Materials Letters 167 (2016) 157–160

Contents lists available at ScienceDirect

Materials Letters

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

Preparation of nanostructure bioactive diopside scaffolds for bone tissue engineering by two near net shape manufacturing techniques

Hamed Ghomi [a,*], Rahmatollah Emadi [a], Shaghayegh Haghjooye Javanmard [b]

[a] Biomaterials Research Group, Department of Materials Engineering, Isfahan University of Technology, Isfahan 84156-83111, Iran
[b] Physiology Research Center, Department of Physiology, Isfahan University of Medical Sciences, Isfahan 81746-73461, Iran

ARTICLE INFO

Article history:
Received 14 August 2015
Received in revised form
23 November 2015
Accepted 29 December 2015
Available online 30 December 2015

Keywords:
Bioceramics
Porous materials
Diopside
Scaffold
Space holder technique
Gelcasting

ABSTRACT

Diopside has been recently investigated as a bioceramic with high mechanical and bioactivity properties, excellent biocompatibility, and appropriate degradation rate. This study has focused on fabrication of novel nanostructure diopside scaffolds by two near net shape manufacturing techniques (space holder and gelcasting methods) and comparison of their properties. The obtained scaffolds were characterized by means of X-ray Diffraction, transmission electron microscopy, scanning electron microscopy, Archimedes method, image analysis, and compression tests. The results demonstrated that both the space holder and gel casting methods led to fabrication of highly porous scaffolds with 82–85% total porosity. The prepared scaffolds were highly interconnected and had appropriate pore size (200–800 μm) and compressive strength (0.98 ± 0.11–1.33 ± 0.13 MPa). Therefore, the prepared nanostructure diopside scaffolds would be appropriate structure for use as bone substitute in bone tissue engineering.

1. Introduction

One of the significant challenges in bone tissue engineering, is the fabrication of highly porous scaffolds ($>80\%$) with interconnected pores and appropriate mechanical strength. Therefore most studies on the scaffolds are focused on two categories. The first group focused on different manufacturing methods and the effective parameters on each process to improve the scaffold properties, and the others focused on scaffold materials to obtain better properties by changing the type (different materials) and structure (micro or nano) of materials or combining materials together (composite materials) [1–6].

Among the materials used for tissue engineering scaffolds, bioactive silicate ceramics such as diopside have been paid much attention in recent researches. According to the literature, diopside has high mechanical and bioactivity properties, excellent biocompatibility, and appropriate degradation rate [7–9]. In addition to, it is recognized that the degradation products of diopside in physiologic fluids, such as silicon, calcium, and magnesium, could promote the proliferation and differentiation of osteoblast cells [9–11]. Despite the benefits mentioned for diopside, there are few articles about building diopside scaffold and most articles have focused on making powder and dense bulk diopside.

Up to now, various techniques have been developed to fabricate tissue engineering scaffolds [4]. In this study, due to the benefits of nanostructured materials, including high surface to volume ratio and faster interaction with biological environment [4–5], efforts were focused on the production of nanostructured diopside scaffolds by using gelcasting and space holder methods and the comparison of the properties of the prepared scaffolds.

2. Experimental

2.1. Preparation of diopside powder and scaffolds

Sol–gel method was used to synthesize nanostructure diopside powder. Appropriate amounts of $Ca(NO_3)_2 \cdot 4H_2O$, $MgCl_2 \cdot 6H_2O$, and $(C_2H_5O)_4Si$ (TEOS) were dissolved in ethanol as a solvent and stirred to form a wet gel solution. The gel after drying was calcined at 800 °C for 2 h. Finally to ensure the preparing of nanoparticle powder, the powder were milled for 10 h (rotational speed of 250 rpm, ball/powder ratio of 5/1) [12–14].

For preparing diopside scaffolds by using space holder method, the appropriate weight ratio of prepared nano powder to NaCl particles (420–600 μm in size) as the spacer was selected to obtain final porosity about 85% and homogeneously mixed together with an amalgamator. The calculated values of the homogeneous mixture were compressed with the applied pressure of 50 MPa in a cylindrical die by universal testing machine (HOUNSFIELD: H50KS) for 2 min. After sintering the compacted samples at 1200 °C for

* Corresponding author.
E-mail addresses: hamed1985.gh@gmail.com, hamed.ghomi@ma.iut.ac.ir (H. Ghomi).

http://dx.doi.org/10.1016/j.matlet.2015.12.161
0167-577X/

150 min, the NaCl particles were removed by immersing the samples in deionized water for 24 h.

For preparing diopside scaffolds by using gelcasting method, appropriate amount of prepared powder, by means of 1 wt% sodium polyacrylate as a dispersant, was dispersed in distilled water to reach a slurry with of 60 wt%. solid loading. The prepared slurry was foamed by severe agitation using a mechanical stirrer at 80 °C with the addition of 1.2 wt% agarose as a gelling agent and 1.5 vol% Tergitol as a surfactant. After casting the highly foamed slurry into the mold, the gelling reaction was conducted by reducing the temperature to 0 °C and the solidifying body was dried in the mold for several days. After de-molding, the green body was further dried at 100 °C in an oven for 24 h and sintered at 1200 °C for 150 min.

2.2. Characterization of diopside powder and scaffolds

The diopside powder and scaffolds were characterized using X-ray diffraction (XRD) to determine their phase structure and grain size. The particle size of the prepared nanostructure diopside powder was characterize by transmission electron microscopy (TEM). The pore size and morphology of the scaffolds were examined using scanning electron microscope (SEM). The pore size distribution in the scaffolds was measured using the image analysis software. The compression tests were performed on cylindrical samples with a ratio of height to diameter of 1.5 using a Hounsfield H50KS universal testing machine at a crosshead speed of 0.5 mm/min. The results were reported based on an average of five scaffolds. The mean values of total and interconnected porosities of the scaffolds were measured according to Archimedes method [15].

Furthermore, the values of total porosity was calculated with the Eq. (1) for checking the results obtained by Archimedes method:

$$\text{Total porosity} = \left[1 - (\rho_{\text{scaffold}}/3.26)\right] \times 100 \quad (1)$$

where 3.26 is the true density of the diopside and ρ_{scaffold} was calculated by dividing the dry weight to volume of the scaffolds.

3. Results and discussion

Fig.1 shows the XRD patterns of the nanostructure diopside powder and scaffolds fabricated by using gelcasting and space holder methods. Only diopside peaks were detected by XRD in the patterns of prepared diopside powder and scaffolds (JCPD 01-086-0932) denoting a pure diopside phase. The measured value of grain size by Williamson–Hall equation for the diopside powder was about 40 ± 3 nm. This value for diopside scaffolds was increase to about 47 ± 2 which is due to sintering the scaffolds at high temperature and releasing the strain.

TEM micrograph of prepared diopside nanopowder that could be used for observation of the shape and size of the powder is shown in Fig.2. The powder particles had frequently spherical shape with sizes less than 50 nm.

In this study the sol–gel processing in organic solvent led to control the structure of diopside on nano-sized scale from the initial stages of processing. In sol–gel method, the formation of integrated 3D network (gel) by either discrete particles arrangement or simultaneous hydrolysis and polymerization of organometallic precursors such as TEOS, provides the possibility to obtain the nanostructured materials in the form of thin film or powder [16]. In nonaqueous sol–gel processes, the slow reaction rates of organic solvents in combination with their stabilizing effect offer the better control of the reaction pathways, enabling the synthesis of highly crystalline nanomaterials with uniform particle morphologies [17]. Furthermore the final ball milling process ensure the production of nanoparticles diopside.

SEM micrographs of the prepared diopside scaffolds are shown in Fig.3. According to SEM micrographs, the prepared scaffolds by space holder method had a uniform pore size distribution (400–600 μm) and a spherical pore morphology. On the other hand, the scaffolds prepared by gelcasting method showed a wide range of pore size distribution (200–800 μm) and an irregular pore morphology. Furthermore, both the scaffolds apart from the macrospores, which provide the potential for tissue ingrowth, exhibit a large amount of microspores which enhance bioactivity and release of ionic products [18]. According to the literature, a porous structure with pore sizes in the range of 150–900 μm allows for nutrient supply and waste removal of cells grown on the scaffold. In addition to, microspores with sizes less than 10 μm are needed for capillary ingrowth and cell-matrix interactions [19].

In this study, Archimedes method was used to measure the total and interconnected porosities of the scaffolds. The interconnected porosity measurements are based on the presence of open pores and infiltration of water to the pore, which are related to properties such as permeability, circulation and exchange of body fluids, and surface area of the porous structure [20].

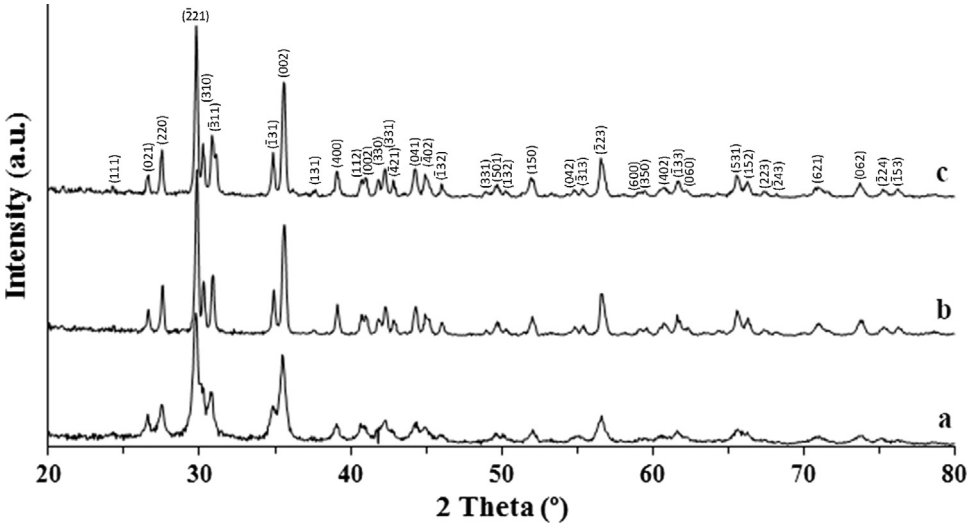

Fig.1. XRD patterns of the nanostructure diopside (a) powder and scaffolds fabricated by using (b) space holder and (c) gelcasting methods.