy with energy dispersive spectroscopy (SEM–EDS) (JEOL JSM-6301). The zeta potential values were measured with a Photal ELS-6000KS (Otsuka Electronics). Samples were in powder form (mass 0.05 g) and suspended in deionized water (50 ml) at pH 7.4. The ion release concentration of the materials after soaking in Tris buffer solution was measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES; Shimadzu ICPS-7000, Kyoto, Japan). Samples with a non-significant difference in the surface area were prepared in triplicate for both Ag-free BG and Ag-doped BG and the experiment was repeated twice. Moreover, the weight loss as a function of the immersion time was measured and the degradation profile was compared to the respective of the ion release concentrations. Finally the pH value was monitored and the changes are reported.

### 2.2. Ecm

The ECM hydrogels were provided by the University of Pittsburgh. They were prepared from decellularized porcine UBM as previously described [45]. In brief, porcine urinary bladders from market weight pigs were harvested, and the urothelial, serosal



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