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Evaluation of electrospun biomimetic substrate surface-decorated with nanohydroxyapatite precipitation for osteoblasts behavior

Sai Zhang [a], Guojun Jiang [b], Molamma P. Prabhakaran [c], Xiaohong Qin [a,*], Seeram Ramakrishna [c]

[a] Key Laboratory of Textile Science & Technology, Ministry of Education, College of Textiles, Donghua University, Shanghai 201620, PR China
[b] Department of Science, Zhijiang College of Zhejiang University of Technology, No.958 Kehua Road, Keqiao, Shaoxing 312000, PR China
[c] Center for Nanofibers and Nanotechnology, Department of Mechanical Engineering, National University of Singapore, 2 Engineering Drive 3, Singapore 117576, Singapore

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ABSTRACT

To engineer bone tissue, it is crucial to design scaffolds with micro- and nano-sized architecture imitating approximate hierarchical structure of native bone, and afford desirable biological properties by introducing biocompatible polymers and bioceramics into the scaffolds. Here, a novel scaffold consisting of poly-3-hydroxybutyrate-*co*-3-hydroxyvalerate (PHBV)/polyaspartic acid (PAA) was fabricated by electrospinning and nano-hydroxyapatite (nHA) was deposited by calcium-phosphate dipping process for bone tissue regeneration. Characterization of the prepared nanofibers revealed the formation of definite nHA crystal, porous structure of membranes, improved wettability with nHA deposition and satisfied mechanical properties. Human fetal osteoblasts were cultured on nanofibers and experienced in vitro evaluations of cell proliferation, adhesion and mineralization confirming the non-cytotoxicity and biocompatibility of scaffolds. Cells proliferation rate and ALP expression on PHBV/PAA-nHA were 36.40% and 40.14% higher than on PHBV/PAA, respectively. The utmost significance of this study is introducing bioactive PAA-nHA on polymeric nanofibers to regulate and improve specific cells adhesion, proliferation and mineralization of osteoblasts. All results indicate PHBV/PAA-nHA nanofibrous scaffolds can be applied as biomimetic platform for bone tissue repairation with appropriate physico-chemical properties, osteoinductivity and osteoconductivity.

1. Introduction

Natural bone is an energetic organ persistently experiencing remodelling to adjust to mechanical stress, to remain healthy and to repair injuries. Belonging to calcified tissues, bone can be considered as a natural anisotropic composite in structure, forming from bio-inorganic component, mostly in the shape of hydroxyapatite (HA), collagen-based hydrogels, other organic materials, and water [1]. The nano-structure of the extracellular matrix (ECM) is a natural assembly involving a sophisticated network of nanofibers, which is fundamental to support cells and assumes a conducive background to guide cell behaviour [2]. Currently, employing an autogenous graft is the optimum choice for surgical repair of bone loss, but existing associated limitations involving significant clinical morbidity, prolonged hospitalization, delayed rehabilitation, and surgical complications [3,4]. Currently, interdisciplinary strategies in tissue regeneration combine the advanced developments in materials science and engineering, cell and molecular biology, as same as bio-chemical and medical sciences, thus presenting fresh paradigms for the recovery of tissue and organ functions [5,6].

Favourable scaffolding materials for bone tissue engineering (BTE) should possess such elementary characteristics like sustainability, reliability, high quality and cost-effectiveness all over the longevity of people and support further medical assistance in therapy and surgery [7]. The nanofibrous matrices fabricated by electrospinning technology have acquired tremendous interest mainly owing to the processing availability to a wide range of materials, as well as simple set-up and operation at low cost [8,9]. Electrospun scaffolds with large surface area-to-volume ratio, high porosity, and mechanical properties and morphology resembling to the ECM of natural tissue can be engineered to act as ideal bone substitutes [10]. Apart from traditional two-dimensional (2D) nanofibrous structures, electrospinning is formidable in fabricating 3D nanofibrous structures for tissue engineering, especially for bone regeneration [11,12].

Researchers have exploited various nanofibrous polymeric compositions to assist bone cell growth with the intention of achieving better cellular adhesion and mineral deposition suitable for the regeneration of bone [13,14]. Some natural polymers have been considered as potential substrates for bone grafts, including collagen, chitosan, gelatin and silk fibroin [15–18]. However, electrospun scaffolds containing poly-(α, β)-DL-aspartic acid (PAA) is less explored in the application of tissue engineering, especially for BTE. It is a reproducible, highly biodegradable and completely water soluble ionic polymer comprised of

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[*] Corresponding author.
E-mail address: xhqin@dhu.edu.cn (X. Qin).

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carboxylate and amino groups within its structure. Polypeptide moieties like PAA are competent to integrate multiple ECM proteins and growth factors in the medium, which benefits cell proliferation and migration [19]. In addition, PAA plays an important role in the process of biomineralization since it was demonstrated that proteins containing aspartic acid-rich sequences were responsible for interacting with crystals of calcium salt in a specific manner, and such sequences appeared in HA-binding proteins [20]. Nevertheless, the inferiority in mechanical properties of natural materials is an intractable problem for BTE. Together with PAA, poly-3-hydroxybutyrate*co*-3-hydroxyvalerate (PHBV) was exploited and electrospun into high surface area fibrous membranes as effective bone tissue scaffolds instead of some prevalent usual polymers involving poly(ε-caprolactone) (PCL), poly(L-lactide) acid (PLLA) and poly(lactide-co-glycolide) (PLGA) [21–23]. PHBV exhibits advantageous mechanical characteristics, good oxygen permeability and high biocompatibility. The degradable period of PHBV is longer than many other biocompatible polymers, which is beneficial for preserving the mechanical and structural integrity of the tissue/substrates further into bone remodelling phase, and its biodegradation products, 3-hydroxybutyrate, are normal mammalian metabolites [24].

Nanophase ceramics, especially nano-hydroxyapatite (nHA), are preferred in bone substitutes, coatings and other filler materials as a result of their documented capability of facilitating mineralization [25]. Tetteh et al. fabricated polyurethane (PU) microfibrous mats without beads and irregularities, and improved the mechanical characteristics of scaffolds through incorporating micro/nano HA particles. The results also indicated that the incorporation of HA particles increased the cell viabilities as well as calcium deposition of electrospun composites [26]. Suslu et al. utilized HAp–PHBV composited suspensions including both an organic salt and a surfactant for electrospinning and proved that HAp/PHBV nanofibrous scaffolds would be a promising candidate for tissue engineering applications [27]. The calcium and phosphate ions dissociating from nHA were absorbed by the body after the bone scaffolds were implanted inside, which consequently contributed to the growth of fresh tissue. HA deposition on the surface of nanofibers in situ was performed by immersing nanofiber sheets into a simulated body fluid in the research of Kung et al. [28]. The HA-mineral reinforced the bioactivity of electrospun membrane. Yet its friability and poor flexural strength heavily confined the broad application of nHA ceramic in biomaterials [29].

In this study, we introduced a compound strategy to preserve the requested cell affinity of PAA as well as osteoconductivity of nHA with the compactible polymer of PHBV to produce a scaffold material that can strengthen osteogenic behaviour of human fetal osteoblasts (hFOB) cells for bone tissue engineering. It may supply a more desirable synthetic microenvironment to better imitate natural bone tissue physiology as well as offer the additional higher mechanical strength that precipitating nHA on a biodegradable polymeric network. In order to obtain thoroughly efficient system for BTE, the targeted system should associate simultaneously multiple functionalities like cell coupling, calcium binding, mineralization capabilities and osteoconductivity. The overall strategy and summary of the work is illustrated in Fig. 1.

2. Materials and methods

2.1. Fabrication of nanofibrous scaffolds

PHBV with hydroxyvalerate (HV) content of 3%, is bountifully afforded by TianAn Enmat chemical company, China. PAA (Mw = 2000–11,000 Da) and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) were purchased from Sigma Aldrich. PHBV pellets were dissolved in HFIP at 8% (w/v) to prepare the pure PHBV solution. PHBV/PAA solution was prepared at a ratio of 80:20 (w/w) in HFIP at the same concentration of 8% (w/v). The polymer solutions were individually loaded into standard syringes with the volume of 5 ml (Becton Dickinson, BD, N.J, US.) and attached to a high voltage electric field of 13 kV (DC high voltage power supply from Gamma High Voltage Research, Florida, US.) to draw the fibres from the spinneret (27G 1/2 needle) onto the collector plate. The solutions were fed at a constant flow rate of 1.0 ml/h through a syringe pump (KD 100 Scientific Inc., Holliston, MA, USA) and the collecting distance between the tip of the spinneret and the collector plate was maintained at 120 mm. Nanofibers were collected on either aluminium foil or 15-mm coverslips, which were dried overnight under vacuum to eliminate residual solvents before using.

Biomineralization procedure was then carried out on the PHBV and PHBV/PAA samples to deposit nHA by calcium-phosphate dipping strategy. The electrospun PHBV and PHBV/PAA nanofibrous membranes were firstly immersed in 0.5 M $CaCl_2$ solution (Sigma) for 15 min, and followed by being rinsed in DI water for 1 min. The samples were then entrapped in 0.3 M of Na_2HPO_4 (Merck & Co. Inc., N.J, US.) for 15 min and rinsed for 1 min in DI water. This entire procedure was considered as 1 cycle. The scaffolds were subjected to 3 cycles of the above treatment. The scaffolds were frozen in refrigerator overnight after

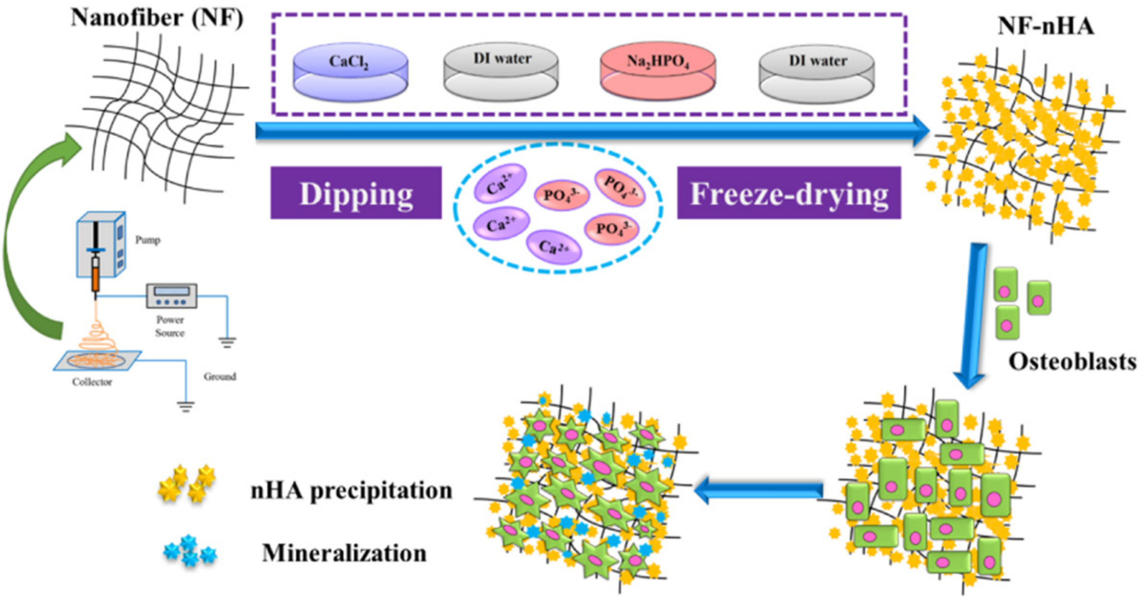

Fig. 1. Schematic illustration of the procedure for preparing nHA-deposited nanofibers and hFOB cultured on nanofibrous scaffolds for bone tissue engineering.