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Enhanced bioactivity, biocompatibility and mechanical behavior of strontium substituted bioactive glasses

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ABSTRACT

Strontium contained biomaterials have been reported as a potential bioactive material for bone regeneration, as it reduces bone resorption and stimulates bone formation. In the present investigation, the bioactive glasses were designed to partially substitute SrO for SiO_2 in Na_2O–CaO–SrO–P_2O_5–SiO_2 system. This work demonstrates that the substitution of SrO for SiO_2 has got significant benefit than substitution for CaO in the bioactive glass. Bioactivity was assessed by the immersion of the samples in simulated body fluid for different intervals. The formation of hydroxy carbonate apatite layer was identified by X-ray diffractometry, scanning electron microscopy (SEM) and energy dispersive spectroscopy. The elastic modulus of the bioactive glasses was measured and found to increase with increasing SrO for SiO_2. The blood compatibility of the samples was evaluated. In vitro cell culture studies of the samples were performed using human osteosarcoma U2-OS cell lines and found a significant improvement in cell viability and proliferation. The investigation showed enhancement in bioactivity, mechanical and biological properties of the strontia substituted for silica in glasses. Thus, these bioactive glasses would be highly potential for bone regeneration.

1. Introduction

A significant challenge is posed for regeneration of large size bone defects generally caused due to infections, trauma, accidents, tumors or genetic malformations in the human body. It necessitates the need for effective materials of bone regeneration and tissue engineering capability. Multifunctional bioactive glasses offer an excellent opportunity in delivering therapeutic ions like strontium (Sr) [1–3], a trace element in the human body. Sr has been found to exert anabolic and anti-catabolic effects on bone metabolism. It is beneficial for biological applications, specially for the formation of healthy bone growth which stimulates the bone formation and reduces the resorption having favorable effects on osteogenesis and angiogenesis [1,4–8]. The amount of strontium in human bone is typically only 3.5% of its calcium content. The majority of the absorbed Sr is localized in bone, particularly at regions of high metabolic turnover [9]. Potential applications of Sr have been reported for treatment of osteoporosis and regaining of bone mass [10–15]. Bioactive glasses are able to form interfacial bonds with living organisms of hard and soft tissues by their degradation in physiological solutions and formation of a stable hydroxyl-carbonate apatite (HCA) layer on the glass surface [16,17]. The SiO_2–Na_2O–CaO–P_2O_5 glass system has higher bioactivity in comparison to HA [18,19]. The bioactive glass (45S5 Bioglass®) has been studied extensively and is being used as bone regenerative material in orthopaedic and dental applications [16,17]. Further, it was reported that the strontium substituted bioactive glasses are superior biomaterial in comparison to 45S5 Bioglass® [4,20,21]. Hence, it authenticates that strontium being an ideal element is positive for substitution in bioactive glasses. However, many reports mentioned that substitution of SrO for CaO retards the formation of HA in simulated body fluid (SBF) and there is lack of a detailed mechanical behavior analysis of those bioglasses (BG). For treatment of large bone defects, mechanical properties need to be considered essentially [22]. A biomaterial must be able to withstand against mechanical stress caused during surgical handling and post implantation to complete HA transformation. Thus, expediting the patient's recovery and improving the clinical consequences. Network connectivity (NC) can be used to predict a number of properties, such as structural, chemical, mechanical, bioactivity and biological [23–25]. NC depicts the average number of bridging oxygen (BOs) atoms per glass forming elements in the glass structure. In general, the glass NC tends to decrease with increasing the concentration of modifiers.

It is still a challenge to develop new biomaterials which would develop HA quickly for providing adequate mechanical strength. Many research reports are available on SrO substitution for CaO in bioactive glasses [8,21,26–30]. To the best of our knowledge, the substitution of SrO for SiO_2 in a bioactive glass has never been reported earlier. The

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new compositions show a significant effect on the glass density and network connectivity and which will have immense effect on bioactivity, cytocompatibility and mechanical behavior. The present authors systematically report the in vitro bioactivity and cell cultural studies (viability, proliferation and cell apoptosis) as well as mechanical behavior of the bioactive glasses.

2. Materials and methods

2.1. Formulation of bioactive glass composition

Four bioactive glass compositions were formulated in a five component system (Na_2O–CaO–SrO–P_2O_5–SiO_2) along with the 45S5 Bioglass® was also prepared for comparison as shown in Table 1. The bioactive glass (Sr-1) was prepared as a reference samples where SrO substituted for CaO and which is very close to Donnell et al. glass composition (Sr7.5) [4]. The new formulations containing partially substituted SrO for SiO_2 on the molar basis. Network Connectivity (NC) was calculated on the basis of general Eq. (1) [23,31] assuming that SiO_2 form the network structure in the glass whereas, P_2O_5 remains in orthophosphate phase.

$$NC = \frac{4 \text{ x } SiO_2 + 6 \text{ x } P_2O_5 - (2 \text{ x CaO} + 2 \text{ x SrO} + 2 \text{ x } Na_2O)}{SiO_2} \quad (1)$$

2.2. Preparation of the bioactive glasses

The bioactive glasses shown in Table 1 were prepared by melting analytical reagent grade calcined quartz (purity 99.9%), sodium carbonate (99.5%), calcium carbonate (98%), strontium carbonate (99.5%) and ammonium dihydrogen orthophosphate (99%) as a source of SiO_2, Na_2O, CaO, SrO and P_2O_5, respectively. The weighed batches were mixed for 30 min in an agate mortar and pestle and melted in a platinum crucible at 1400 °C for 2 h in an electrical furnace. In order to ensure homogeneity, the glass melts were taken out of the furnace, poured on a preheated aluminum plate, cooled, crushed and re-melted in the furnace for an another period of 2 h. The bulk glass samples were annealed in a pre-heated furnace at 450 °C and after 1 h of annealing, the furnace was cooled to room temperature. The bulk glass samples were cut and polished into required dimensions. The polished glass samples were ultrasonically cleaned in an acetone bath. The densities of the glasses were determined by ASTM B962-14 method.

2.3. Assessment of bioactivity in SBF

The bioactivity of the samples was assessed by immersing in SBF. The SBF was prepared according to Kokubo et al. [32] method having inorganic ion concentrations similar to those of human body fluid. The SBF was prepared at 37 °C by dissolving analytical reagent grade NaCl, KCl, $NaHCO_3$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$ and KH_2PO_4 into double distilled water and it was buffered at pH = 7.4 with TRIS (trishydroxy methyl aminomethane) and 1N HCl. The powdered samples (<75 μm) were immersed in SBF in sterilized plastic container and incubated for 7 days at 37 °C. The pH behavior of SBF was recorded continuously for 7 days using a pH meter (Universal Bio-microprocessor, India). After immersion, the samples were filtered, rinsed with double distilled water and dried in an electric oven at 100 °C for 2 h. The glass powder was examined by X-ray diffractometry (Rigaku, Miniflex II, Japan) to observe the HA formation. A thin glass sheets of the samples were immersed in SBF for 7 days and they were dried, gold sputtered and examined by SEM (Inspect S50, FEI) as well as energy dispersive spectroscopy (EDS) (Oxford Instrument, X-act, Germany) for surface morphology and elemental analysis, respectively.

Table 1
Chemical composition of the bioactive glasses (mol%).

Glass code	SiO_2	P_2O_5	CaO	Na_2O	SrO	NC
Sr-0 (45S5)	46.10	2.60	26.90	24.40	0.00	2.11
Sr-1	46.10	2.60	24.22	24.40	2.00	2.11
Sr-2	45.10	2.60	26.90	24.40	1.00	2.03
Sr-3	44.10	2.60	26.90	24.40	2.00	1.94
Sr-4	43.10	2.60	26.90	24.40	3.00	1.84

2.4. Mechanical properties

The Young's, shear and bulk moduli of the polished bulk glass samples were determined by ultrasonic measurement gauge (45MG, Olympus, USA) according to our previous publication [33]. Briefly, the ultrasonic wave velocities were recorded as longitudinal (VL) and transverse wave (VT). The velocities of sound wave propagated in the polished bioactive glass samples were measured using ultrasonic pulse-echo technique. The test was performed using two transducers as one was V112 for longitudinal wave (10 MHz) and another was V156 for the transverse wave (5 MHz). The elastic properties such as Young's modulus (E), shear modulus (S) and bulk modulus (K) were calculated. Five samples from each group were measured and the mean and standard deviation were calculated.

2.5. Biological evaluation

2.5.1. Cell lines and cell culture

Human osteosarcoma cell U2-OS was purchased from American Type Culture Collection (ATCC), Manassas, USA. The cells were maintained in RPMI 1640 (Invitrogen, Carlsbad, CA), supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), 100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen, Carlsbad, CA), henceforth, called as complete medium. The cell line used in the study was free from mycoplasma.

2.5.2. In-vitro cell viability assay

Effect of Sr-contained bioactive glasses (Sr-1, Sr-2, Sr-3 and Sr-4) on the viability of tumor cells was evaluated by a colorimetric XTT (sodium 3-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzene sulfonic acid hydrate) assay (Roche Molecular Biochemicals, Indianapolis, IN). Tumor cells were plated (5×10^3 cells/well) in a 96-well plate and exposed to serial concentrations of strontium (5, 10, 25, 50, 100, 250 and 500 μM) contained bioactive glasses (Sr-1, 2, 3, and 4) and incubated at 37 °C and 5% CO_2 for 18 h. Optical Density was measured at 450 nm using Synergy HT Multi-Mode Micro plate Reader BioTek, USA [34]. The data was presented as the percentage of viable cell calculated from the following Eq. (2):

$$\%\text{Cell Viability} = \frac{\text{Experimental OD450}}{\text{Control OD450}} \times 100 \quad (2)$$

2.5.3. Cell proliferation assay

Growth inhibitory potential of strontium contained bioactive glass samples against the tumor cells were studied by MTT assay. In a 96-well tissue culture plate, 5×10^3 cells/well were added and exposed to Sr-contained samples. Plates were incubated at 37 °C and 5% CO_2 for 48 h. The cell proliferation was measured by Cell Titer 96 Non-Radioactive Cell Proliferation Assay (MTT) Kit from Promega, USA. The data was presented as the percentage of inhibition of tumor cells and it was calculated from the following Eq. (3):

$$\%\text{Growth Inhibition} = \left[1 - \frac{\text{Experimental OD570}}{\text{Target OD570}}\right] \times 100 \quad (3)$$