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Osteogenesis of human adipose-derived stem cells on poly(dopamine)-coated electrospun poly(lactic acid) fiber mats

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ABSTRACT

Electrospinning is a versatile technique to generate large quantities of micro- or nano-fibers from a wide variety of shapes and sizes of polymer. The aim of this study is to develop functionalized electrospun nano-fibers and use a mussel-inspired surface coating to regulate adhesion, proliferation and differentiation of human adipose-derived stem cells (hADSCs). We prepared poly(lactic acid) (PLA) fibers coated with polydopamine (PDA). The morphology, chemical composition, and surface properties of PDA/PLA were characterized by SEM and XPS. PDA/PLA modulated hADSCs' responses in several ways. Firstly, adhesion and proliferation of hADSCs cultured on PDA/PLA were significantly enhanced relative to those on PLA. Increased focal adhesion kinase (FAK) and collagen I levels and enhanced cell attachment and cell cycle progression were observed upon an increase in PDA content. In addition, the ALP activity and osteocalcin of hADSCs cultured on PDA/PLA were significantly higher than seen in those cultured on a pure PLA mat. Moreover, hADSCs cultured on PDA/PLA showed up-regulation of the ang-1 and vWF proteins associated with angiogenesis differentiation. Our results demonstrate that the bio-inspired coating synthetic degradable PLA polymer can be used as a simple technique to render the surfaces of synthetic biodegradable fibers, thus enabling them to direct the specific responses of hADSCs.

1. Introduction

Tissue engineering with the aim of developing biological materials that restore, maintain, or enhance harmed tissue and organ regeneration, has been intensively studied in the past few decades [1]. Electrospinning is a versatile technique that can be used to generate large quantities of micro- or nano-scale fibers from a wide variety of shapes and sizes of polymers, as well as composites containing inorganic materials such as hydroxyapatite [2,3]. Many studies focus on the biodegradable electrospun nanofiber polymers, as noted in a recent review of the literature [4], including synthetic polymers such as polyglycolide (PGA), poly(ε-caprolactone) (PCL) [5], poly(lactic acid) (PLA) [2,6] and their copolymers, P(LLA-CL) and PLGA [7], that have been approved by FDA for clinical use; blends of these [8]; and natural proteins, such as collagen, gelatin, silk, chitosan and alginate [9,10]. Among these, thermoplastic and biocompatible PLA is one of the most promising bio-based polymers, finding applications in textiles, drug-carriers and implants. More recently, PLA-based materials have found more durable applications in automotive, communication and electronic industries. However, pure PLA is a typical hydrophobic polymer, which has a lack of cell-recognition signals and limited use in biomaterials [11].

Several studies inspired by the adhesion of mussels to rocks in wet environments have reported that the adhesive proteins secreted by mussels mainly contain dihydroxyphenylalanine (DOPA) and lysine, and this has attracted great attention in the field of biomaterials [12]. Similarly, dopamine (DA) contains the same catechol functional group as that of the side chain of DOPA residues, as well as the same amine functional group, and a unique feature of polydopamine (PDA) is its ability to deposit on various hydrophobic or hydrophilic surfaces via self-polymerization by the oxidation of dopamine (DA) in a weak alkaline buffer solution [13]. Recently, a simple method for surface modification based on the mussel-inspired polydopamine (PDA) was demonstrated by Messersmith's group, and it has since been applied in wide range of biomedical applications [14,15]. The material-independent PDA coating can be easily and quickly obtained by base-triggered oxidation and polymerization of DA, and the PDA adlayer serves as a platform for post-modification, including spontaneous deposition of metal and bioceramic, as well as covalent immobilization of several serum adhesive proteins [13,16–20].

The objective of this work was to develop a simple procedure for DA-assisted coating on a PLA electrospun substrate. The polymer was incorporated into dopamine coatings, resulting in a simple one-step coating procedure. The deposited DA films were examined by X-ray photoelectron spectroscopy (XPS), and their efficacy in accelerating protein adsorption and short-term cell adhesion (1 and 3 h) was evaluated. Finally, the adhesion, proliferation, osteogenesis and angiogenesis of

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human adipose-derived stem cells were investigated to evaluate the efficacy of the surface modification.

2. Materials and methods

2.1. Fabrication of PLA nanofiber mat

PLA was constructed into nanofibers similar to our previously described electrospinning process with some modifications [2,21]. Briefly, PLA (Mn = 146,000, Nature Works LLC, Minnetonka, MN) was dissolved in a chloroform/DMSO mixture (75:25, v/v) by stirring overnight. For the electrospinning process, the system consisted of a power supply (ES30P, Gamma High Voltage Research Inc., USA), a syringe pump (LSP04-1A, Baoding Longer Precision Pump Co., Ltd., China), and a metal board used for collection. The resulting polymer solution (6%, w/v) within plastic syringe (5-mL) was then directly electrospun onto the aluminum foil-covered metal collector with the operating condition of 21 kV, 23 G injection needle (inside diameter of 0.17 mm), 0.5 mL/h injection rate, and a gap of 18 cm between the needle and the collector.

2.2. PDA coating

The deposition of PDA onto electrospun PLA nanofiber mats was conducted via direct immersion coating. All the nanofiber mats were rinsed with deionized water before PDA deposition. For the PDA coating, the substrates were immersed into a dopamine solution (1 and 2 mg/mL in 10 mM Tris, pH 8.5) at room temperature. PLA nanofiber mats were soaked in 0.3 mL of DA solution at room temperature for 12 h, followed by several rinses with deionized water. The elemental compositions of the PDA-coated polymer films were characterized with an electron spectroscope for chemical analysis (ESCA, PHI 5000 VersaProbe, ULVAC-PHI, Kanagawa, Japan) and the concentration of measured elements were given in atomic percent.

2.3. Antibacterial activity

To investigate the anti-bacterial effects of a PDA-coated PLA mat, all specimens were sterilized by soaking in 75% ethanol and exposure to UV light for 2 h. After washing three times with phosphate-buffered saline (PBS; Caisson Laboratories, North Logan, UT, USA), the specimens were placed in 24-well culture plates and mixed with 1 mL *Staphylococcus aureus* in LB culture media (4.0×10^4 bacteria per mL) and cultured for 3 and 12 h. At end time-points, aliquot of 0.1 mL from each group was mixed with 0.9 mL PrestoBlue® for 30 min. The solution in each well was then transferred to a new 96-well plate. Plates were read in a multiwell spectrophotometer (Hitachi, Tokyo, Japan) at 570 nm, with a reference wavelength of 600 nm. Bacteria cultured on the plate without specimens was used as a negative control, while referring to the $Ca(OH)_2$ group as a positive control. The results were obtained in triplicate from three separate experiments in terms of optical density (OD). In addition, the pH of the LB media was also monitored with an IQ120 miniLab pH meter (IQ Scientific Instruments, San Diego, CA). Triplicate measurements were used.

2.4. Human adipose-derived stem cell culture

The prepared nanofiber sheets and roughened membranes (15 mm in diameter) were sterilized under UV light for 1 h, and then immersed in 70% ethanol for 10 min for further sterilization. The materials were washed with phosphate buffer solution (Invitrogen, Grand Island, NY, USA) three times to eliminate any residual ethanol. The human adipose-derived stem cells (hADSCs) were obtained from Invitrogen at passage 3, and cells were expanded in culture medium until passages 3–8 (P3–P8) and seeded on various types of electrospun fiber sheet and roughened casted membranes with/without mineralized HA at a cell concentration of 10^4 cells/sample. The sample size for all material groups and the tissue culture plastic control (Ctrl) was three. The culture medium consisted of Dulbecco's modified Eagle's medium (DMEM, Caisson) with 20% fetal bovine serum (FBS; Caisson), 1% penicillin (10,000 U/mL)/streptomycin (10,000 mg/mL) (PS, Caisson) and kept in a humidified atmosphere with 5% CO_2 at 37 °C; the medium was changed every three days. The osteogenic differentiation medium was DMEM supplemented with 10^{-8} M dexamethasone (Sigma-Aldrich), 0.05 g/L L-ascorbic acid (Sigma-Aldrich) and 2.16 g/L glycerol 2-phosphate disodium salt hydrate (Sigma-Aldrich). The angiogenic induction reagent contained 2% fetal bovine serum, 1% penicillin (10,000 U/mL)/streptomycin (10,000 mg/mL), and 50 ng/mL vascular endothelial growth factor (Prospec, East Brunswick, NJ) were mixed with DMEM.

2.5. Cell adhesion and proliferation

hADSCs were routinely cultured onto the nanofibrous mats at different time periods at a density of 10^4 cells/mL Cell cultures were incubated at 37 °C in a 5% CO_2 atmosphere. After different culturing times, cell viability was evaluated using the PrestoBlue® (Invitrogen, Grand Island, NY) assay. Briefly, at the end of the culture period, the medium was discarded and the specimens were washed with cold PBS. Each well was then filled with the medium with a 1:9 ratio of PrestoBlue® in fresh DMEM and incubated at 37 °C for 30 min. Finally, the solutions were transferred to a multiwell spectrophotometer (Hitachi, Tokyo, Japan) for absorbance measurement at 570 nm. Cells cultured on the tissue culture plates were used as a control (Ctl). The results were obtained in triplicate from three separate experiments in terms of optical density (OD).

2.6. Cell morphology

After cell seeding for 3 h, the specimens were washed three times with cold PBS and fixed in 1.5% glutaraldehyde (Sigma) for 2 h at room temperature. The specimens were further dehydrated using a serious of graded ethanol solutions for 20 min at each concentration, and finally dried with liquid CO_2 using a critical point dryer device (LADD 28000; LADD, Williston, VT). The dried specimens were mounted on stubs, coated with gold, and viewed using a scanning electron microscope (JEOL JSM-7401 F, Tokyo, Japan). For immunofluorescent staining, cells were seeded on different substrates for the same length of time. Cells were fixed by 4% paraformaldehyde for 30 min and permeabilized by 0.1% Triton X-100 for 15 min. Specimens was then blocked with 2% BSA for 1 h. These cells were incubated with AlexaFluor-594-conjugated phalloidin (F-actin, red color) for 1 h at room temperature. The nuclei were stained with DAPI (4′,6-diamidino-2-phenylindole, dilactate) for 1 h at room temperature. The samples were then washed with TBS-T three times and the cells were photographed under indirect immunofluorescence using a Zeiss Axioskop 2 microscope (Carl Zeiss, Thornwood, NY).

2.7. Collagen adsorption on substrates

After being cultured for different periods of time, the amounts of collagen (COL) secreted from cells onto the fiber mat's surface were analyzed using ELISA assay. The cells were detached using a trypsin-EDTA solution (Cassion) after being washed three times with cold PBS. Specimens were then washed three times with PBS-T (PBS containing 0.1% TWEEN-20), followed by blocking with 5% bovine serum albumin (BSA; Gibco) in PBS-T for 1 h. Dilutions of primary antibodies were set at 1:500. Following this procedure, samples were incubated with anti-human β-actin or anti-human COL I antibody (GeneTex, San Antonio, TX) for 3 h at room temperature. Afterwards, samples were washed three times with PBS-T for 5 min and incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h at room