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Imparting electroactivity to polycaprolactone fibers with heparin-doped polypyrrole: Modulation of hemocompatibility and inflammatory responses

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ABSTRACT

Hemocompatibility, anti-inflammation and anti-thrombogenicity of acellular synthetic vascular grafts remains a challenge in biomaterials design. Using electrospun polycaprolactone (PCL) fibers as a template, a coating of polypyrrole (PPy) was successfully polymerized onto the fiber surface. The fibers coated with heparin-doped PPy (PPy-HEP) demonstrated better electroactivity, lower surface resistivity (9–10-fold) and better anti-coagulation response (non-observable plasma recalcification after 30 min vs. recalcification at 8–9 min) as compared to fibers coated with pristine PPy. Red blood cell compatibility, measured by% hemolysis, was greatly improved on PPy-HEP-coated PCL in comparison to uncoated PCL ($3.9 \pm 2.1\%$ vs. $22.1 \pm 4.1\%$). PPy-HEP-coated PCL fibers also exhibited higher stiffness values (6.8 ± 0.9 MPa vs. 4.2 ± 0.8 MPa) as compared to PCL fibers, but similar tensile strengths. It was also observed that the application of a low alternating current led to a 4-fold reduction of platelet activation (as quantitated by CD62p expression) for the PPy-HEP-coated fibers as compared to non-stimulated conditions. In parallel, a reduction in the leukocyte adhesion to both pristine PPy-coated and PPy-HEP-coated fibers was observable with AC stimulation. Overall, a new strategy involving the use of hemocompatible conducting polymers and electrical stimulation to control thrombogenicity and inflammatory responses for synthetic vascular graft designs was demonstrated.

1. Introduction

The replacement of vascular tissue often becomes a clinical necessity during arterial diseases [1]. In cases where vascular allografts are not readily available or unsuitable for use, synthetic grafts, most commonly of polytetrafluoroethylene (PTFE), are often used [1]. However, due to the body's natural defense mechanisms against foreign materials, synthetic grafts are often susceptible to infection. Blood vessel injury, thrombus formation and intimal hyperplasia are typically the other main reasons for the failure of these synthetic grafts [2]. Both decellularized vascular matrices [3,4] and decellularized pre-seeded synthetic constructs [2] comprising the extracellular matrix (ECM) material have shown promising results in pre-clinical studies. These vascular grafts were able to support endothelial cell growth and best matches the compliance values of native vascular tissues [5]. However, the disadvantages of these patient-specific grafts include the time and cost required for the decellularization process [5]. Hence, alternative strategies that involve simple processing steps to fabricate synthetic materials with not only desirable bulk properties, but also surface properties that allow for the amelioration of immune response and thrombogenicity are highly desirable.

Polycaprolactone (PCL) is currently being investigated extensively for biomedical applications owing to its flexibility, biocompatibility and long-term degradation *in vivo* [6]. PCL is already used as a bioresorbable material for surgical sutures [6] and has recently gained approval by US FDA for use in a load-bearing bone

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implant fabricated by 3D printing [7]. Leveraging on its good biocompatibility, various groups have performed surface modifications on PCL with various techniques such as hydrolysis [8], plasma treatment [9], aminolysis [10] and radical polymerization [11,12] in order to improve its cell-supporting capabilities and to explore its efficacy as a material for tissue-engineered vascular grafts (TEVG). The presence of a confluent and healthy layer of endothelial cells on the graft's inner surface is generally a positive indication of the anti-thrombogenic nature of the graft. Hence, a variety of engineering strategies have been applied to materials in order to induce rapid endothelial cell coverage [13–15]. Great strides have been made in the area of TEGVs with pre-seeded grafts, where clinical trials have demonstrated remarkable increases in long-term patency of large-diameter grafts [16,17]. Seeded small-diameter vascular grafts (< 6 mm), however, continue to be beset by high failure rates mainly associated with anastomotic hyperplasia [2,18] (thickening tissue at junction between graft and native tissue, due to hyperproliferation of cells and deposition of fibrotic tissue). Our group has recently evaluated the effects of surface modification of PCL on the regulation of thrombogenicity by endothelial cells [19]. The thrombogenicity of the endothelial cells, indicated by the production of cellular thrombogenic or anti-thrombogenic factors, appeared to be partly mediated by cellular responses to the material itself [19,20].

Early work using electrospun polyurethane mats for vascular prosthesis took place in the late 1970s [21]. Electrospinning allows for the control of the mechanical properties, microfiber diameter and porosity of tissue-engineered vascular grafts [22], and in previous studies, various electrospun polymer nanofibers have been used as templates for the *in situ* polymerization of conducting polymers such as polypyrrole (PPy) [23,24]. PPy, a versatile conducting polymer that has been found to possess good conductivity and biocompatibility, has been explored for biomedical applications [25,26]. Anionic dopants used during the polymerization process confer dopant-specific electrochemical properties to the surface of PPy, while the deposition of PPy would confer electrical conductivity and hence allows for the transmission of currents along the electrospun fibers. External electric fields have already been exploited for controlling epithelial cell orientation, migration and proliferation [27,28]. While these studies have effectively demonstrated biological responses in other non-excitable cells to external electric fields, [27–31], no study has yet examined the effects of electrically-stimulating a material on its thrombogenic and inflammatory properties.

In this current study, fibrous PCL substrates were prepared using electrospinning and a layer of PPy material was subsequently deposited onto the individual fibers. Using heparin, an anti-thrombogenic biomolecule [32], as a dopant for the PPy coating, electroactivity and hemocompatibility was conferred on the PCL mesh. We then demonstrate for the first time the reduction of thrombogenicity and inflammatory responses using an alternating current which was passed directly through the PPy-HEP-coated PCL mesh.

2. Materials and methods

2.1. Chemical reagents and cells

Polycaprolactone pellets (PCL, average Mn 80,000), heparin sodium salt (Grade 1-A, 17–19 kDa), (+/−)-10-Camphorsulfonic acid (CSA, 98%), ammonium persulfate (APS, 98%), pyrrole (98%), 1,1,1,3,3,3-hexafluoro-2-propanol (HFP,>99%), toluidine blue reagent, sodium chloride and hydrochloride acid were obtained from Sigma–Aldrich Chemical Co, and were used without further purification.

2.2. Electrospinning of PCL fibrous meshes

PCL (10% w/v) was dissolved in HFP and electrospun from a blunt-ended 22G needle (inner tip diameter of 0.4 mm) at 11 kV using a high-voltage power supply (Gamma High-Voltage Research). A syringe pump was used to feed the polymer solution at a rate of 3 mL/h and the PCL fibers were collected on an aluminum foil set 8 cm from the needle. A single mesh of 14–15 cm in diameter and 150–200 μm in thickness was obtained by spinning the PCL solution (3 mL). After fabrication, the meshes were dried in a vacuum oven overnight and cut into required sizes in subsequent experiments.

2.3. Template polymerization of doped PPy on PCL fibrous meshes

The polymerization reactions of PPy on PCL fibers were carried out with no dopant for the pristine PPy coating, and with heparin, or chloride dopants for the doped PPy coatings. Hydrochloric acid (1 M) or heparin (in heparin/pyrrole weight ratio of 1) was dissolved with pyrrole monomers (15 mM) in dH_2O. The electrospun PCL scaffolds were immersed in the solutions and ultrasonicated for 10 s (250 W, 5 s pulses) on ice. The PCL scaffolds were then incubated in the polymerization mixture on ice for 1 h before APS (15 mM) was added dropwise under vigorous stirring. The polymerization reactions on the PCL scaffolds proceeded for either 4 h or 16 h at 4 °C depending on sample group. After polymerization, the coated PCL scaffolds were washed twice in fresh dH_2O for 10 min under stirring and followed by 10 s ultrasonication (250 W, 5 s pulses) on ice to remove unreacted pyrrole monomers on the surface.

2.4. X-ray photoelectron spectroscopy (XPS) characterization

For XPS measurements, an AXIS HSi spectrometer (Kratos Analytical Ltd.) with a monochromatized Al Kα X-ray source (1486.6 eV photons) was employed at a constant dwell time of 100 ms and a pass energy of 40 eV. The anode voltage was 15 kV and the anode current was 10 mA. The pressure in the analysis chamber was maintained at 10^{-7} to 10^{-9} Torr during each measurement. All the wide scans and the core-level spectra were obtained at a photoelectron take-off angle of 90° without Argon ion etching. Wide scans were recorded within a range of 0–1100 eV. To compensate for the surface charging effect, all of the core-level spectra were referenced to the C 1s hydrocarbon peak at 284.6 eV. In the spectral curve-fitting, the line width (i.e. full width at half-maximum) of the Gaussian peaks was maintained constant for all components in a particular spectrum range. The peak area ratios for the various elements were correlated with the experimentally-determined instrumental sensitivity factors.

2.5. Tensile testing

The PCL and PPy-coated PCL scaffolds were cut into dumbdell-shaped samples in accordance to ASTM D638 standards and stretched using a 10 N load cell (Instron 5567, Instron) to obtain stress-stain curves, from which Young's modulus and tensile strength could be found.

2.6. Toluidine blue (TB) assay

The amount of heparin exposed on the PPy nanocoating on the PCL fibrous meshes was quantified using the Toluidine blue (TB) assay [33]. The TB solution was prepared by dissolving TB reagent (2.5 mg) and NaCl (0.2 wt%) in HCl solution (0.01 M, 50 mL). Standard heparin solutions were made by dissolving known amounts of heparin sodium salt in sodium chloride solution

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