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Fabrication of a novel nanostructured calcium zirconium silicate scaffolds prepared by a freeze-casting method for bone tissue engineering

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Abstract

In this research mechanical activation derived nanostructured calcium zirconium silicate($Ca_3ZrSi_2O_9$ or Baghdadite)-based scaffolds were fabricated by a water-based freeze casting method. By varying the solid loading of the mixture and the cooling rate, a range of structures with different pore sizes and strength characteristics were achieved and the effects of cooling rate and solid loading on pore sizes and mechanical characteristics of scaffolds were studied. Increasing the solid loading from 12.5% to 20% (v) led to a decrease in porosity and an increase in the compressive strength for both cooling rates. In addition, with the increase in cooling rate at the constant solid loading, parameters of linear shrinkage and also porosity decreased whereas strength increased significantly. According to the obtained mechanical results, the best mechanical properties were achieved when the scaffold was prepared at cooling rate and solid loading of 4 °C/min and 20% (v), respectively, (E=59.8 MPa and σ= 2.1 MPa).

Keywords: Baghdadite; Bioceramics; Scaffold; Freeze-casting method

1. Introduction

Nano-crystalline materials with an average crystalline size of a few nanometers have been of much interest to many investigators [1–3]. Nanomaterials exhibit increased strength/hardness, enhanced diffusivity, improved ductility/toughness, reduced density, reduced elastic modulus, increased specific heat, etc. They have high potentials for use in structural and device applications in which enhanced mechanical and physical properties are required [4–6].

Aging of population together with the prolonged life expectancy bring forth the increasing demand for artificial materials with ability to regenerate/replace diseased or lost bones [7–10]. Over the past 20 years, calcium silicate (Ca–Si) based ceramics have been introduced as an ideal bone graft substitute due to their bioactivity [10–12], mechanical properties [13,14] and ability to stimulate bone growth. However, main drawback of the calcium silicate bioceramics is their chemical instability [10,14]. Chen et al. [15] demonstrated that quadrivalent anions such as Si and Ti may improve the stability of material which is attributed to the ability of such anions to form an ionic-binded network with Ca ions. Ramaswamy et al. [16] have pioneered incorporation of Zr ions –as a quadrivalent anion– into the Ca–Si system and developed a stable Ca–Si based material.

It has been demonstrated that incorporating Zr ions into a Ca–Si system will develop the Baghdadite ceramic which did not have any toxic effects on osteoblasts and it could be used for bone tissue engineering [16]. Based on our knowledge, up to now, there are only a few reports on the synthesis and biological response of Baghdadite. Roohani-Esfahani et al. [14] and Ramaswamy et al. [16] synthesized Baghdadite powders by a sol–gel method using zirconia oxide nitrate ($ZrO(NO_3)_2$), calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$) and tetraethyl orthosilicate ($TEOS,(C_2H_5O)_4Si$) as raw materials with potential applications in bone tissue regeneration. Liang et al. [17] prepared $Ca_3ZrSi_2O_9$ powder using a high-temperature solid-state reaction and then the powder was

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sprayed onto Ti–6Al–4V substrate and it was found that this coating possessed both excellent chemical stability and good apatite-formation ability.

Although there are numerous fabrication techniques for manufacturing porous bioceramics [18–20], only a few of them provide pore interconnectivity which is as essential as porosity for bone in-growth, especially in the early stages of bone regeneration and penetration in the scaffold [21,22]. Recently, freeze casting process has been introduced as an alternative approach to produce porous bioceramics [23,24]. This has been established to be an attractive manufacturing method as it enables construction of reticulated porous ceramics on a finer scale and without the polymer burnout stage.

In this study, Baghdadite powder was synthesized by a mechanical activation method and a porous calcium zirconium silicate based scaffold with aligned and interconnected porosity was produced by freeze casting. In order to optimize the scaffold manufacturing process, pertinent factors namely cooling rate and solid loading on mechanical properties and their structure were investigated. Also, in order to study the biological response of these scaffolds, in vitro evaluations were conducted.

2. Materials and methods

2.1. Synthesis of Baghdadite powder

Appropriate amounts of silica gel, zirconium oxide and calcium oxide (all purchased from Merck) were mixed to adjust the Ca:Si:Zr molar ratio to 3:2:1 which corresponds to the stoichiometric composition of pure Baghdadite. The powder mixture was milled in planetary ball mill for 20 h with 300 rpm. The milling media consisted of a zirconia vial and balls with ball to powder ratio of 10. Synthesized powders were characterized by simultaneous thermal analysis (STA). Then milled powders were calcinated at 950 °C for 3 h at a heating rate of 1 °C/min to form Baghdadite phase. Synthesized powders were characterized by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and transmission electron microscopy (TEM).

2.2. Scaffold fabrication

In order to prepare Baghdadite's suspension, Baghdadite powder and deionized water were mixed at two different solid loading conditions (12.5% and 20% w/v) (Table 1). Adequate amount of Dolapix P62 dispersant (3 wt% of solid component) was added to the samples and in order to obtain a homogenous suspension, samples were stirred for 20 min by a magnetic stirrer. To provide an initial strength to the scaffold, 5 wt% of PVA was added to the rotating samples. The suspensions were degassed in a vacuum desiccator for 10 min to remove possible trapped air bubbles after blending.

In order to study the effect of cooling rate, 5 mL of the prepared suspension was poured into a cylindrical polyethylene mold with 10 mm diameter. The mold was directly in contact with copper plate (cooling part of the apparatus) and its temperature was controlled by liquid nitrogen. Suspensions were cooled at two different cooling rates (1 and 4 °C/min) (Table 1). It is worthy to note that the suspension was thoroughly frozen at −45 °C then sample was separated from the mold and cooled at −55 °C in a freeze dryer (vacuum pressure of 2.1 Pa) for 72 h. The scaffolds were heated to 300 °C in an electrical furnace at the heating rate of 1 °C/min and kept for 1 h. This heating process continued by sintering at 600 °C at the same rate. After that, the temperature elevated to 1350 °C at 2 °C/min and remained for 3 h then sample was cooled to room temperature.

Table 1
Levels of different parameters for fabrication of Baghdadite's suspension.

Solid loading (%v)	Cooling rate (°C/min)
12.5	1
	4
20	1
	4

2.3. Powder characterization

2.3.1. X-ray diffraction

Phase analysis was performed by an X-ray diffractometer (Philips, model PW3710). This instrument worked with voltage and current settings of 40 kV and 30 mA, respectively. A Cu-Ka radiation was also utilized for Baghdadite powder (1.54 Å). For qualitative analysis, XRD diagrams were recorded in the interval $20^\circ \leq 2\theta \leq 60^\circ$ at the scan speed of 2°/s.

2.3.2. Simultaneous thermal analysis

In order to evaluate the thermal behavior of Baghdadite powder, simultaneous thermal analysis was performed on the powder including thermogravimetry (TG) and differential thermal analysis (DTA) using a thermo-analyzer (Netzsch, model STA409PC/PG, Germany) working in the temperature range of ambient temperature to 1200 °C with the heating rate of 10 °C/min.

2.3.3. Transmission electron microscopy

Transmission electron microscopy (Philips, model GM200 PEG, Netherland, working at 200 kV) was used for observing the morphology of Baghdadite. For this purpose, the particles were deposited on Cu grids, which support a carbon film by deposition from a dilute suspension in ethanol.

2.3.4. Fourier transform infrared spectroscopy analysis

In order to determine the functional groups of the synthesized powder FTIR analysis was used. FTIR spectra of the synthesized powders were recorded in the frequency range of 400–4000 cm^{-1}.

2.4. Scaffold characterization

2.4.1. Scanning electron microscopy (SEM)

The pores and walls thickness morphology of the scaffolds were observed using scanning electron microscopy (Stereoscan,