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Synthesis of RGD-peptide modified poly(ester-urethane) urea electrospun nanofibers as a potential application for vascular tissue engineering

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HIGHLIGHTS

- A novel PEUU-RGD electrospun mats for vascular application were fabricated.
- PEUU-RGD mat was a good intima for prevent the formation of thrombi or hyperplasia.
- The fabricated mats showed excellent mechanical properties and high biocompatibility.
- Inhibition of platelet adhesion of the mats were also tuned.

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ABSTRACT

The development of a biomimetic surface which is able to promote endothelialization is fundamental in the research for blood vessel substitutes to overcome the formation of thrombi or hyperplasia. In the present work, the fabrication of acrylamide-terminated glycine-arginine-glycine-aspartic peptides (Ac-GRGD) modified poly(ester-urethane) urea (PEUU) nanofibrous mats via electrospinning technique followed by covalent immobilizing method for improving its endothelialization was successfully achieved. Series of PEUU based polymers including PEUU, PEUU with t-butoxycarbonyl group (PEUU-Boc), and PEUU-amino group (PEUU-NH_2) were synthesized by a two-step solution polymerization and a de-protection process. The PEUU-RGD as-prepared nanofibrous mat was characterized using different techniques, such as, scanning electron microscopy, solide-state ^{13}C CP-MAS nuclear magnetic resonance, and stress-strain test. In addition, to motivate cell adhesion and proliferation, PEUU nanofibers mat was immobilized by coupling of Ac-GRGD. The results present that incorporation of Ac-GRGD peptide improved the mechanical properties and does not have negative effect on the morphology and the structure of PEUU nanofibers. From cell viability and cell morphology results, the prepared PEUU-RGD nanofiber mats are cytocompatible. Interestingly, the immobilized PEUU-RGD nanofibers possess lower hemolysis rate and an improved inhibition of platelet adhesion. Overall, Ac-GRGD peptides immobilized PEUU nanofibrous mats may have a potential application for vascular tissue engineering.

1. Introduction

For blood vessel tissue engineering, an ideal vascular replacement should possess excellent biocompatibility and mechanical properties [1,2]. It is also well known that native blood vessels consist of three different layers: intima, media, and adventitia. The intima, consists of a continuous monolayer of endothelial cells

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(ECs) [3]. A healthy endothelial layer is the only fully blood compatible surface that completely avoids thrombus development [4–6]. However, clinically employed vascular scaffolds do not spontaneously endothelialize; endothelial cells typically cover only a small percentage of the luminal surface, leaving a portion of the scaffolds without a complete endothelial cell layer [7,8]. However, tissue engineering offers a potential "ideal" small diameter vascular replacement with one concept creating biodegradable polymeric structures that would be used to provide mechanical support and continuous blood supply while promoting vascular tissue development in situ [9–12]. For this reason, how to promote endothelial cell growth, adhesion and proliferation on vascular scaffolds are an active area of research [13–15].

Electrospinning is a versatile technique, which can produce fibrous scaffolds with fiber diameters ranging from tens of nanometers to a few micrometers [16–18]. This method has been widely used to prepare tubular scaffolds with a rotation mandrel to collect the fibers [19]. The fibrous structures produced by the electrospinning could mimic the natural extracellular matrix (ECM) and contribute to the cell proliferation process by the electrospinning could mimic the natural extracellular matrix (ECM) and contribute to the cell proliferation process [20–24].

Segmented polyurethanes have been employed as elastomeric biomaterials because of their tunable mechanical properties, processability, and good biocompatibility in a variety of applications [25–27]. Although our group previous studies have shown that a novel method to developed PEUU based scaffold for the subsequent non-interfering modification with heparin and TPS peptide, but the patency of 20% at 8 weeks after implanted in rabbit carotid artery [28,29]. In this paper, the biodegradable PEUU-NH_2 elastomers were synthesized by a de-protection process from PEUU-Boc polymers. PEUU-NH_2 nanofibers immobilized with Ac-GRGD peptides were fabricated through carboxyl-amino condensation reaction. Additionally, in vitro assays with endothelial cells (human umbilical vein endothelial cells, HUVECs) were performed to test the potential of PEUU-RGD nanofibers to promote adhesion and control proliferation.

2. Materials and methods

2.1. Materials

Polycaprolactone diol (PCL2000, Mn = 2000), N-Boc-serinol (97%), hexamethylene dissocyanate (HDI), stannous octotate (Sn $(Oct)_2$), butanediamine were provided by Sigma-Aldrich and used as received unless specified. Acrylamido-terminated glycine-arginine-glycine-aspartic peptides (Ac-GRGD) and fluorescein isothiocyanate labeled acrylamido-terminated glycine-arginine-glycine-aspartic peptides (Ac-GRGD-FITC) were obtained from China Peptides Co., Ltd. (Shanghai, China). Chloroform (CF), trifluoroacetic acid (TFA), N-(3-dimethylaminopropyl)-N′-ethylcaobodiimide hydrochloride (EDC), and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich Trading Co., Ltd. (Shanghai, China). 1, 1, 1, 3, 3, 3-hexafluoro-2-propanol (HFIP) was acquired from Shanghai Darui Finechemical Co., Ltd. (Shanghai, China). Anhydrous ethanol (EtOH) and Anhydrous dimethylsulfoxide (DMSO) were purchased from Changshu Hongsheng Chemical Reagent Co., Ltd. (Changshu, China). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), phosphate buffer saline (PBS, pH = 7.4), paraformaldehyde (POM), 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumbromide (MTT), penicillin–streptomycin and trypsin were purchased from Shanghai Yuanxiang Medical Equipment Co. Ltd (Shanghai, China). 4′, 6′-diamidino-2-phenylindole (DAPI) and rhodamine-conjugated phalloidin were obtained from Invitrogen (USA). All chemicals were used without further purification. Water used in all experiments was purified using a Milli-Q Plus 185 water purification system (Millipore, Bedford, MA) with resistivity higher than 18 MΩ cm.

2.2. Synthesis of PEUU based polymer

PCL diol (PCL2000) and N-Boc-serinol were dissolved in DMSO in a three-necked flask with the concentration of 10% (w/v) and HDI was added under nitrogen gas for protection. Followed this process, the addition of $Sn(Oct)_2$ (0.05 wt% based on the monomer concentration) and the reaction was carried out for 3 h at 80 °C. Butanediamine/DMSO solution at 2% (w/v) was added drop wise to the pre-polymer solution and the molar ratio of PCL2000 with N-Boc-serinol, HDI and butanediamine was 1:2:1. The reaction continued with stirring for 18 h at 40 °C. Then the polymer solution was extruded into the deionized water for precipitation. PEUU-Boc was eventually obtained after being dried in a vacuum oven at 60 °C for 48 h. PEUU was also synthesized in a similar method with the exceptional of addition N-Boc-serinol.

The synthesized PEUU-Boc was dissolved in CF/TFA solvent (v: v = 50/50) at the concentration of 5% (w/v, PEUU-Boc/solvent) in a round bottom flask and stirred for 1 h at 25 °C to remove the Boc-preotcted groups. The excess CF and TFA were moved by rotary evaporation and the polymer was precipitated and neutralized in 2% (w/v) Na_2CO_3 aqueous solution (pH = 11.4) to remove residual TFA. The product was then washed with distilled water and rinsed in isopropanol for 1 day, followed by drying in a vacuum oven at 60 °C for 2 days [29].

2.3. Fabrication of electrospun nanofibers

1.2 g of polymers (PEUU, PEUU-Boc, PEUU-NH_2) were dissolved in 10 mL of HFIP at room temperature for 24 h with vigorous magnetic stirring to prepare the solution for electrospinning.

The polymers (PEUU, PEUU-Boc, PEUU-NH_2) in 1, 1, 1, 3, 3, 3-hexafluoro-2-propanol (HFIP) solution (12%, w/v) were fed at 1.0 mL/h, 0.5 mL/h, 0.55 mL/h from a capillary charged at 9 kV, 10 kV, 8.45 kV, respectively, and perpendicularly located 14 cm from the target thin aluminum foil which acting as a collector which was positioned horizontally and grounded. All of the electrospinning processes were carried out at around 25 °C and 50% ± 2% relative humidity.

2.4. Nanofibers surface immobilization with Ac-GRGD peptide

Ac-GRGD molecules were covalently attached to the PEUU-NH_2 nanofibrous mats using EDC/NHS chemistry as previously described with modification [30]. Briefly, the PEUU-NH_2 nanofibrous mats were saturated with EtOH for 5 h prior to immobilization reaction. After this step, the PEUU-NH_2 nanofibrous mats were collected and washed first with PBS 3 time. At the same time, Ac-GRGD (8.98 mmol, 4 mg), EDC (100 mmol, 19.17 mg), and NHS (250 mmol, 28.77 mg) were dissolved in 2 mL DMSO, 2 mL PBS, 2 mL PBS, respectively. Then all the three solutions were mixed and stirred for 3 h to activate the carboxyl group of Ac-GRGD. The activated Ac-GRGD was added dropwise into the above PBS solution of the PEUU-NH_2 nanofibrous mats (10 mL) under stirring for 2 days. After 2 days crosslinking reaction at room temperature, mats were washed with copious amounts of distilled water to remove the byproducts, and then were lyophilized for 2 days. For comparison, PEUU-NH_2 with Ac-GRGD-FITC conjugation was also prepared in a manner similar to that used to form the PEUU-RGD-FITC nanofibrous mats. The only difference is the use of Ac-GRGD-FITC instead of the use of Ac-GRGD (Fig. 1(c)).