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Osteogenesis of human adipose-derived stem cells on hydroxyapatite-mineralized poly(lactic acid) nanofiber sheets

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ABSTRACT

Electrospun fiber sheets with various orientations (random, partially aligned, and aligned) and smooth and roughened casted membranes were prepared. Hydroxyapatite (HA) crystals were in situ formed on these material surfaces via immersion in 10× simulated body fluid solution. The size and morphology of the resulting fibers were examined using scanning electron microscopy. The average diameter of the fibers ranged from 225 ± 25 to 1050 ± 150 nm depending on the electrospinning parameters. Biological experiment results show that human adipose-derived stem cells exhibit different adhesion and osteogenic differentiation on the three types of fiber. The cell proliferation and osteogenic differentiation were best on the aligned fibers. Similar results were found for phosphorylated focal adhesion kinase expression. Electrospun poly(lactic acid) aligned fibers mineralized with HA crystals provide a good environment for cell growth and osteogenic differentiation and thus have great potential in the tissue engineering field.

1. Introduction

Bone fractures, osteoporosis, malformation, and tumors are clinically important bone diseases [1,2]. The current medical need is to solve the problems of traditional bone graft systems such as implant failure caused by the leakage of regenerated tissue around the implant surface, which results in poor bone remodeling and the loosening of implants [3]. Tissue engineering has received a lot of interest, with many fabrication techniques proposed for the fabrication of nanoscale materials such as nanofibers and nanofibrous sheets. The ideal scaffold for tissue engineering should be bioactive, biodegradable, biocompatible, and highly porous to mimic natural microenvironments [4]. In the natural bone matrix, parallel collagen fibers and apatite crystals arrange into a staggered pattern to form fibrils. Such mineralized collagen-based materials have a variety of structures that meet various mechanical requirements and can be used to bridge microcracks [5,6]. Parallel collagen fibers and apatite crystals give the composite high tensile mechanical strength and resistance [7]. Aligned nanofibers mineralized with apatite crystals are potential structures for bone tissue substitutes [8,9].

Electrospinning can be used for making micro- or nanometer-sized non-woven fiber scaffolds with an extremely high surface-to-volume ratio, tunable porosity, and malleability to conform to a wide variety of sizes and shapes [10]. Several reports founded that electrospun nanofiber scaffolds may be suitable substrates for biomedical or tissue engineering [11–13], drug carriers, wound dressing, immobilized enzymes, and catalysts [14,15]. Previous studies on electrospun nanofibers focused on biodegradable polymers such as poly(ε-caprolactone) (PCL) [1,16], poly(lactic acid) (PLA) [17,18], poly(glycolic acid) (PGA), and poly(lactic-co-glycolic acid) (PLGA) [19,20], and natural materials such as collagen, gelatin, silk protein, and fibrinogen [20,21]. Using ceramic–polymer composite materials can increase the integration between graft materials and native bone tissue. The addition of nanoscale inorganic particles into a polymer matrix improves the biomineralization capability of the composite scaffolds and increases the stiffness of the material without reducing its mechanical strength [22]. Lee reported a nanocomposite system made of PCL-grafted hydroxyapatite (HA) and found that it had better adhesion and proliferation of fibroblasts

Abbreviations: HA, hydroxyapatite; PCL, poly(ε-caprolactone); PLA, poly(lactic acid); PGA, poly(glycolic acid); PLGA, poly(lactic-co-glycolic acid); SBF, simulated body fluid; PBS, phosphate-buffered saline; SMCs, smooth muscle cells; FAK, focal adhesion kinase; pFAK, phosphorylated FAK; HRP, horseradish peroxidase; OC, osteocalcin; ALP, alkaline phosphatase; hADSCs, human adipose-derived stem cells; DMSO, dimethyl sulfoxide; PBS, phosphate-buffered saline; FA group, aligned fiber group; FP group, partially aligned fiber group; FR group, random fiber group; MC group, membrane made of chloroform; MD group, membrane made of chloroform/DMSO; MCHA, HA-mineralized MC group; MDHA, HA-mineralized MD group; FAHA, HA-mineralized FA group; FPHA, HA-mineralized FP group; FRHA, HA-mineralized FR group; Ctrl, tissue culture plastic control group; SEM, scanning electron microscopy; EDS, energy-dispersive X-ray spectroscopy; XRD, X-ray diffraction; TGA, thermogravimetric analysis; UV, ultraviolet; DMEM, Dulbecco's modified Eagle's medium; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; PVDF, polyvinylidene difluoride; TBS-T, Tris-buffered saline and Tween 20; *p*NP, *p*-nitrophenol; *p*NPP, *p*-nitrophenyl phosphate.

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and protein adsorption compared to those for unmodified HA and pure PCL control groups. As the HA content increased, improved cell anchorage and proliferation were obtained because more HA nanocrystals were exposed on the nanocomposite surface, which may lead to protein pre-adsorption and cell adhesion and proliferation [23].

Many reviews have concluded that fibrous nanocomposites of HA and synthetic materials (e.g., PLA, PCL, and PLGA) are potential tissue growth scaffolds with interconnected pores, ensuring accommodation of a large number and a uniform distribution of bone cells. Several strategies have been developed for the fabrication of nanofibrous composites of HA and PLA, such as blending β-tertiary calcium phosphate in a PLA matrix, the use of a surfactant as mediator, and the use of modified HA nanoparticles with low-molecular-weight PLA [24,25]. Yang et al. found that the differentiation rate of neural stem cells is higher on PLLA nanofibers than on microfibers and that it is independent of the fiber alignment [17].

Biomineralization is a process whereby a substrate after immersion in a simulated body fluid (SBF) forms a bone-like apatite layer. The SBF solution composition is similar to that of human blood plasma to mimic the microenvironment for bone cell growth. A bone-like mineral, apatite crystal can be observed on material surfaces after soaking in SBF. Previous studies demonstrated that apatite crystal can be deposited on the surface of materials such as ceramics [26,27], metals [27,28], and polymers [29,30] after immersion in SBF [31,32]. Although apatite precipitates on the material surface, the effective factors such as the chemical composition of the substrate, ionic concentration, and the components of the SBF solution on the growth rate of the apatite layer are still unclear [1,33]. Xu et al. studied the attachment and migration behaviors of human smooth muscle cells (SMCs) on aligned poly(*L*-lactic-*co*-ε-caprolactone) nanofibers. Their results showed that the SMCs displayed a spindle-like contractile shape on the axis of the aligned nanofibers and that smooth muscle actin fibers that formed inside SMCs were parallel to the direction of the nanofibers. Moreover, the aligned nanofibrous scaffold significantly enhanced the attachment and proliferation behavior of SMCs [34].

The purpose of this work is to study the effect of mineralization on PLA nanofibrous surfaces with random, partially aligned, and aligned structures. Two casted membrane groups (smooth and roughened) were used for comparisons. The effects of the preparation conditions of the materials and the precipitation parameters on the mineral HA particle growth rate were determined. Biological experiments on cell adhesion and proliferation, the expression of focal adhesion kinase (FAK) and actin filaments, and osteogenic properties were conducted using human adipose-derived stem cells (hADSCs).

2. Materials and methods

2.1. Materials

PLA (Mw = 13,000), methylene chloride, dimethyl sulfoxide (DMSO), sodium chloride, calcium chloride dihydrate, sodium hydrogen carbonate, magnesium chloride hexahydrate, sodium sulfate, potassium chloride, potassium phosphate dibasic heptahydrate, tris-(hydroxymethyl) aminomethane (Tris), 1.0 mol/L hydrochloric acid, 1.0 mol/L NaOH, and citric acid monohydrate were purchased from Sigma (St. Louis, MO, USA). Phosphate-buffered saline (PBS, pH 7.2–7.4) was prepared by dissolving a PBS tablet (Sigma) in MilliQ water (highly purified and deionized water, 18.2 MΩ·cm). All other chemicals were used directly as received.

2.2. Fabrication of electrospun fibers

PLA was constructed into nanofibers using a previously described electrospinning process with some modifications [18,35]. Briefly, PLA (6%) was dissolved overnight in a chloroform/DMSO mixture (75:25 v/v) under stirring to obtain a homogeneous solution. The system for the electrospinning process consisted of a power supply (ES30P, Gamma High Voltage Research, Ormond Beach, FL, USA), a syringe pump (LSP04-1A, Baoding Longer Precision Pump Co., Ltd., China), and a collecting metal board (or roller). The polymer solution (6%) was placed in a 5-mL plastic syringe fitted to a needle with a needle spinneret (ID: 0.17 mm). An electrospinning voltage of 21 kV was applied to the needle using a high-voltage power supply (Chargemaster CH50 Electrostatic Generating Power Supplies, SIMCO Industrial Static Control, Hatfield, PA, USA). A syringe pump was used to feed the polymer solution into the needle tip at a feed rate of 0.5 mL/h. The solution formed a Taylor cone upon exit and was collected onto a metal board (for random type fiber sheet collection, denoted as the FR group) or a self-made wire-based roller collector (roller diameter: 10 cm) with the distance from the needle to the metal board/roller surface set at 18 cm to form nanofiber sheets. The PLA fiber diameters can be controlled via the operating conditions. The rotation speed and collection time were set at 1800 rpm × 6 h and 900 rpm × 3 h for the aligned group (FA group) and the partially aligned group (FP group), respectively. For comparison, two PLA membranes fabricated by dissolving PLA in chloroform (membrane made of chloroform, denoted as the MC group) and chloroform/DMSO (75:25 v/v) (membrane made of chloroform/DMSO, denoted as the MD group) were prepared by a direct polymer casting method, with the PLA solution cast on glass and dried at room temperature for 8 min. PLA film (MD) with a porous morphology was obtained after the glass with PLA film was immersed in distilled water for 3 h and dried overnight at room temperature. All solvents used for the preparation of casted membrane groups and electrospun fiber groups were evaporated in a vacuum oven for 24 h and then immersed in distilled water. In total, five types of pure PLA were prepared, namely casted membranes MC and MD and electrospun fiber sheets FR, FP, and FA.

2.3. Mineralization of hydroxyapatite

All prepared pure materials were surface-mineralized with HA crystals. Briefly, the mineralization of HA nanocrystals was achieved by subjecting the prepared materials to a series of calcium and phosphate treatments, as described previously [31]. Briefly, 10× SBF was chosen to trigger homogeneous nucleation and growth. The electrospun PLA fiber sheets and casted roughened membranes with/without mineralized HA were sectioned into pieces 15 mm in diameter and 0.5 mm thick. A stable stock solution containing NaCl (58.44 g), KCl (0.375 g), $CaCl_2 \cdot 2H_2O$ (1.016 g), $MgCl_2 \cdot 6H_2O$ (3.675 g), and $NaH_2PO_4 \cdot H_2O$ (1.1998 g) with a pH value of about 4.1 was prepared in advance. In each step, the complete dissolution of the reagent was observed. At the beginning of a coating process, $NaHCO_3$ (0.84 g) was added at room temperature and the pH value rose to about 6.3. Each specimen was placed in a 24-well plate and immersed in 10× SBF at room temperature for 6 h (solution was changed every 2 h). All materials were subjected to the mineralization process with various immersion times. Upon removal from SBF at predetermined intervals, all materials were gently rinsed with d_2H_2O three times and dried in a vacuum oven overnight. Here, five types of HA-mineralized PLA were prepared: two casted membranes, denoted as MCHA and MDHA and three electrospun fiber sheets, denoted as FRHA, FPHA, and FAHA.

2.4. Contact angle and water content

The water contact angle on each film was determined at room temperature. Briefly, a nanofiber sheet was placed on the top of a stainless steel base. A drop of MilliQ water (1 μL) was placed on the surface of the film, and an image was taken by a CCD camera after an elapsed time of 30 s. The image was analyzed using ImageJ software (National Institutes of Health) to determine the water contact angle. The water content measurements were performed at 37 °C in PBS. Each sample was weighed (W_0) and immersed in PBS overnight (at least 12 h).