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Original Research

Electrospun composites of PHBV/pearl powder for bone repairing

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Abstract

Electrospun fiber has highly structural similarity with natural bone extracelluar matrix (ECM). Many researches about fabricating organic–inorganic composite materials have been carried out in order to mimic the natural composition of bone and enhance the biocompatibility of materials. In this work, pearl powder was added to the poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and the composite nanofiber scaffold was prepared by electrospinning. Mineralization ability of the composite scaffolds can be evaluated by analyzing hydroxyapatite (HA) formation on the surface of nanofiber scaffolds. The obtained composite nanofiber scaffolds showed an enhanced mineralization capacity due to incorporation of pearl powder. The HA formed amount of the composite scaffolds was raised as the increase of pearl powder in composite scaffolds. Therefore, the prepared PHBV/pearl composite nanofiber scaffolds would be a promising candidate as an osteoconductive composite material for bone repairing.

Keywords: Hydroxyapatite; Pearl; PHBV; Mineralization; Composite nanofibers scaffolds

1. Introduction

Bone extracelluar matrix (ECM) is a type of nanocomposite on which cells adhere, proliferate and differentiate [1]. The structure of bone ECM is organic collagenous fibers embedded by inorganic hydroxyapatite (HA) nanocrystals. Many processing techniques have been developed to mimic bone ECM. Electrospinning is a simple method to fabricate nanofiber. This electrospun fiber has highly structural similarity with natural bone ECM [2–6]. The fibrous surfaces fabricated by electrospinning can improve cell adhesion when compared with smooth surfaces [7]. Recently many synthetic polymers have been used in electrospinning, such as, poly lactic acid (PLA) [8,9], polycaprolactone (PCL) [10], and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV)[11–14].

PHBV produced by microbial fermentation is one of the biomaterials applied in bone tissue engineering. It has being researched as a substitute for natural bone because of its biodegradability and biocompatibility. The degradation time of PHBV can be controlled by adding inorganic materials in PHBV matrix such as HA [15]. Besides, the ultimate degradation product of PHBV is hydroxybutyric acid which is a constituent of human blood [16–18].

The method using inorganic materials to improve both mechanical properties and biocompatibility of synthetic ECM has attracted much attention [19–21]. Using Pearl powder to enhance biological and mechanical performances has been studied recently [22]. Pearls contain growth factors that are able to increase osteoblast proliferation [23]. Furthermore, it is approved that pearl shows great biocompatible and biodegrade ability [24].

In this study, we selected PHBV as the main matrix and pearl powder as inorganic additive. The PHBV/Pearl powder composite scaffolds were prepared by electrospinning. HA would deposit on PHBV/pearl nanofiber scaffold by mineralization method in vitro. The effects of pearl powder on the surface morphology of electrospun fibers and mineralization rate were discussed here.

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2. Materials and methods

2.1. Materials

PHBV (PHV content: 2 wt%) and pearl powder (100 nm) were purchased from Tianan biomaterials LTD (Ningbo, China) and Fenix Pearl-biotech CO.,LTD. (Zhejiang, China), respectively. 1,1,1,3,3,3-hexafluoro-2-isopropanol (HFIP) were purchased from Darui LTD. (Shanghai China).

2.2. Pretreatment of pearl

Before added in PHBV solution, pearl powder was pretreated in polyethylene glycol (PEG) solution with the aim at raising compatibility. First, suspensions were obtained by dispersing pearl powder in deionized water by stirring, then PEG powder was added to the suspensions (mass ratio of PEG/pearl = 1/20) combing ultrasonic and stiring. Each process lasted for 1 h. The suspensions were frozen overnight, and then were freeze-dried for two days.

2.3. Production of PHBV/pearl nanofibers

Pearl powder and PHBV were used to produce composite nanofibers. First, polymer solution was obtained by dissolving PHBV in HFIP at room temperature, and then pearl powder was added to the solution with sufficient stirring. In order to examine the effect of pearl powder content on fiber morphology, the polymer solution was prepared by adding varied mass ratio of pearl/PHBV in the range of 0%, 2%, 4%, and 6%.

2.4. Mineralization ability

The osteogenetic activity of the nanofibers scaffolds were generally characterized by forming bonelike HA on its surface. The nanofiber scaffolds were soaked in 1.5 simulated body fluid (1.5 SBF) for a few days and 1.5 SBF was prepared according to the method described in reference [25]. After mineralization, the nanofibers scaffolds were washed with deionized water to remove residual salts and dried before further analysis.

2.5. Mineralized sample characterization

The morphology of nanofibers scaffolds before and after soaked in SBF were characterized by scanning electron microscopy (SEM, JSM-5600 LV, JEOL, Japan). Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) was performed by a Nicolet-670 FTIR spectrometer (NIcolet-Thermo, USA). All spectra were measured in the wavelength range of 500–4000 cm^{-1} with a resolution of 4 cm^{-1}. X-ray diffraction (XRD) Patterns were obtained with a D/max-2500 PC diffractometer (Rigaku co., Japan) using Cu/k_α radiation with wavelength of 0.154 nm at 40 kV and 200 Ma over the range of 0–60°. The thermogravimetric analysis (TGA) was employed to evaluate the weight loss of the samples in nitrogen from room temperature to 900 °C at a heating rate of 10 °C/min using a thermal analyzer (TG 209 F1, Germany).

2.6. Cell culture

MC3T3 cells were used in this work. The cells were incubated with α-MEM medium supplemented with 10% PBS, 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. Samples were placed in 24-well dishes. Before seeding cells ($2*10^4$ per well), the samples were soaked in 75% ethanol aqueous solution for at least 1 day.

2.7. MTT assay

MTT assay was applied to evaluate the cell proliferation on samples. After incubating for a certain time, the old media was

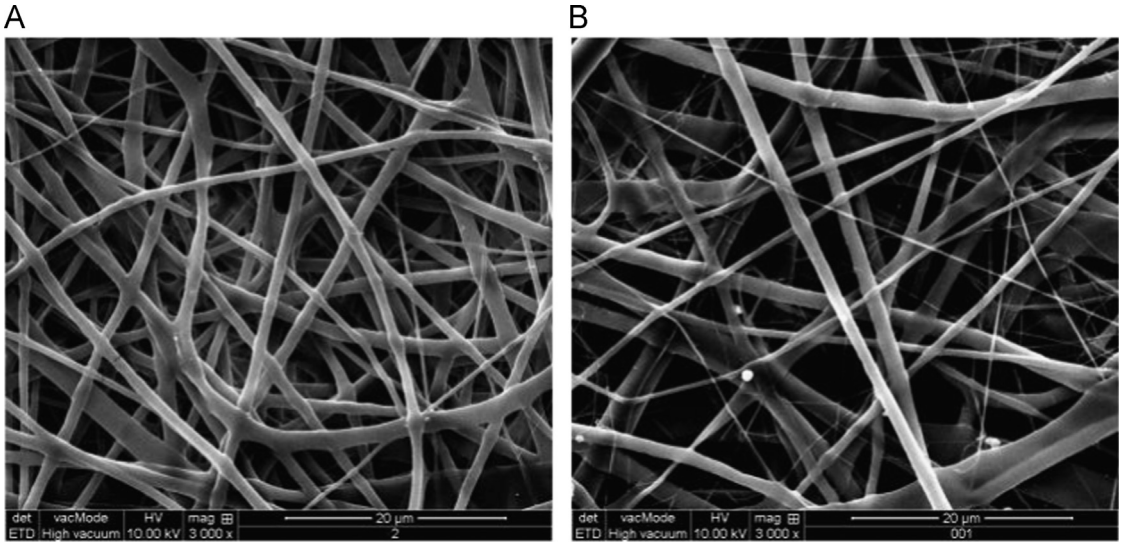

Fig. 1. SEM micrographs of electrospun fibers using PHBV solution in HFIP.(A) 4 wt% and (B) 5 wt%.