



Synthesis, characterization and *in vitro* study of magnetic biphasic calcium sulfate-bioactive glass



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ABSTRACT

Calcium sulfate-bioactive glass (CSBG) composites doped with 5, 10 and 20 mol% Fe were synthesized using quick alkali sol–gel method. X-ray diffraction (XRD) data of samples heated at 700 °C revealed the presence of anhydrite, while field emission scanning electron microscopy (FESEM) and energy dispersive X-ray (EDX) characterization confirmed the formation of nano-sized CSBGs. The UV–vis studies confirmed that the main iron species in 5% Fe and 10% Fe doped CSBGs were tetrahedral Fe(III) whereas that in 20% Fe doped CSBG were extra-framework FeO_x oligomers or iron oxide phases. Measurement of magnetic properties of the samples by vibrating sample magnetometer (VSM) showed very narrow hysteresis loop with zero coercivity and remanence for 10% Fe and 20% Fe doped CSBG, indicating that they are superparamagnetic in nature. All samples induced the formation of apatite layer with Ca/P ratio close to the stoichiometric HA in simulated body fluid (SBF) assessment.

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1. Introduction

Calcium sulfate (CS) has been used as a synthetic bone graft for more than 100 years [1]. It has also been used to treat periodontal disease [2], alveolar bone loss [3], maxillary sinus augmentation [4], endodontic surgery [5] as well as distal radial fracture [6]. CS possesses good biocompatibility and undergoes complete in-vivo resorption with minimal inflammatory response [7]. On the other hand, bioactive glass (BG) has attracted numerous interests among biomaterial scientists due to its excellent ability to bond to live hard tissue [8]. Its structure is composed of SiO₂ as main network, P₂O₅ as partner network, and also some network modifiers such as CaO [9]. It is highly bioactive as it easily induces apatite formation on the surface upon immersion in physiological fluid. This has rendered wide application to BGs in bone regeneration field, such as implant coatings [10], bone fillers [11] and scaffolds in tissue engineering [12].

Cancer is characterized by uncontrolled growth of cells. Once a single cell mutates to become cancerous, it divides uncontrollably at an accelerated rate to form tumors. Tumors will compete with the healthy tissue for nutrients from the blood stream and gradually displace them [13]. Eventually, the function of organ and in turn the body is compromised. To grow further, new blood cells will be formed around the tumor to provide nutrients in a process called angiogenesis. The problem worsens when cancerous cells spread to other parts of the body from their origin through a process called metastasis [14]. More than 20 million new cancer cases and 13.2 million deaths related to cancer are

expected by 2030 [15]. Therefore, a tremendous amount of scientific resources have been directed towards combating this fatal disease. Currently, the gold standard treatments for cancer are chemotherapy and radiation therapy. However, researchers are constantly developing new methods to improve efficiency of therapy; this includes additive treatment such as hyperthermia.

Hyperthermia involves providing heat to the local environment of the cancerous tissues. When the local temperature reaches 43 °C, cancerous cells die, whereas healthy cells survive. Cancer cells are characterized by poorly developed nervous system and have lesser blood vessels. They generally perish around 43 °C due to stasis, hemorrhage and vascular occlusion. On the other hand, normal healthy cells are only damaged above 45 °C. Therefore, hyperthermia treatment is expected to be most effective between 43 and 45 °C [16]. Furthermore, commendable synergistic effects can be attained by combining radiotherapy or chemotherapy with hyperthermia. For instance, cells in the hypoxic (low oxygen) and low pH areas tend to develop radioresistance, making radiotherapy ineffective. Hyperthermia can increase radiosensitivity of tumor cells because it can cause an increased blood flow, thus improving tissue oxygenation [17]. Such blood flow increase is known to enhance intracellular drug uptake, improved DNA damage of the cancerous cells and higher intratumor drug concentrations in chemotherapy. Many anticancer drugs e.g. doxorubicin, mitoxantrone, cisplatin etc. have shown enhanced results in the combined therapy [17]. Methods to increase local temperatures include ultrasound, electromagnetic radiation (microwaves, radiowaves) and hot water [18]. The use of magnetic materials remains a promising method in the area of hyperthermia, especially with the rapid advancement of nanotechnology [19].

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Biomaterials incorporated with iron can be applied in a patient's body in order to treat bone cancer. When the magnetic BG is subjected to an alternating magnetic field, the heat is generated due to magnetic hysteresis loss [20]. To ensure that the optimum temperature is safely reached, several factors must be considered which include suitable intensity of magnetic field, volume and composition of material used as thermoseed, as well as the distance from the thermoseed to the affected zone [21]. However, it should be noted that the incorporation of iron oxide in BG has been found to reduce apatite forming ability [22]. Therefore, in this study calcium sulfate which promotes faster bone formation due to more rapid resorption [23] was selected to synergize with BG. Here, a simple sol–gel synthesis was used to synthesize magnetic biphasic composite nanoparticles consisting of CS and BG.

2. Experimental

2.1. Materials

All chemicals used for sol–gel synthesis were reagent-grade, which included tetraethyl orthosilicate (TEOS, Fluka, Switzerland), calcium nitrate tetrahydrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, QREC, New Zealand), triethyl phosphate (TEP, Fluka, Switzerland), iron (II) sulfate (FeSO_4 , QREC, New Zealand) and iron (III) chloride (FeCl_3 , QREC, New Zealand). The 2 M ammonia solution and 2 M nitric acid were prepared from 37% ammonia (NH_3 , QREC, New Zealand) and 65% concentrated nitric acid (HNO_3 , Fluka, Switzerland). Ethanol ($\text{CH}_3\text{CH}_2\text{OH}$, Fluka, Switzerland) and deionized water were used as solvents in this investigation.

2.2. Sol–gel synthesis of biphasic calcium sulfate-bioactive glass (CSBG)

Quick alkali sol–gel method was used to synthesize CSBG [16]. Around 10 mL of TEOS was mixed with water/ethanol solution before few drops of 2 M nitric acid were added to undergo acid hydrolysis for 1 h. This was followed by the addition of TEP, FeSO_4 , FeCl_3 and $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ with a 30 min interval. The resulting mixture was stirred further for 1 h to obtain a clear sol. The ratio of number of moles for $\text{Fe}^{2+}:\text{Fe}^{3+}$ was always maintained at 1:2 (Table 1). Excess NH_3 (2 M) solution was added slowly to the resulting reaction mixture in an ultrasonic water bath until gelation occurred [17]. The formed gel was dried at 75 °C for 48 h followed by calcinations at 700 °C for 2 h with heating rate of 5 °C/min in a muffle furnace. Different compositions of BGs prepared in this study are listed in Table 1.

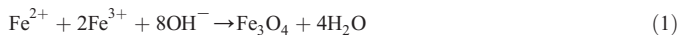
2.3. Characterization

Phase composition of synthesized CSBGs was studied using X-ray diffractometer (XRD, Bruker, D8 Advance) at 40 kV and 30 mA utilizing $\text{CuK}\alpha$ radiation. The range of 2θ angles was from 20 to 80°, at a step size of 0.02° and step time of 1 s. Diffuse reflectance UV–vis (DRUV–vis) spectra of CSBGs were recorded in the range of 200–800 nm under ambient conditions on a Perkin Elmer Lambda 900 UV/VIS/NIR spectrometer using barium sulfate (BaSO_4) as reference. The functional groups of the CSBG powder samples were identified by Fourier transform infrared spectrophotometer (FTIR, Jasco, FT/IR-6100), using the KBr pellet technique. The infrared spectra were recorded in a wavenumber range of 450–4000 cm^{-1} in transmission mode with 32 scans and resolution

of 4 cm^{-1} . The morphology and composition of calcined CSBG samples were studied using field emission scanning electron microscopy (FESEM, JEOL, JSM-6710F) at an operating voltage of 15 kV and energy dispersive X-ray spectrometer (EDX, Hitachi, SwiftED3000) respectively. To evaluate particle size distribution, measurements of particles size were made at 100 random locations in FESEM images using image analysis software ImageJ (National Institutes of Health, USA). The magnetic properties of all samples were studied using vibrating sample magnetometer (VSM, DMS, model 8810) under ambient conditions. The applied external magnetic field was in the range of -30 k Oe to $+30$ k Oe. The *in vitro* bioactivity of samples was evaluated by immersing them in SBF solution prepared according to the method reported by Kokubo et al. [9]. The samples were immersed in SBF solution for 3, 7 and 14 days. The scanning electron microscope (SEM, Hitachi, TM3000) at an operating voltage of 15 kV and EDX were used to study the morphology and the Ca/P ratio of the apatite formed on the surface of CSBG samples respectively.

3. Results and discussion

Calcium sulfate–BG (CSBG) composites containing varying amount of iron (5, 10 and 20 mol% Fe) were synthesized in this study. Nominal compositions of the synthesized BGs are listed in Table 1. The formation of magnetic iron oxide is shown in Eq. (1) [24]. Ammonia, which acted as a base was added to an aqueous mixture of Fe^{2+} and Fe^{3+} at a 1:2 molar ratio to furnish a magnetic phase of iron oxide.



Agglomeration of spherical particles having a mean diameter of 31, 36 and 60 nm was observed for 5Fe, 10Fe and 20Fe respectively (Fig. 1). The particle sizes as well as their size distribution became bigger and wider in range with increasing contents of Fe. Fig. 2 shows the elemental composition obtained from EDX analysis of Fe doped CSBG. The intensity of peak due to Fe increased with the increased doping concentration, confirming higher degree of metal substitution in the prepared BG as intended. The homogeneity of elemental distribution for representative sample was confirmed by the EDX elemental mapping analysis (Fig. 3).

The XRD patterns of all prepared samples are shown in Fig. 4. The XRD of 20Fe contained distinct diffraction peaks at 25.44°, 31.37°, 38.64°, 40.82°, 43.33°, 45.45°, 48.67°, 52.23°, 62.24°, 65.44° and 66.97° which were respectively assigned to the (0 2 0), (0 1 2), (2 0 2), (2 1 2), (1 3 1), (1 0 3), (0 3 2), (0 4 0), (4 1 2), (0 2 4) and (2 1 4) planes of anhydrite calcium sulfate [JCPDS File #37-1496]. Furthermore, a peak only observed for 20Fe at around 35° was assigned to the (3 1 1) plane of magnetite, Fe_3O_4 [24]. No peaks due to the silicate crystalline phases were observed because the phase transition temperature for BG is generally above 800 °C [9]. The XRD results revealed that the prepared samples were composite of crystalline CaSO_4 phase and amorphous BG phases. The formation of CaSO_4 involved the reaction between sulfate ions from iron (II) sulfate and calcium ions from calcium nitrate during the synthesis (Eq. (2)). The combination of CaSO_4 and BG is known to promote faster *in vivo* bone formation [23].



The UV–vis spectrum of all Fe doped CSBG samples contained an absorption band at 200–300 nm and 400–600 nm (Fig. 5). The intense peak at 200–300 nm was attributed to the $d\pi\text{--}\pi\pi$ charge transfer between Fe and O, indicating that iron was bound to oxygen atoms (Si–O–Fe) in the form of Fe(III) cations isolated in a tetrahedral environment [25]. The peaks at 200–300 nm for 5Fe and 10Fe increased in intensity because Fe in these samples mainly existed in ionic form and thus formed part of the silicate network whereas Fe in 20Fe was present in the form of iron oxide, which was also supported by XRD data shown in Fig. 4. Small band observed at around 470 nm was assigned to

Table 1
Nominal composition of samples.

Sample code	Composition (mol%)				
	SiO_2	CaO	P_2O_5	SO_4^{2-}	$\text{Fe}^{2+}/\text{Fe}^{3+}$
50Si	50	45	5	0	0
5Fe	50	38.33	5	1.67	5
10Fe	50	31.67	5	3.33	10
20Fe	50	18.33	5	6.67	20

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