Materials Science and Engineering C 68 (2016) 350–357

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Fabrication and characterization of strontium incorporated 3-D bioactive glass scaffolds for bone tissue from biosilica

Ali Can Özarslan, Sevil Yücel *

Department of Bioengineering, Faculty of Chemistry and Metallurgy, Yildiz Technical University, Esenler, 34210, Istanbul, Turkey

ARTICLE INFO

Article history:
Received 3 March 2016
Received in revised form 7 May 2016
Accepted 1 June 2016
Available online 2 June 2016

Keywords:
Bioactive glass
Rice hull ash
Scaffold
Silica
Strontium

ABSTRACT

Bioactive glass scaffolds that contain silica are high viable biomaterials as bone supporters for bone tissue engineering due to their bioactive behaviour in simulated body fluid (SBF). In the human body, these materials help inorganic bone structure formation due to a combination of the particular ratio of elements such as silicon (Si), calcium (Ca), sodium (Na) and phosphorus (P), and the doping of strontium (Sr) into the scaffold structure increases their bioactive behaviour. In this study, bioactive glass scaffolds were produced by using rice hull ash (RHA) silica and commercial silica based bioactive glasses. The structural properties of scaffolds such as pore size, porosity and also the bioactive behaviour were investigated. The results showed that undoped and Sr-doped RHA silica-based bioactive glass scaffolds have better bioactivity than that of commercial silica based bioactive glass scaffolds. Moreover, undoped and Sr-doped RHA silica-based bioactive glass scaffolds will be able to be used instead of undoped and Sr-doped commercial silica based bioactive glass scaffolds for bone regeneration applications. Scaffolds that are produced from undoped or Sr-doped RHA silica have high potential to form new bone for bone defects in tissue engineering.

1. Introduction

Artificial extracellular matrix (ECM) which mimics the properties of three-dimensional tissue structure and supports the new tissue formation is called scaffold [1]. These artificial structures should have three main properties; (i) biocompatibility, (ii) biodegradability, (iii) bioactivity [2]. Likewise, pores are necessary for scaffolds [3], porosity and pore size both are important morphological properties at the macroscopic and the microscopic level for bone regeneration [4]. Scaffolds should have an extremely porous structure to allow cell proliferation, adhesion [5], infiltration and diffusion of fluids-nutrients [4]. The aforementioned excellent properties of them make them an essential component for tissue engineering applications. Scaffolds are produced by a significant number of different methods as solvent casting & particulate leaching, gas foaming, thermally induced phase separation, polymer foam replication, scaffold coating using polymer, ceramics (bioactive glasses) etc. [6–12].

Bioactive glasses were first produced by Hench in 1969 [13] with the general formula (wt%: $45SiO_2$–$24.5CaO$–$24.5Na_2O$–$6P_2O_5$) [14]. Bioactive glasses are fabricated by sol-gel method or melt-derived method [15] which can be effectively used in bone tissue engineering as a scaffold material thanks to their biocompatibility, biodegradability and bioactivity [16]. An attractive feature of bioactive glass is that it can easily bond to bone tissue in either bulk or particular form via formation of hydroxyapatite (HA) on the surface layer (because of silica (SiO_2) formation interface) [17] without any inflammatory effects. Additionally, bioactive glasses support enzyme activity [18,19] and vascularization [20,21]. When they dissolve, the elements regulate gene expression that controls osteogenesis and growth factors [22]. These materials gain more biofunctional properties thanks to the features of some trace elements such as silver “Ag” (antibacterial), copper “Cu” and “Sr” (osteogenesis) [23]. Sr is also a doping material for scaffolds that are made of bioactive glass and improves its properties such as bioactivity.

Strontium shows similar chemical and biological behaviour to calcium [24]. Besides, Sr is a bone-seeking element, which is correlated with bone compressive strength improves the bone density and stimulates bone formation like Ca [25]. Sr can also be substituted with Ca as the atomic radius of (1.16 Å) Sr is larger than atomic radius of (0.94 Å) Ca [26], Ca-Sr substitution causes lattice expansion [27]. As a result, the solubility of the bioglass changes in biological fluids [28]. In addition, if the quantity of Sr increases in the composition of bioactive glass, silica network becomes more polymerized and this results in more solubility and bioactivity [29]. Some studies showed that Sr-containing ceramics have higher dissolution rate in SBF, and these studies showed a more pronounced HA layer formation than undoped ceramics [14,30]. Previous studies reported that the best ratio is 4% among various ratios of Sr such as 1, 2, 3, 4(%) because of high bioactivity [31]. Various bioactive

Abbreviations: UDC, undoped commercial silica-based; UDRHA, undoped RHA silica-based; Sr-DC, 4% Sr-doped commercial silica-based; Sr-DRHA, 4% Sr-doped RHA silica-based.

* Corresponding author.
E-mail addresses: alicanozarslan@gmail.com (A.C. Özarslan), syucel@yildiz.edu.tr (S. Yücel).

http://dx.doi.org/10.1016/j.msec.2016.06.004
0928-4931/

glass compositions have been studied and developed. One of them is in a (wt%: 44.08SiO_2–24Na_2O–21.60CaO–4.43SrO–5.88P_2O_5) system that is called stone bone [31].

Various agricultural wastes contain silica in high amounts [32–35]. The most important one is rice hull. Silica is obtained through a process that starts with the burning of rice hull and continues with the alkaline extraction of burnt rice hull that is called rice hull ash (RHA). There are different studies using RHA-based silica [36]. RHA-based silica slightly contains some trace elements such as Cu (angiogenesis and osteogenesis) [37], iron-"Fe" (bioactivity enhancer) [38], magnesium-"Mg" (proliferation enhancer) [35]. These elements provide several biological and chemical features to materials that are produced from RHA-based silica [39].

The purpose of this study is to use RHA, which is rich in silica (about 60%) as a cheap alternative source of amorphous silica, to manufacture bioactive glass and to fabricate polymer/composite RHA-based silica bioactive glass scaffold. It aims to prepare and characterize three-dimensional tissue scaffolds for bone regeneration by using commercial silica-based bioactive glass, Sr-doped commercial silica-based bioactive glass and RHA silica-based bioactive glass, Sr-doped RHA silica-based bioactive glass. Doping ratio of Sr was chosen as 4% (wt) in accordance with the reviewed literature. The effects of RHA silica on bioactivity and biodegradation and the effect of Sr doping on scaffolds were investigated. The results were compared with those of commercial silica.

2. Materials and methods

2.1. Materials

Polyurethane (PU) sponges (porosity; 60 PPI) were supplied from Urosan Chemical Industry. Silicon dioxide (SiO_2) was obtained from Riedel-deHaën and gelatin from AppliChem (Analytical grade, ≥99%). Polyvinyl alcohol (MA = 72,000 g/mol), di-sodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$), calcium carbonate ($CaCO_3$), sodium bicarbonate ($NaHCO_3$) were purchased from Merck (Analytical grade, ≥99%) and strontium oxide (SrO) was purchased from Sigma Aldrich (Analytical grade, 99.9%). Rice hull ash was supplied from Yetis Food Industry. The ash is composed of 97.9% Si, 1.5% Na and 0.6% minor elements (Ca, Mg, Fe, K, Cu, Al) after acid leaching.

2.2. Sodium silicate solution and silica production

Rice hull ash was burned at 600 °C in muffle furnace for 5 h. The obtained 40 g ash was added in 240 mL distilled water and pH of the solution was decreased to 1 with hydrogen chloride (HCl). The solution was boiled for 1 h and then filtered. Acid leached ash was mixed with 240 mL sodium hydroxide (NaOH) for 1 h and filtered. Thus, sodium silicate solution was obtained. Silica gel was produced by sodium silicate solution upon treatment with strong acid. Obtained silica gel was aged for 18 h and subsequently washed three times and then dried overnight in drying oven. After drying, silica was ground and silica powder was obtained [39].

2.3. Bioactive glass production

Bioactive glasses were produced with four different compositions (Table 1). A mixture consists of SiO_2 (RHA-based or commercial) and other chemical components which are $CaCO_3$-$Na_2HPO_4 \cdot 2H_2O$-$NaHCO_3$-SrO used in melt-quenching technique for the production of bioactive glasses.

Prepared mixtures were put in platinum pots and kept at 1400°C in a muffle furnace (Protherm, TURKEY) for 1 h. The melted bioactive glasses were cast into cold water. Solid and cold bioactive glasses were ground and put again in platinum pots. Bioactive glasses were kept again at 1450 °C in a muffle furnace for 2 h. At the end of 2 h, samples were cast again onto the counter and annealed at 550 °C for 24 h in a furnace. Thus, bioactive glasses were obtained [39].

2.4. Scaffold production

Scaffolds were manufactured by using bioactive glass powders with polymer foam replica method [14]. Briefly, bioactive glasses were ground. After that, PU sponges (1 cm × 1 cm × 1 cm) were immersed in a slurry obtained by mixing 0.7 g powder (particle size; <90 μm) and PVA binder. Burning and sintering process were performed at 600 °C and 950 °C, respectively, at a heating rate of 2 °C/min. Finally, obtained scaffolds were coated with gelatin using 2% (*w*/*v*) gelatin solution obtained by mixing gelatin powder in distilled water at 37 °C for 1 h. Scaffolds were immersed in gelatin solution and dried overnight in an oven at 37 °C.

2.5. Characterization of produced scaffold samples

The presence of chemical functional groups in scaffolds, before and after SBF immersion, was evaluated by FT-IR (Shimadzu) with an ATR (attenuated total reflection) unit, in the range of 4000 cm^{-1}–650 cm^{-1}. Data were collected in transmittance mode.

In this study, porosity percentage was calculated by using the following equation (Eq. 1):

$$P\% = \left[1 - \left\{\left(W_1 / \rho_{solid}\right) + \left(W_2 - W_1 / \rho_{gelatin}\right)\right\} \Big/ V\right] \times 100 \quad (1)$$

where P% is total porosity (volume, %), W_1 and W_2 are the weights of the scaffolds before and after coating with gelatin, respectively. V is the volume of the scaffolds, $\rho_{gelatin}$ is the density of gelatin ($\rho_{gelatin}$ = 1.2 g cm^{-3}) and ρ_{solid} is the theoretical density of 45S5 Bioglass® (ρ_{solid} = 2.78 g cm^{-3}) [40,41].

Previously, structural characterization of different scaffolds was studied using SEM image analysis. In this study, pore sizes were measured by SEM image analysis and determined according to F2450 and F2883 ASTM Standards.

The morphology of scaffolds, before and after SBF immersion, was analyzed with scanning electron microscope (SEM, Zeiss Evo® Ls 10) images. Scaffolds were coated with gold prior to the SEM analysis by using a Sputter Coater device (Emitech K 550X).

X-ray diffraction patterns of scaffolds were obtained by XRD device (Rigaku Smartlab). Acceleration voltage and current used in the analysis were 40 kV and 30 mA, respectively. 2θ diffraction angle range was 2°–100° with a step of 0.01° and a scanning rate of 0.5°/min.

Each scaffold was put in falcon tubes at 37 ± 1 °C containing SBF prepared by the method of Kokubo et al. [42]. For degradation and water absorption studies, initial weight (W_i) of all scaffolds were measured

Table 1
Compositions of prepared bioactive glass samples.

Prepared bioactive glasses	SiO_2, wt%	CaO, wt%	Na_2O, wt%	P_2O_5, wt%	SrO, wt%
Undoped commercial silica-based	45	24.5	24.5	6	0
Undoped RHA silica-based	45	24.5	24.5	6	0
4% Sr-doped commercial silica-based	45	20.5	24.5	6	4
4% Sr-doped RHA silica-based	45	20.5	24.5	6	4