Materials Science and Engineering C 69 (2016) 208–220

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Physicochemical and mechanical properties of freeze cast hydroxyapatite-gelatin scaffolds with dexamethasone loaded PLGA microspheres for hard tissue engineering applications

Farnaz Ghorbani [a], Hanieh Nojehdehian [b,*], Ali Zamanian [c]

[a] Department of Biomedical Engineering, Tehran Science and Research Branch, Islamic Azad University, P. O. Box: 4515/775, Tehran, Iran
[b] Department of Dental Materials, School of Dentistry, Shahid Beheshti University of Medical Sciences, P.O. Box: 1983963113, Tehran, Iran
[c] Biomaterials Research Group, Department of Nanotechnology and Advanced Materials, Materials and Energy Research Center, P.O. Box: 14155-4777, Tehran, Iran

ARTICLE INFO

Article history:
Received 27 December 2015
Received in revised form 8 June 2016
Accepted 25 June 2016
Available online 27 June 2016

Keywords:
Scaffold
Microsphere
Dexamethasone
Hard tissue engineering
Freeze casting
Drug delivery

ABSTRACT

Hydroxyapatite (HA)-gelatin scaffolds incorporated with dexamethasone-loaded polylactic-*co*-glycolic acid (PLGA) microspheres were synthesized by freeze casting technique. Scanning electron microscopy (SEM) micrographs demonstrated a unidirectional microstructure and a decrease in the pore size as a function of temperature gradient. Higher amounts of HA resulted in a decrease in the pore size. According to the results, at lower cooling rates, the formation of a lamellar structure decreased the mechanical strength, but at the same time, enhanced the swelling ratio, biodegradation rate and drug release level. On the other hand, higher weight ratios of HA increased the compressive strength, and reduced the swelling ratio, biodegradation rate and drug release level. The results obtained by furrier transform infrared spectroscopy (FTIR) and bioactivity analysis illustrated that the interactions of the materials support the apatite formation in the simulated body fluid (SBF) solution. Based on the obtained results, the synthesized composite scaffolds have the necessary mechanical and physicochemical features to support the regeneration of defects and to maintain their stability during the neo-tissue formation.

1. Introduction

Every year, a large number of people suffer from bone defects mainly caused by age-related conditions, disease or trauma. Due to the complications such as immune rejection and resource constraints arising from autograft and allograft sources, tissue engineering was developed to replace the failing or malfunctioning body tissues by employing a combination of desirable cells, scaffolds and signaling molecules [1]. The main focus of tissue engineering is mimicking natural extracellular matrix (ECM), composed of collagen matrix and HA as the dispersed phase in the skeletal system [2]. However, functionality of the repaired tissue is the critical issue of the regeneration process.

Various biocompatible materials with non-toxic by-products are employed to fabricate interconnected porous scaffolds [1]. A vast number of cellular scaffolds are obtained from mixtures of ceramic materials (hydroxyapatite [3–7] and bioglass [8]) and polymers (chitosan [9–12], elastin [13], alginate [14], gelatin [6,15–19] and collagen [20,21], polylactic-*co*-glycolic acid [19,22,23], polycaprolactone [24]). Biodegradable polymers have been widely used as drug delivery systems [25–27]. PLGA is the most well-known and extensively studied biodegradable polymer used as a delivery vehicle. Due to their resemblance to ECM, biocompatible and biodegradable natural proteins, such as collagen the main structural protein found in the bone extracellular matrix, support cellular proliferation with the highest efficiency. Gelatin, the denatured form of collagen, is biocompatible, biodegradable, non-immunogenic and cost-effective and has been used in research [28]. Calcium phosphate ceramics, particularly macroporous HA, are ideal for bone tissue engineering due to their similarity to the mineral parts of the bone [29,30].

There are several methods to fabricate tissue engineering scaffolds; such as freeze casting [3,4,6,18,31–34], freeze drying [17,35,36], electrospinning [24,37–41], gas foaming [42], and phase separation [43–45]. Freeze casting is a useful technique to produce unidirectional porous ceramic or/and polymeric constructs. This method uses rapid freezing of a liquid suspension in an isolated mold by applying liquid nitrogen, followed by the sublimation of the frozen liquid phase under vacuum to fabricate an anisotropic porous microstructure. Fabrication of a lamellar structure is possible by controlling the growth direction of the ice crystals [7].

Deville et al. demonstrated that porous scaffolds with at least 40% to 65% porosity can be obtained by targeted freezing of HA suspensions and ice sublimation. The resultant porosity was open and unidirectional, exhibiting a lamellar morphology. Freezing rate and concentration of suspension affect pore size distribution; additionally, due to their lamellar architecture and the pore shape anisotropy, the processed scaffolds

* Corresponding author.
E-mail addresses: Farnaz_ghorbani.1991@yahoo.com (F. Ghorbani), hanieh.nojehdehyan@gmail.com (H. Nojehdehian), a-zamanian@merc.ac.ir (A. Zamanian).

http://dx.doi.org/10.1016/j.msec.2016.06.079
0928-4931/

Table 1
Abbreviation of synthesis scaffolds.

Code	Types of scaffolds
15R1	Hydroxyapatite (15%)-gelatin scaffolds-dexamethasone loaded PLGA microspheres freezing rate = 1 °C/min
30R1	Hydroxyapatite (30%)-gelatin scaffolds, dexamethasone loaded PLGA microspheres freezing rate = 1 °C/min
50R1	Hydroxyapatite (50%)-gelatin scaffolds, dexamethasone loaded PLGA microspheres freezing rate = 1 °C/min
15R4	Hydroxyapatite (15%)-gelatin scaffolds, dexamethasone loaded PLGA microspheres freezing rate = 4 °C/min
30R4	Hydroxyapatite (30%)-gelatin scaffolds, dexamethasone loaded PLGA microspheres freezing rate = 4 °C/min
50R4	Hydroxyapatite (50%)-gelatin scaffolds, dexamethasone loaded PLGA microspheres freezing rate = 4 °C/min

exhibited unusually high compressive strength [7]. Since the freeze casting can be used for any type of ceramic or polymeric material, freeze-cast structures can support the regeneration of a wide range of damaged tissues. Moreover, with water as the solvent, the process is environmentally-friendly. Regarding mechanical properties, the compressive strength values can be extremely high along the ice growth direction. Furthermore, a favorable biological response to these types of scaffolds has been reported (the proliferation of preosteoblastic cells in pore channels with diameters of 25 and 100 μm; the regeneration of femoral bone cavities of rabbits after implantation of HA scaffolds) [46]. Arabi and Zamanian employed freeze casting method to fabricate highly porous gelatin scaffolds using different gelatin concentrations and freezing rates. They reported that pore shapes changed from oblate and polygon to almost round at higher gelatin contents, and that the cooling rate had no obvious effect on the pore morphology. The compressive strength of the scaffolds, in contradistinction to their swelling behavior, increased as a function of cooling rate and gelatin concentration [6]. Farhangdoust et al. studied the behavior of HA freeze-cast scaffolds using different initial concentrations, cooling rates and sintering conditions. They reported that the porosities were aligned along the freezing direction, which was parallel with the length of the samples. The formation of lamellar type porosities can be controlled via the initial concentrations of the HA slurry and the cooling rate; in addition, this microstructure improves the mechanical strength of the scaffolds by bridging the frozen layers. Therefore, this method can be easily utilized to synthesize scaffolds with other calcium phosphates or any other material [3]. Tığlı and Gümüşderelioğlu, investigated the anti-inflammatory efficiency of dexamethasone-loaded chitosan scaffolds in reducing the side effects associated with the systemic delivery in cartilage development [47]. Martins et al. demonstrated the osteogenic influence of dexamethasone on bone marrow mesenchymal stem cells differentiation. Increasing the dexamethasone content of the nanofibers resulted in an increase in the concentration of alkaline phosphatase and deposition of mineralized matrix. In addition, the expression of osteoblastic markers proved the osteogenic inducing potential of nanofibrous scaffolds [48]. Son et al. demonstrated the osteoinductivity of HA/DEX-loaded PLA biphasic combination scaffolds. An increased alkaline phosphatase concentration and higher amounts of proteins and calcified bone tissue were observed in dexamethasone-loaded samples in comparison with the control groups (free of dexamethasone), suggesting that the drug-loaded biphasic scaffolds might be applicable as scaffolds for bone regeneration [49]. Also, based on Das, Son and Hickey investigations both in-vitro and in-vivo analysis showed that freezing operation have no negative effect on bioactivity of DEX, due to formation of new bone in defect site [50–52].

In this study, dexamethasone-containing PLGA microspheres were fabricated by solvent evaporation technique. Then, HA-gelatin scaffolds incorporated with dexamethasone-loaded PLGA microspheres were constructed by freeze casting method. The mechanical and physicochemical features were then evaluated. Freezing of the samples was carried out using different cooling rates and varying amounts of HA in order to study the resultant properties and to determine the structure which possesses the necessary features for producing a favorable biological response and supporting ossification and bone regeneration.

2. Materials and methods

2.1. Materials

Hydroxyapatite (HA, M_w = 502.32 g/mol) and Gelatin (M_w = 40–50 kDa) were purchased from Merck Co. Ltd. (Germany). Polylactic acid-*co*-glycolic (PLGA, RG 504 H, M_w 38,000–54,000) and polyvinyl alcohol (PVA) (87–89% hydrolyzed, mol. wt. 31,000–50,000 g/mol) were purchased from Sigma Co. Ltd. (USA). Dexamethasone was purchased from Sina Drau Co. Ltd. (Iran). All chemicals were used directly without further purification.

2.2. Preparation of PLGA microspheres

The most commonly used method for preparing PLGA microspheres is the solvent evaporation technique based on the formation of a double emulsion (water-in-oil-in-water). Therefore, in this study, PLGA microspheres were prepared by solvent evaporation, water-in-oil-in-water technique. Briefly 100 mg of PLGA were dissolved in 500 μl chloroform with 10 mg dexamethasone. This polymer solution was combined with 2 ml of a 2% (w/v) solution of PVA in double-distilled water (ddH_2O) and homogenized (homogenizer, Silent Crusher M, SilentCrusher M, Heidolph, Germany) for 2 min. This emulsion was then added to a 30 ml of stirring 0.2% (w/v) PVA/ddH_2O and continued to be stirred for 4 h. The microspheres were then collected via centrifugation using a centrifuge set at 11,000 rpm, 4 °C, for 10 min. The PLGA microspheres were then washed twice with ddH_2O and centrifuged

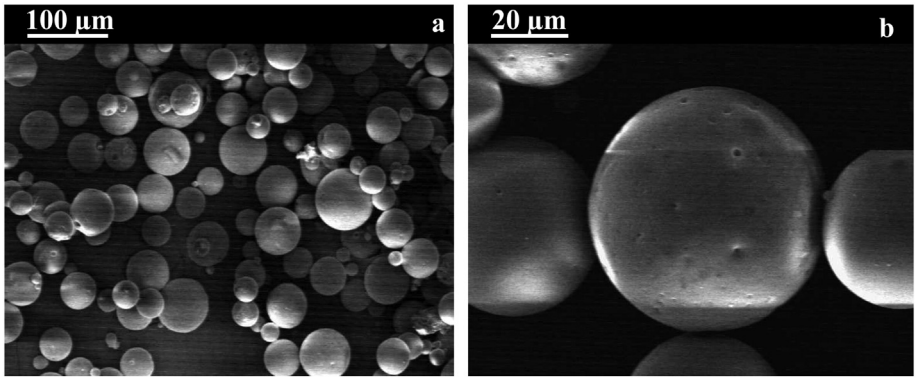

Fig. 1. SEM micrographs of dexamethasone-loaded PLGA microspheres.