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# Synthesis, physico-chemical and biological characterization of strontium and cobalt substituted bioactive glasses for bone tissue engineering

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ABSTRACT

Due to their vast potential for repair and regeneration, different types of bioactive glasses (BGs) have been widely studied for bone tissue engineering. In this study, different groups of melt-derived bioactive glasses containing strontium ($Sr^{2+}$) and cobalt ($Co^{2+}$) ions have been designed to investigate their potential effect on increasing cell osteogenic activity. After full characterization of the synthesized bioactive glasses, they were evaluated for apatite forming ability in simulated body fluid (SBF) after different time intervals. The glasses have been examined for cell attachment and cell cytotoxicity plus their influence on osteogenic activity of the cells was analyzed by alkaline phosphatase assay (ALP) and alizarin red staining. The results show that the samples are in glassy state before immersion in SBF and an apatite-like layer has formed on the surface of SBF-immersed samples after 3, 7 and 14 days. *In vitro* experiments demonstrated that the incorporation of $Sr^{2+}$ and $Co^{2+}$ in the glass composition significantly promote osteogenic activity of human osteosarcoma cells without any cytotoxicity effect.



## 1. Introduction

Based on the worldwide spread of bone injuries and disorders, bone regeneration has become increasingly important. It is predicted that prevalence of bone defects requiring treatment will double by 2020, due to an aging population, accidents, congenital genetic abnormalities and obesity [1]. Traditional approaches including autograft, allograft and xenograft are coupled with many restrictions such as donor site morbidity, rejection and disease transmission [2,3]. Therefore, many types of natural and synthetic biomaterials have been proposed as bone substitutes to reconstruct and restore the native function [4–6]. Among the different biomaterials, bioactive glasses have gained much attention for bone tissue engineering applications due to their superior advantages, such as osteoinductivity and osteoconductivity over conventional bioceramics [7–12]. Today bioactive glasses are used in various applications as a bone blocks, cements and bone void fillers for treatment of disorders such as fractures, bone cysts and tumors [13–15].

In recent years, various inorganic ionic species are added to the initial composition of bioactive glasses ($SiO_2$, CaO, $Na_2O$, $P_2O_5$) to further improve their biological properties, such as better angiogenesis (blood vessel formation) and accelerating wound healing [16]. Among ionic species, strontium ($Sr^{2+}$) is one of the most attractive ions used to improve osteogenic activity of BGs. This metal ion has been used for many years for treatment of osteoporosis, in the form of Strontium Ranelate (SrR) [17–20]. In several prior studies, it has been shown that $Sr^{2+}$ has a significant role in increasing bone density and reducing fracture risk in humans and other mammals [21–24]. $Sr^{2+}$ ions also accelerates bone regeneration through a binary approach in which deposition of new bone increased by osteoblasts and simultaneously resorption of bone is reduced by osteoclasts [25,26]. Furthermore,

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angiogenesis plays a key role in expediting new bone formation, which is regulated by several growth factors (such as vascular endothelial growth factor) and especially conditions (such as Hypoxia) [27]. To induce angiogenesis through hypoxia, several studies have been conducted *in vitro* and *in vivo* [28–31]. Cobalt ($Co^{2+}$) as a metal ion is frequently considered for induction hypoxia condition. It has been previously documented that $Co^{2+}$ induces hypoxia through up-regulation of HIF-1α and vascular endothelial growth factor (VEGF) genes in primary osteoblasts and osteoblastic cell lines [32–36]. Meanwhile, it has been shown that the HIF-1 pathways accelerate bone regeneration by increasing angiogenesis, stem cell differentiation, and fracture repair [20,36]. As a result, it has been suggested that $Co^{2+}$-releasing bioactive glasses can simulate a hypoxic condition which is then used for activation angiogenesis-related genes and thereby promoting bone tissue regeneration [29,30,37,38]. Previously, Azevedo et al. [31] synthesized $Co^{2+}$-containing bioactive glass particles with focus on angiogenesis. Although recent studies provide new information about the effect of $Sr^{2+}$ and $Co^{2+}$ *in vitro* and *in vivo*, the influence of the simultaneous use of these two ions on structural, physico-chemical and biological activity of glasses is still not well understood. Therefore, the main goal of this study was to synthesize bioactive glasses using these two ions in the composition for the improvement of biological and osteogenesis activities. In addition, more multicomponent BGs may inhibit the crystallization of the glasses and facilitate the fabrication of these glasses into porous scaffolds by viscous flow sintering.

## 2. Materials and methods

### 2.1. Glass synthesis

The BGs were synthesized based on a multi-component $SiO_2$–$P2O_5$–CaO–SrO–$Na_2O$-MgO-ZnO-$K_2O$ system using a melt–quench approach. All of the components were purchased as analytical grade (Sigma-Aldrich, UK). The components were melted in a platinum–rhodium crucible for 1.5 h at 1400 °C in an electric furnace (Lenton, Hope Valley, UK). After melting, the glasses were immediately quenched rapidly into water to prevent crystallization. The dried glasses were ground using a vibratory puck mill (Gyro Mill, Glen Creston, London, UK) for 15 min and sieved to a particle size below 38 μm. The different groups were named as Ca, Sr, Ca-Co and Sr-Co and their compositions are given in Table 1.

### 2.2. Glass characterization

#### 2.2.1. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) was used to evaluate glass transition temperature of the samples. For this purpose, the glass powder samples (50 mg) were placed in an inert platinum crucible. An analytical grade alumina powder was used as a reference material. The experiments were carried out in air, using a Stanton-Redcroft DSC 1500 (PL Thermal Sciences, Epsom, UK) at a constant heating rate of 20 K $min^{-1}$ up to a maximum temperature of 1050 °C.

#### 2.2.2. X-ray diffraction (XRD)

The crystal structure of the samples was determined by X-ray diffraction (XRD) (Philips PW1700 series Automated Powder Diffractometer). The samples were evaluated before and after immersion in simulated body fluid (SBF) fusing an X-ray powder diffractometer equipped with a monochromatized Cu-Kα radiation (Θ = 1.54056 Å) in the 2Θ range [39] with a step size of 0.04°.

**Table 1**
The composition and transition glass (Tg) temperatures of the synthesized bioactive glass samples (mol%).

| Sample | $SiO_2$ | $P_2O_5$ | CaO | SrO | $Na_2O$ | MgO | $K_2O$ | CoO | $Tg_1$ (°C) | $Tg_2$ (°C) |
|---|---|---|---|---|---|---|---|---|---|---|
| Ca | 41.2 | 5.06 | 36.14 | 0 | 7.17 | 3.26 | 7.17 | 0 | 590 | 670 |
| Sr | 41.2 | 5.06 | 30.14 | 6 | 7.17 | 3.26 | 7.17 | 0 | 580 | 645 |
| Ca-Co | 41.2 | 5.06 | 35.64 | 0 | 7.17 | 3.26 | 7.17 | 0.5 | 585 | 670 |
| Sr-Co | 41.2 | 5.06 | 29.64 | 6 | 7.17 | 3.26 | 7.17 | 0.5 | 580 | 650 |

**Table 2**
Ion concentrations of the prepared SBF compared to the human blood plasma (volume: 1000 ml, pH: 7.2–7.4).

| Ion | Plasma (mmol/l) | SBF (mmol/l) |
|---|---|---|
| $Na^+$ | 142.0 | 142.0 |
| $K^+$ | 5.0 | 5.0 |
| $Mg^{+2}$ | 1.5 | 1.5 |
| $Ca^{+2}$ | 2.5 | 2.5 |
| $Cl^-$ | 103.0 | 147.8 |
| $HCO_3^-$ | 27 | 4.2 |
| $HPO_4^{-2}$ | 1.0 | 1.0 |
| $SO_4^{-2}$ | 0.5 | 0.5 |

#### 2.2.3. FTIR analysis

To analyze the samples before and after soaking in SBF, the dried powders were analyzed using Fourier-transform infrared spectroscopy (FTIR) (NICOLET IS10 FT-IR SPEC, Thermofisher, USA). The FTIR spectra were recorded from 400 to 4400 $cm^{-1}$ with a resolution of 4 $cm^{-1}$.

#### 2.2.4. Microscopic observations

The shape and surface morphology of the glasses and also the formation of hydroxyapatite layer on the surface of the samples after soaking in SBF solution was evaluated using scanning electron microscope (SEM) (Tescan, Vega ts5136MM, CZ). The samples were coated before microscopy by a gold layer.

#### 2.2.5. Particle size analysis

The particle size distribution of the powders was determined using a laser particle size analyzer device (Mastersizer 2000, Malvern Instruments, UK).

### 2.3. Bioactivity assessment

For bioactivity assessment of the samples, a simulated body fluid (SBF) was prepared according to the method described by Kokubo and Takadama [40]. Briefly, 75 mg of each sample was immersed in 50 ml of the SBF solution and incubated at 37 °C on an orbital shaker (KS 4000i control, IKA, Germany) at a constant agitation of 60 RPM for 8 h, 3, 7 and 14 days. At the end of each time period, pH was measured and the solutions filtered through medium porosity filter paper (5 μm particle retention, Whatman, USA). Finally, the glass powders treated in SBF were rinsed with acetone (Merck, Germany) to stop any further reaction. Any precipitation or changes in the content was observed during the preparation and preservation of this solution. Table 2 shows the amount of various chemicals used to SBF preparation.

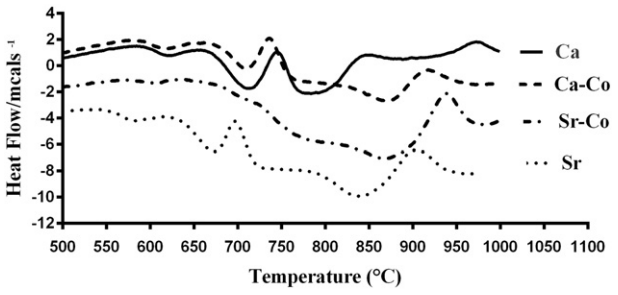


**Fig. 1.** Glass transition temperature (Tg) of the BGs determined by DSC analysis. A slight delay is visible in the Sr-containing glasses (Sr and Sr-Co).