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Characterization, in vitro bioactivity and biological studies of sol-gel synthesized SrO substituted 58S bioactive glass

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ABSTRACT

Bioactive glasses (BGs) are considered as a high potential candidate in bone repair and replacement. In the present study, sol–gel derived BGs based on 60% SiO_2-(36%-x) CaO-4%P_2O_5-x SrO (where x = 0, 5 and 10 mol%) quaternary system were synthesized and characterized. The effect of Sr substitutions on bioactivity, proliferation, alkaline phosphatase activity of osteoblast cell line MC3T3-E1 and antibacterial activity were investigated. Dried gels were stabilized at 700 °C to eliminate the nitrates and prevent the crystallization of bioactive glasses. X-ray diffraction, Fourier transform infrared spectroscopy and scanning electron microscopy results confirmed the formation of hydroxycarbonate apatite on the BG surfaces. The 3-(4,5dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and alkaline phosphate activity results showed that 5% SrO increased both differentiation and proliferation of MC3T3-E1 cells, while 10% SrO resulted in a decrease in bioactivity. Live/Dead and DAPI/Actin staining exhibited viable cell and the morphology of actin fibers and nuclei of MC3T3 cells treated with BG-0 and BG-5. The result of antibacterial test showed that strontium substituted 58S BG exhibited antibacterial effect against methicillin-resistant Staphylococcus aureus bacteria. Taken together, results suggest that 58S BG with 5 mol% SrO is a good candidate for bone tissue engineering with maximum cell proliferation and ALP activity, good bioactivity and high antibacterial efficiency.

1. Introduction

Bioactive glasses (BGs) are a class of biomaterials, generally based on amorphous silicate compositions, which are developed and used as bone grafts or fillers in the field of bone repair and regeneration [1–3]. BGs can bond to living tissues because of the precipitation of a carbonated hydroxyapatite (HAC) layer on their surface in contact with body fluids and have been clinically utilized as hard tissue regenerative materials in orthopedics treatments [4,5].

The synthesis of the BGs by the sol–gel method provides a flexible low temperature route that provides better compositional control and higher homogeneity and purity in comparison with conventional melt quenching route [4–6].

In recent years several studies have been conducted on the synthesis [7], in vitro dissolution rate [8] and characterization [9] of the sol–gel derived bioactive glasses in the system of SiO_2–CaO–P_2O_5 such as 45S5 bioglass and 58S bioglass.

The osteoconduction and antibacterial properties may be enhanced by incorporation of metallic ions in bioactive glasses [3,10–13]. For instance, strontium-doped BG decreases osteoclast activity and enhances osteoblast proliferation and activity [14,15] as well as increase antibacterial activity [16].

Strontium is an alkaline earth metal that plays a similar role as calcium in bone formation [17]. Previous animal studies revealed that Sr has a potential to use in osteoporosis treatment because of its ability to increase the bone formation and prevent osteoporosis [9,18]. Moreover, the presence of Sr in BG composition (Sr-BG) can inhibit the formation of the calcium phosphate (Ca-P) layer on their surfaces [19], but has a stimulatory effect on the proliferation and differentiation of osteoblasts [20,21].

The effect of Sr incorporating in BG composition on formation of the apatite layer and in vitro bioactivity has not been elucidated exactly. Some reports show that Sr can enhance the bioactivity by formation of a new bone and reduction of the bone resorption [22–29]. However, other reports indicate that Sr can decrease bioactivity by inhibiting the formation of the calcium phosphate layer [19,21,28,30–32] or inhibiting the crystallization of the hydroxyapatite [33,34].

On the other hand, Christi et al. recently reported that substitution of CaO with SrO in strontium-containing phosphate

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glasses, causes little or no change in the dissolution rate of and that the bioactivity remains constant [35].

Another unclear issue is the optimal amount of substituted SrO for CaO in BG composition which has a positive effect on cell proliferation and activity in BGs. Previous reports show a range of SrO substitution for CaO for a variety of bioglasses which caused cell proliferation; e.g., 2.9 mol% [36], 2.5 and 5 mol% [37], 10 mol% [26,38] and even 20 mol % [39].

Orthopedic implant infections are considerable due to their morbidity and usually involve the removal or replacement of implanted biomaterials [40]. A bacterial infection can potentially lead to surgical failure due to a delay in the process of wound healing [41]. Therefore, Anti-infective biomaterials need to be designed according to the specific clinical application [42]. Staphylococcus aureus are common bacterial. Methicillin-resistant Staphylococcus aureus (MRSA), a type of staphylococcus, has been associated with preventable hospital-acquired infections, which are resistant to the antibacterial activity of methicillin and other related antibiotics of the penicillin class [43].

The aim of this study was to elucidate the effect of SrO substituting for CaO on the bioactivity, biocompatibility, alkaline phosphatase and antibacterial activity of the sol-gel derived 58S-BG and to suggest the optimal content of SrO in BG-58S composition to achieve the highest cell proliferation and activity as well as antibacterial efficiency against MRSA. For this purpose, we synthesized the sol-gel derived strontium-containing glasses with a composition range of 0–10% SrO. Moreover, the effect of strontium on the reactivity of Sr-BGs was studied in vitro by immersing samples in SBF and observing the change of the morphology and the structure of Sr-BGs due to formation of hydroxycarbonate apatite (HCA) on their surfaces. Inductively coupled plasma atomic emission spectrometry (ICP-AES), Fourier transform infrared (FT-IR), X-ray diffraction (XRD) and scanning electron microscopy (SEM) were used for characterization of the formed HCA. Also, Live/Dead and DAPI/Actin staining techniques were performed in order to qualitatively assess cell viability and visualize the morphology of actin fibers and nuclei of MC3T3 cells in presence of strontium, respectively. Finally, based on the in vitro bioactivity, cell proliferation, ALP and antibacterial activity results, the optimal SrO content in the bioactive glass composition was reported.

2. Materials and method

2.1. Materials

Tetraethylorthosilicate (TEOS: $Si(OC_2H_5)_4$), triethylphosphate (TEP: $PO(OC_2H_5)_3$), calcium nitrate $Ca(NO_3)_2$,$4H_2O$ and strontium nitrate $Sr(NO_3)_2$ were used as a source of silicon, phosphorus, calcium and strontium, respectively. Moreover, NaCl, KCl, $K_2HPO_4{\cdot}3H_2O$, $MgCl_26H_2O$, $CaCl_2$, Na_2SO_4 reagents, tris (hydroxymethyl) aminomethane $(HOCH_2)_3CNH_2$, and HCl were used for preparing SBF solution. All the reagents were purchased from Merck Company (Darmstadt, Germany).

A mouse osteoblast-like cell line, MC3T3-E1, was purchased from Sigma-Aldrich (Poole, UK) for in-vitro biological investigation. Cells cultured at 37 °C in a humidified, 5% CO_2, 95% air atmosphere in α-MEM supplemented with 10% fetal bovine serum (FBS), (Sigma-Aldrich, UK), 1% antibiotic, 2 mM glutamine and 0.1% penicillin-streptomycin.

2.2. Bioactive glass synthesis

Bioactive glasses in the SiO_2–CaO–P_2O_5–SrO system were prepared using the sol-gel method. CaO was partially replaced by the SrO 0–10% on molar basis; Table 1).

Distilled water was mixed with 0.1 M HNO_3 for 15 min at room temperature using magnetic stirrer. Then TEOS added into the solution and stirred at room temperature for 1 h. TEP, $Ca(NO_3)_2{\cdot}4H_2O$ and $Sr(NO_3)_2$ were added in sequence and reacted for 45 min intervals. The final mixture was stirred for 60 min to complete the hydrolysis process. The prepared sol was poured into Teflon container and kept sealed at 37 °C for 3 days and dried at 75 °C for 24 h. The dried gel was then calcined in a furnace at 700 °C for 3 h to eliminate the nitrates and organic substances.

Table 1
Elemental compositions of the various Sr-BGs (in mol%).

Glass	Label	SiO_2 mol%	CaO mol%	P_2O_5 mol%	SrO mol%
58S-0% SrO	(BG-0)	60	36	4	0
58S-5% SrO	(BG-5)	60	31	4	5
58S-10% SrO	(BG-10)	60	26	4	10

The final product was ground into a fine powder using a zirconia planetary ball mill (Retsch, Germany) with a final particle size of below 50 μm. Finally, disc-shaped samples (Ø10×3 mm) were prepared for in vitro evaluation by forming the powders with a hydraulic press under 9 MPa pressure.

2.3. Preparation of SBF

The standard SBF solution was prepared according to the procedure described by Kokubo [44]. Reagent-grade sodium chloride (NaOH), potassium chloride (KCl), sodium bicarbonate ($NaHCO_3$), magnesium chloride hexahedrate ($MgCl_2{\cdot}6H_2O$), calcium choloride ($CaCl_2$), and monopotassium phosphate (KH_2PO_4) dissolved in distilled water and buffered at pH = 7.25 with TRIS (tris hydroxymethyl aminomethane) and hydrochloric acid (HCl) 1 N at 37 °C.

2.4. Characterization of BGs

2.4.1. Thermal analysis

The differential thermal (DTA) and thermogravimetric (TGA) analyses were done for BG-0 and BG-10 to determine the stabilization temperatures for dried gels. DTA/TGA curves were obtained in a Shimadzu TGA-50 apparatus, under a nitrogen atmosphere starting from room temperature up to 1100 °C with the heating rate of 10 °C/min.

2.4.2. X-ray diffraction analysis

The surface of the bioactive glasses were studied before and after immersion in SBF by X-ray diffraction (XRD, INEL-Equinox-3000, France) with a Cu-Kα radiation source (λ = 1.5405 A°) operating at 40 kV in a 2θ range of 20–50°.

2.4.3. FTIR analysis

The BG sample surfaces were characterized before and after soaking in SBF by Fourier transform infrared spectroscopy (FT-IR, Nicolet Avatar 660 (Nicolet, USA)). For this purpose, 1 mg of material scarped from BG surface was mixed with 100 mg of spectroscopy grade KBr and palletized under vacuum. Then the pellets were analyzed in the range of 400–4000 cm^{-1} with a resolution of 8 cm^{-1}.

2.4.4. Inductively Coupled Plasma-atomic emission spectroscopy

For in vitro study, samples were soaked in the SBF at 37 °C for 1, 3, 7 and 14 days and remained in an incubator at 37 °C. Before the immersion in the SBF, every sample was washed in pure acetone, rinsed with distilled water and air-dried. The ratio of disc surface area to solution volume of the SBF was 0.1 cm^{-1}. At the end of each time period, samples were taken out from the SBF, gently rinsed with distilled water and dried at room temperature. All the reacted solutions were saved for inductively coupled plasma atomic emission spectroscopy (ICP-OES; Varian Vista Pro, Palo Alto, USA) analysis of Ca, Si, P and Sr to measure ionic concentration in the SBF solutions.