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Attachment, proliferation and differentiation of BMSCs on gas-jet/electrospun nHAP/PHB fibrous scaffolds

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ABSTRACT

In this study, poly(3-hydroxybutyrate) (PHB)-based scaffolds containing nanosized hydroxyapatite (nHAP) were manufactured by gas-jet/electrospinning. The morphologies of the scaffolds were characterized. The effect of the scaffolds on attachment, proliferation and differentiation of the bone marrow stroma cells (BMSCs) were accessed by using scanning electron microscopy (SEM), methylthiazol tetrazolium (MTT) assay and alkaline phosphatase (ALP) activity. The results show that the gas-jet/electrospun scaffolds possess an extracellular matrix-like topography. In vitro studies describe that the scaffolds have positive effects on attachment, proliferation and differentiation of BMSCs in vitro. It can be concluded that the scaffolds combing the unique structural features generated by gas-jet/electrospinning with functional factors, have the potential to be used in bone tissue engineering.

1. Introduction

In bone tissue engineering, ideal scaffolds should approximately mimic the structural morphology of the extracellular matrix (ECM), so they must present appropriate porosity, sufficient surface area and suitable surface chemistries that encourage cell adhesion, proliferation, and differentiation [1]. Many techniques have been developed to fabricate scaffolds to satisfy these demands. Among them, electrospinning has attracted much attention, which is a unique process for producing continuous fibers with nanoscale diameters [2,3]. In order to improve the performance of the conventional electrospinning, Professor Wu et al. designed a gas-jet/electrospinning apparatus, which combine the conventional electrospinning apparatus with a gas-jet device. Their experimental results show that it can make finer nanofibers and express higher efficiency in the process than the conventional electrospinning apparatus [4].

Poly(3-hydroxybutyrate) (PHB), as a member of polyhydroxyalkanoates (PHA) family, has attracted much attention for a variety of medical applications because of its biodegradation and excellent biocompatibility [5–7]. Nanoparticle hydroxyapatite (nHAP) has composition and crystallographic property very close to nature bone mineral particle and is considered for potential enhanced bone outcomes [8–10].

Therefore, in this study, in order to mimic the natural bone structure, which is a biocomposite of hydroxyapatite (HA) mineral crystals arranged in an organic collagen matrix, PHB-based scaffolds containing 10 wt% nHAP particles were fabricated through a gas-jet/electrospinning process. The effect of the gas-jet/electrospun nHAP/PHB scaffolds on attachment, proliferation and differentiation of the rat bone marrow stroma cells (BMSCs) in vitro were investigated and compared with the gas-jet/electrospun pure PHB scaffold and cell culture plate.

2. Experiment

2.1. Materials

All the chemical reagents used in the present study were of analytical grade. PHB was purchased from Chengdu Institute of Biology, Academy of Science, China. Hydrothermally synthesized nHAP with average size of 50–100 nm diameter were provided by College of Material and Engineering, Sichuan University, China.

2.2. Preparation of spinning solution

4 wt% pure PHB solutions were prepared by dissolved PHB in chloroform at 60 °C. 4 wt% gas-jet/electrospinning nHAP/PHB opalescent suspension containing 10% weight percentages nHAP

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was prepared by adding nHAP into the PHB/chloroform solution. In order to well disperse the nHAP particles, the mixture was stirred by magnetic force for at least 12 h at room temperature and subsequently ultrasonic agitated for 30 min.

2.3. Gas-jet/electrospinning process

The gas-jet/electrospinning was carried out in air. The prepared spinning solution was extruded to the spinneret (inner diameter is 0.24 mm) at the flow rate of 0.5 ml min^{-1}. The applied voltage used in electrospinning was 29.0 kV and the gas flow rate was 10.0 l min^{-1}. The fibers were collected on a clear stainless steel plate under the spinneret and the spinneret-collector distance was 20 cm.

2.4. Morphologies of gas-jet/electrospun scaffolds

The morphologies of the electrospun fibrous scaffolds were examined using scanning electron microscopy (SEM JEOL JSM-5900 LV Japan) at an accelerating voltage of 20 kV after being coated with a thin layer of gold.

2.5. Rat bone mesenchymal stem cells (BMSCs) isolation, seed and culture

Total bone marrow cells were isolated from the femurs of young (4–6 weeks old) SD rat. Whole bone marrow cells were plated in cell culture flasks (Falcon, BD, New Jersey, USA) and the medium was changed after 3 days. Scaffold samples (2.4 cm × 2.4 cm) were sterilized by autoclaved and pretreated with culture medium for 4 h prior to cells implant. Passage two isolated BMSCs were seeded at a density of 1×10^5 cells/sample on pretreated samples and cell culture plate. Cells were incubated in 2 ml of the same medium as stated previously at 37 °C and 5% CO_2 for the duration of the experiment.

2.6. Attachment, proliferation and alkaline phosphatase (ALP) activity of BMSCs

SEM was used to determine the morphology of BMSCs cultured on the scaffolds after cell seeding for 1, 4 and 72 h. Cell proliferation was assessed using a methylthiazol tetrazolium (MTT) assay after cell seeding for 1, 3, 5 and 7 days. The level of ALP activity of the BMSCs cultured on the fibrous scaffold and cell culture plate was detected after cell seeding for 3, 7 and 10 days.

2.7. Statistical analysis

Data were presented as means ± standard error of mean. Statistical analysis was performed using one-way ANOVA. *P*-values <0.05 were considered statistically significant.

3. Results

3.1. Character of gas-jet/electrospun PHB-based scaffolds

Fig. 1 shows SEM images of gas-jet/electrospun PHB-based nanofiber mats with or without nHAP particles. From the images, we can see that both groups were successfully electrospun into long and continuous nanofibers. Compared with the pure PHB fibers, which were smooth and uniform, the surfaces of the nHAP/PHB composite fibers were rougher, because nHAP nanoparticles protruded from the surface layer of the composite fibers and even in several regions of the fibers, the nanoparticles aggregated and came to the fibers surfaces.

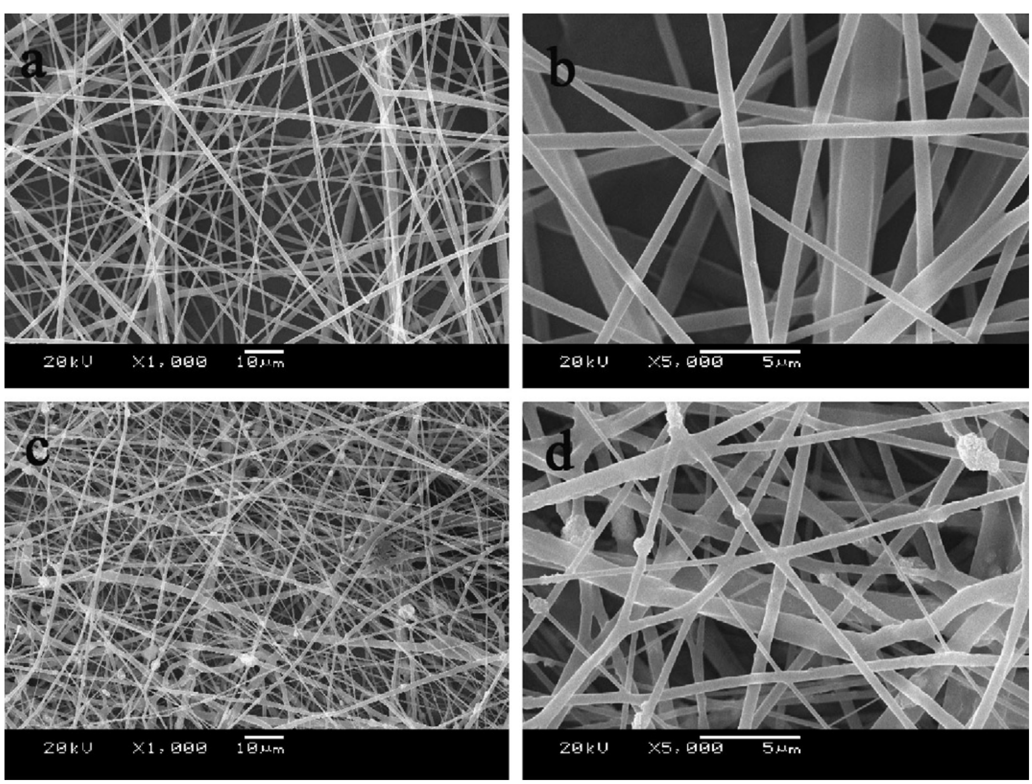

Fig. 1. SEM images of the gas-jet/electrospun PHB-based scaffolds: (a and b) pure PHB; (c and d) nHAP/PHB.