Composites Science and Technology 121 (2015) 115–122

Contents lists available at ScienceDirect

Composites Science and Technology

journal homepage: http://www.elsevier.com/locate/compscitech

Electrospun PHBV nanofibers containing HA and bredigite nanoparticles: Fabrication, characterization and evaluation of mechanical properties and bioactivity

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ARTICLE INFO

Article history:
Received 2 January 2015
Received in revised form
8 October 2015
Accepted 5 November 2015
Available online 10 November 2015

Keywords:
Nano composite
Mechanical properties
Differential scanning calorimetry (DSC)
Electro-spinning

ABSTRACT

In this study, hydroxyapatite (HA), bredigite (BR) and hydroxyapatite/bredigite (HABR) (50/50) nanoparticles were synthesized using sol–gel method and characterized by X-ray diffractometer (XRD) and Transmission electron microscopy (TEM). Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) or PHBV nanofibers containing different concentrations (0, 5, 10 and 15%) of HA or BR or HABR nanoparticles were prepared by electrospinning process. Physiochemical properties of the prepared nanofibers were evaluated by scanning electron microscopy (SEM), Fourier transform infrared (FT-IR) and differential scanning calorimetry (DSC). Evaluation of their mechanical properties showed that the addition of 10% of any one of the above mentioned nanoparticles to PHBV produced composite nanofibers with regard to their tensile strength and Young's modulus. PHBV containing either 10% HA or 10% HABR showed higher mechanical strength and Young's modulus than the PHBV fibers incorporated with 10% BR. At the same time, studies on the ability of bone formation of the nanofibers in simulated body fluid (SBF) confirmed higher bone-like apatite formation on PHBV fibers containing either 10% HABR or BR compared to the HA composite. We concluded that the 10% HABR incorporated PHBV nanofibers possess optimized mechanical properties with high ability for apatite formation, thus potentially suitable as a novel substrate for bone regeneration application compared to the most commonly studied HA composite fibers.

1. Introduction

Bone is considered as a unique “living nanocomposite” that consists of a fibrous protein, collagen, stiffened by an extremely dense filling and surrounded by mineral crystals. The major minerals present in bone are calcium and phosphate, and it exists mainly in the form of hydroxyapatite (HA) ($Ca_{10}(PO_4)_6(OH)_2$). Other minerals include trace elements such as the carbonate, citrate, sodium, magnesium, fluoride, chloride, and potassium that support various metabolic functions of bone [1,2]. The collagen present in bone has fibrous structure with diameter in the range of 100–2000 nm. Similarly, HA in the bone mineral is in the form of nanocrystals, with dimensions of about 4 nm × 50 nm × 50 nm. In recent years, special attention has been paid to articulate the biomimetic properties of bone. Various studies have been reported on the fabrication of different inorganic nanoparticles (NPs) such as bioactive glass [3], tricalcium phosphate [4] and hydroxyapatite [5] and their incorporation in natural or synthetic polymer such as the collagen [6], chitosan [5], polycaprolactone [7], poly lactic acid [8], polyhydroxyalkanoates [9], etc. Among these inorganics, synthetic HA is the most widely used bioceramic material having similar composition and morphology to the inorganic component of natural bone. In addition, it can provide a favorable environment for cell adhesion, osteoconduction and osteoinduction [10]. Bredigite

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http://dx.doi.org/10.1016/j.compscitech.2015.11.006
0266-3538/

($Ca_7MgSi_4O_{16}$, BR), is a Ca, Si and Mg-containing ceramic which has been reported to possess apatite forming ability in the simulated body fluid (SBF) and can stimulate cell proliferation and differentiation [11,12].

Nanofibrous materials have been widely used as desired scaffolds for bone tissue engineering applications. Besides the small fiber diameter, nanofibrous scaffolds offer high surface to volume ratio, high porosity with interconnected pores and the enhanced attachment and proliferation of cells, due to their nanoscale biomimetic structure of natural extracellular matrix (ECM) [1–3]. There are several techniques used for the preparation of polymeric nanofiber based scaffolds including multicomponent fiber spinning, centrifugal spinning, modular melt blowing, pressurized gyration process, infusion gyration and electrospinning [13–16]. Among these methods, electrospinning is the most used technique due to its simplicity and inexpensive instrumental setup and it does not require any sophisticated equipments, thus used for electrospinning various polymeric materials, with potential to scale-up [1,15]. Moreover, it enables the incorporation of various ceramic particles, thus capable of tailoring the chemical and mechanical properties of the prepared scaffolds.

Little is known about BR and hydroxyapatite/bredigite (HABR) composites, and here we attempt for the electrospinning of polyhydroxybutyrate-co-hydroxyvalerate (PHBV) fibers reinforced with HA, HABR or BR nanoparticles for the first time, facilitating the fabrication of scaffolds suitable for bone tissue regeneration. The chemical and mechanical properties of scaffolds were characterized, along with the *in vitro* bioactivity were evaluated to investigate their potential as substrates suitable for bone tissue engineering.

2. Materials and methods

2.1. Synthesize of the nanoparticles

BR particles were prepared by sol–gel method using tetraethyl orthosilicate ($(C_2H_5O)_4$ Si, TEOS, Merck), magnesium nitrate hexahydrate ($Mg(NO_3)_2.6H_2O$, Merck) and calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$, Merck) as raw materials, as described before by Wu et al. [17]. The particles were ball-milled in a zirconia mechanical ball mill to obtain BR nanoparticles. Pure HA nanoparticles were prepared by sol gel method as described before by Fathi et al. [18]. Briefly, a stoichiometric amount of calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$, Merck) and phosphoric pentoxide (P_2O_5, Merck) with the molar ratio of 10:3, were dissolved in absolute ethanol (C_2H_5OH, Merck). The as-prepared sol transforms into a gel after 24 h under continuous stirring at ambient temperature. As-formed gel was aged for 24 h, dried at 80 °C in air for 24 h and finally sintered at a heating rate of 5 °C/min at 600 °C in a muffle furnace. In order to obtain HA/bredigite(50/50) nanoparticles (termed as HABR throughout this manuscript), BR nanoparticles with a ratio of 50 wt% was sonicated in 5 ml ethanol for 30 min and added to HA sol and the above described HA synthesis procedure was followed.

2.2. Fabrication of PHBV-NP nanofibers

Composite PHBV nanofibers were fabricated using 8% (wt/v) PHBV solution added with different amounts (5, 10 and 15 wt% of PHBV) of either one of the NPs (HA, BR, HABR) in HFP (1,1,1,3,3,3 hexafluoro-2-propanol, Sigma). In short, the desired amounts of NPs were sonicated for 30 min in HFP; further PHBV was added to this mixture and stirred overnight. The solution was fed into a 3 ml syringe fitted with a needle of 0.5 mm inner diameter. During electrospinning, the applied voltage, flow rate and distance between the needle and the collector were held constant at 14 kV, 1 ml/h and 18 cm, respectively. The scaffold names are abbreviated as PHBV-XY, where X represent HA or BR or HABR and Y represent the % of loaded NPs.

2.3. Characterization of nanoparticles and nanofibers

Phase structure analysis of HA, BR and HABR composite NPs were performed using X-ray diffractometer (XRD) (Philips X'Pert-MPD, Cu Kα radiation at 30 mA and 40 kV, at a scan rate of 3°/min). Transmission electron microscopy (TEM, Philips CM-120) was used to determine the morphology and size of the prepared particles. The average particle size of the particles were determined by counting 100 particles from the TEM image using the image J software. The surface morphology of the electrospun scaffolds was observed by field-emission scanning electron microscopy (FE-SEM S-7400; Hitachi, Japan) and processed by image J software (National Institutes of Health, Bethesda, Maryland, USA). To investigate the chemical composition, Fourier transform infrared (FTIR) spectra of electrospun scaffolds were measured using Avatar 380 (ThermoQ14 Nicolet, MA), at a resolution of 4 cm^{-1} and a spectral range of 4000–400 cm^{-1}. Thermal properties of the scaffolds were studied by DSC822e Differential Scanning Calorimeter (METTLER Q15 TOLEDO, Switzerland) and thermograms were analyzed using STARe version 9.10 software. Samples weighing 5–7 mg each was sealed in aluminum crucibles with lids and the experiments were performed at a heating rate of 10 °C/min under nitrogen atmosphere, at a temperature range of 20–350 °C.

Degree of crystallinity (Xc) of each sample was calculated as follows:

$$X_c = (\Delta H_m(j/g))/(\Delta H_{m^\circ}(j/g)) \times 100 \quad (1)$$

Where ΔH_m (j/g) is the enthalpy of melting, derived from DSC curves, and ΔH_{m°(j/g) is the enthalpy of melting 100% of the crystalline polymer. For PHBV, ΔH_{m° is 109 J/g [19].

2.4. Mechanical properties of nanofibers

Scaffolds were tested for their mechanical strength using a tabletop tensile tester (Instron 5943, USA). The pure and composite PHBV nanofibrous mats were cut into rectangular strips (10 mm × 20 mm) mounted vertically on the gripping unit and tested at a crosshead speed of 10 mm/min and a load cell capacity of 50 N was used.

2.5. In vitro biodegradation and bioactivity study

Electrospun samples were cut into square pieces of 10 × 10 mm, weighed, and soaked in 10 ml of 1× phosphate buffer solution PBS (pH 7.3, Sigma–Aldrich) at 37 °C. PBS was changed every 3 days. The morphology of the nanofibrous mats during degradation was observed by SEM and the mass loss was calculated from the equation: $((W_0-W_t)/W_0) \times 100$, where W_0 is the initial weight and W_t is the weight of sample after degradation for predicted period of time. The ability of the prepared composite nanofibers to induce the formation of bone-like apatite was assessed in SBF, by the method as previously described by Kokubo et al. [20]. The nanofibrous mats (10 × 10 mm) were individually soaked in 30 ml of the SBF in polyethylene containers at 36.5 °C using a thermostatic bath. After designated time period, the samples were rinsed with distilled water, dried at 100 °C and observed under SEM. The pH of the SBF was also recorded within a designed period of times using a pH meter.