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In-vitro characterization of antibacterial bioactive glass containing ceria

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Abstract

Several compositions of bioactive glass (BG) containing ceria were synthesized from chloride precursor using quick alkali sol–gel method. XRD data revealed the presence of ceria in 5 and 10 mol% Ce samples. SEM and EDX characterization confirmed the nano-size and elemental composition of all samples, while FTIR data indicated that high Ce content has disrupted the silicate network of BG. UV absorption spectrum showed that ceria in BG samples is present in +3 and +4 oxidation states, depending on the initial cerium content. Nitrogen adsorption–desorption isotherm confirmed the mesoporosity of the samples. 5 and 10 mol% Ce samples exhibited significant antibacterial properties compared to 1Ce and 50Si samples. All samples induced the formation of apatite particles with Ca/P ratio close to 1.67 upon immersion in simulated body fluid (SBF), confirming their good bioactivity. For the first time, this study has demonstrated that cerium is a promising candidate to impart BG with excellent antibacterial properties without compromising its bioactivity.

Keywords: B. Electron microscopy; Rare earth biomaterials; Sol–gel growth; Nanostructures; Antibacterial properties

1. Introduction

Bioactive glass (BG) is a group of biomaterials with a silicate structure of chemical formula SiO_2, CaO with or without P_2O_5 [1,2]. Since its invention about 40 years ago, it has aroused great interest among material scientists because of its excellent bioactivity that leads to osteointegration [3]. Its bioactivity is characterized by the apatite forming ability on the surface upon immersion in physiological fluid [4]. This has rendered BG its wide use in bone regeneration field, for example periodontal disease [5], coatings of implants [6] and scaffolds [7]. Clinically, bacterial colonization or infections pose a serious threat to the usability of implants and often leads to their failure [8]. Although some BG have been reported to possess antibacterial properties, it is more advantageous to modify BG by incorporating some useful elements into its structure in order to control the release of these ions which are responsible for the antibacterial activity [9].

Cerium, a rare earth element has found numerous industrial applications such as catalysts, fuel additives and colored component doped in glass, however, its potential role in biomedical application has been undervalued. Cerium has been known to exist in different valencies in glass due to its interesting electronic structure, which can be investigated using UV spectroscopy. Cerium has been used in the treatment of severe burns; however, its antimicrobial potential is open to debate since contrasting *in vitro* results are always reported [10]. On the other hand, materials incorporated with Cerium have shown promising antimicrobial activities [11,12], possibly due to its ability to dissociate

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outer membrane of bacterial cells from cytoplasmic membrane [13]. Cerium in the form of ceria has been shown to exert toxicity toward bacterial cells through interaction with the cell membrane [14,15]. In previous study of Ce doped BG no CeO_2 phases were detected in the XRD even though CeO_2 was directly mixed in BG [16]. In another study the preparation of Ce doped BG from cerium (III) nitrate also showed no presence of CeO_2 phases, which led them to conclude that the Ce existed as Ce_2O_3 in BG [17], however, phase attributed to Ce_2O_3 was not detected in their XRD. To best of our knowledge, there is still no report of antibacterial effects of BG containing ceria. Therefore, in this study, *in vitro* bioactivity and antibacterial response towards *Escherichia coli* via quantitative method are studied for BG containing ceria synthesized from chloride precursor.

2. Material and methods

2.1. Materials

All chemicals used for sol–gel synthesis were reagent-grade, tetraethyl orthosilicate (TEOS, Fluka, Switzerland), calcium nitrate tetrahydrate ($Ca(NO_3)\cdot 4H_2O$, QREC, New Zealand), triethyl phosphate (TEP, Fluka, Switzerland) and cerium chloride heptahydrate ($CeCl_3\cdot 7H_2O$, QREC, New Zealand). 2 M ammonia solution and 2 M nitric acid were prepared by using 37% ammonia solution (NH_4OH, QREC, New Zealand) and 65% concentrated nitric acid (HNO_3, Fluka, Switzerland). Ethanol (CH_3CH_2OH, Fluka, Switzerland) and deionized water were used as solvents in this investigation.

2.2. Sol–gel synthesis

Quick alkali mediated sol–gel method [18] was used to synthesize BG containing ceria. TEOS was mixed with 2 M nitric acid to undergo acid hydrolysis for 1 h. This was followed by the addition of TEP, $CaCO_3.4H_2O$, and $CeCl_3\cdot 7H_2O$ with 30 min interval between each addition to allow each reagent to react completely. Finally, the whole mixture was stirred for 1 h to obtain a clear sol. Excess NH_4OH (2 M) solution was added to the resulting solution in an ultrasonic water bath until gelation occurred [19]. The formed gel was dried at 75 °C for 48 h followed by calcinations at 700 °C for 2 h with heating rate 5 °C/min in a muffle furnace. Different compositions of BGs are listed in Table 1

Table 1
Nominal composition of BG.

Sample code	Composition (mol%)			
	SiO_2	CaO	P_2O_5	CeO_2
50Si	50	45	5	
1Ce	50	44	5	1
5Ce	50	40	5	5
10Ce	50	35	5	10

2.3. Characterization techniques

2.3.1. X-ray diffraction

Phase composition of synthesized BGs was studied using X-Ray Diffractometer (XRD, Bruker, D8 Advance) at 40 kV and 30 mA utilizing CuKα radiation. The range of 2θ angles was from 20° to 80°, at a step size of 0.02°and step time of 1 s.

2.3.2. UV-NIR spectroscopy

Ultra violet–visible absorption spectra were measured for all samples in the 200–800 nm spectral range, by using the diffuse reflectance technique (UV–vis, Shimadzu, UV-3101PC).

2.3.3. Fourier transform infrared spectroscopy

The infrared spectra of the samples were recorded in a wavenumber range of 450–4000 cm^{-1} using a Fourier Transform Infrared spectrophotometer (FTIR, Jasco, FT/IR-6100).

2.3.4. Low vacuum scanning electron microscope and energy dispersive X-ray spectroscopy

The morphology and composition of calcined BG samples were studied using SEM (SEM, JEOL, JSM-6390) at an operating voltage of 15 kV and Energy Dispersive X-ray Spectrometer (EDX, Hitachi, SwiftED3000) respectively. Before examination, samples were coated with platinum at 20 mA for 40 s. To evaluate particle size distribution, measurements of particles size were made on 100 random locations in SEM image using image analysis software ImageJ (National Institutes of Health, USA).

2.3.5. Textural characterization

N_2 adsorption and desorption isotherms were obtained at 77 K on a Quantachrome Autosorb 1 sorption analyzer. All samples were outgassed for 16 h at 200 °C under high vacuum in the degas port of the adsorption analyzer. The specific surface area of the prepared samples was calculated from the N_2 adsorption isotherms using the multipoint Brunauer–Emmett–Teller (BET) technique. Textural pore size distribution and the mean pore diameter were derived using the Barrett–Joyner–Halenda (BJH) method. Total pore volumes were estimated from the adsorbed amount of N_2 at relative pressure of 0.995.

2.3.6. Antibacterial tests

Antibacterial properties of all samples were investigated using the quantitative viable count method. The stock solution was prepared by mixing 1 mL *E. coli* with 9 mL of LB (Luria-Bertani) broth and incubated at 37 °C for 24 h with shaking at 250 rpm. 0.1 g BG powder was autoclaved and mixed with the stock solution. 0.1 mL of the prepared mixture was then inoculated on LB agar plates followed by incubation at 37 °C for 24 h. Finally, the number of colony-forming units were counted. The tests were carried out in triplicate. Student's *t*-test was used to evaluate the statistical significance amongst the data.