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Controlled delivery of antimicrobial gallium ions from phosphate-based glasses

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Abstract

Gallium-doped phosphate-based glasses (PBGs) have been recently shown to have antibacterial activity. However, the delivery of gallium ions from these glasses can be improved by altering the calcium ion concentration to control the degradation rate of the glasses. In the present study, the effect of increasing calcium content in novel gallium (Ga_2O_3)-doped PBGs on the susceptibility of *Pseudomonas aeruginosa* is examined. The lack of new antibiotics in development makes gallium-doped PBG potentially a highly promising new therapeutic agent. The results show that an increase in calcium content (14, 15 and 16 mol.% CaO) cause a decrease in degradation rate (17.6, 13.5 and 7.3 $\mu g\ mm^{-2}\ h^{-1}$), gallium ion release and antimicrobial activity against planktonic *P. aeruginosa*. The most potent glass composition (containing 14 mol.% CaO) was then evaluated for its ability to prevent the growth of biofilms of *P. aeruginosa*. Gallium release was found to reduce biofilm growth of *P. aeruginosa* with a maximum effect (0.86 $\log_{10}$ CFU reduction compared to Ga_2O_3-free glasses) after 48 h. Analysis of the biofilms by confocal microscopy confirmed the anti-biofilm effect of these glasses as it showed both viable and non-viable bacteria on the glass surface. Results of the solubility and ion release studies show that this glass system is suitable for controlled delivery of Ga^{3+}. ^{71}Ga NMR and Ga K-edge XANES measurements indicate that the gallium is octahedrally coordinated by oxygen atoms in all samples. The results presented here suggest that PBGs may be useful in controlled drug delivery applications, to deliver gallium ions in order to prevent infections due to *P. aeruginosa* biofilms.

Keywords: Glass; Drug delivery; Microbiology; Antimicrobial; Biofilm

1. Introduction

Advances in medicine and surgery have led to increasing reliance on a variety of medical devices. However, the non-shedding surfaces of medical devices, such as catheters, frequently become colonized by members of the indigenous microbiota and opportunistic pathogens such as *Pseudomonas aeruginosa* which can cause hospital-acquired infections (HAIs). Many of the diseases caused by *P. aeruginosa* (such as airway infections in cystic fibrosis (CF) patients, chronic wound and sinus infections) appear to be associated with biofilm formation and are responsible for significant mortality [1–4]. Biofilm formation occurs as a result of a sequence of events: microbial surface attachment, cell proliferation, matrix production and detachment [5]. Biofilm-associated bacteria show a decreased susceptibility to antibiotics [6], disinfectants [7] and clearance by host defences [3,8]. A recent study has found that Ga^{3+} ions inhibit *P. aeruginosa* growth and biofilm formation in vitro by decreasing bacterial Fe uptake and interfering

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with Fe signalling via the transcriptional regulator *pvdS* [9]. Other studies have demonstrated that gallium is effective against the organisms causing tuberculosis [10] and malaria [11] in human beings, and in the treatment of pneumonia due to *Rhodococcus equi* in foals [12]. Further to this, we have recently shown that Ga_2O_3-doped phosphate-based glasses (PBGs) containing Ga^{3+} exhibit a potent antibacterial effect against planktonic bacteria including *P. aeruginosa*, methicillin-resistant *Staphylococcus aureus* and *Clostridium difficile* [13]. However, bacteria grown planktonically are known to be far more susceptible to antibacterial agents compared to their biofilm counterpart [14].

Ga_2O_3-doped PBGs are durable materials which can act as a unique system for the delivery of gallium ions in a controlled way [13]. Ions incorporated into the glass structures are not a separate phase, and thus their rate of release is defined by the overall degradation rate of the glass. In the past copper and silver have been incorporated into PBGs and have then been used as wound dressings to prevent infections [15] and also to control urinary tract infections in patients needing long-term indwelling catheters [15,16]. However, there is an underlying need to improve the properties of existing biomaterials due to the incidence of HAIs, which often lead to revision surgery and the growing resistance to antibiotics exhibited by these bacteria [17]. Despite the recent increase in the number of reported HAIs, there are very few new antibacterial drugs with an entirely new mechanism of action that has been introduced in the past three decades or are present in the advanced stages of development [18]. Our recent work showed the potential of Ga_2O_3-doped PBGs as a novel drug delivery system in combating bacteria associated with HAIs, especially *P. aeruginosa* [13]. However, in that study, it was found that increasing the gallium content of the glasses decreased the rate of degradation and subsequent release of gallium ions which highlighted the need to improve the controlled delivery of Ga^{3+} for antimicrobial applications.

The aim of the study reported here is to investigate the effect of increasing calcium content in Ga_2O_3-doped PBGs on their structure, properties and antibacterial activity against both planktonic cells and biofilms of *P. aeruginosa*.

2. Materials and methods

2.1. Preparation of Ga_2O_3-doped PBGs

PBGs were produced using NaH_2PO_4 (BDH, $\geqslant$98%), P_2O_5 (Sigma, $\geqslant$97%) and $CaCO_3$ (BDH, $\geqslant$98.5%). For the preparation of gallium-containing PBGs, Ga_2O_3 (Sigma, 99.99%) was also used, as shown in Table 1. The required amount of each reagent was weighed and added to a Pt/10% Rh crucible (Johnson Matthey, Royston, UK). The crucible was then placed in a preheated furnace at 1100 °C for 1 h, after which the molten glass was poured into graphite moulds, which had been preheated to 350 °C. The glass samples were allowed to cool to room temperature, and the resulting glass rods cut into discs by using a rotary diamond saw (Testbourne Ltd., Basingstoke, UK). Density measurements were conducted in triplicate on samples using Archimedes' principle.

Gallium-doped glasses of general composition $(CaO)_x(Na_2O)_{52-x}(P_2O_5)_{45}(Ga_2O_3)_3$, where $x = 14$, 15 and 16, hereafter given the abbreviations C14, C15 and C16 respectively, were prepared along with a sample containing no gallium, hereafter given the abbreviation Ga0, of composition $(CaO)_{16}(Na_2O)_{39}(P_2O_5)_{45}$.

2.2. Degradation study

Ga_2O_3-doped PBG rods (5 mm diameter and 2 mm thickness) with different CaO contents were put in plastic containers, filled with 50 ml of deionized water (pH 7 ± 0.5), and placed in an incubator at 37 °C. At various time points (6, 24, 48, 72 and 120 h), the three discs were taken out of their respective containers, and excess moisture removed by blotting the samples dry with tissue prior to weighing them. All the discs were put into a fresh solution of deionized water and placed back into the incubator. To obtain the rate of mass loss, the initial weight (M_0) of each sample was measured as well as the mass at time t (M_t) to give a mass loss per unit area thus: mass loss $= (M_0 - M_t)/A$, where A is the surface area (mm^2). The measurements were carried out in triplicate, and the weight loss per unit area plotted against time. The slope of this graph gives a degradation rate value in units of $mg\,mm^{-2}\,h^{-1}$, determined by fitting a straight-line of the form $y = mx$.

2.3. pH measurements

The pH measurements of the medium in which the glass discs had been soaked were taken at each time point (6, 24, 48, 72 and 120 h) using a Hanna Instruments pH 211 Microprocessor pH meter (BDH, UK) with an attached glass combination pH electrode (BDH, UK). The pH electrode was calibrated using pH calibration standards (Colourkey Buffer Solutions, BDH, UK).

Both dissolution studies and standards, for ion release study, were prepared using high purity water. This was obtained from a PURELAB UHQ-PS system (Elga Labwater, UK) with a purity level of 18.2 $M\Omega\,cm^{-1}$ resistivity.

2.4. Ion release study

Ion release studies were simultaneously conducted, and the medium was analysed for Na^+ and Ca^{2+} using ion chromatography (Dionex, UK). ICP-MS (inductively coupled plasma mass spectrometry, Spectromass 2000 by SPECTRO) was used to determine the amount of both gallium and phosphorus ions released from all glass compositions at the previously mentioned time points. The instrument was calibrated for the concentration range 0.1–1000 ppb by mixing single element standards obtained from Sigma and diluting in ultra pure water.

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