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Biomimetic modification of polyurethane-based nanofibrous vascular grafts: A promising approach towards stable endothelial lining

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ABSTRACT

The emerging demand for small caliber vascular grafts to replace damaged vessels has attracted research attention. However, there is no perfect replacement in clinical use yet, mainly due to low patency rate of synthetic small caliber grafts. The main pathology behind low patency rate include thrombosis and graft/vessel hemodynamic mismatch, leading to intimal hyperplasia. Rapid in-situ endothelialization of vascular grafts is considered as one of the best strategies to overcome these complications. In the present study, Heparin and VEGF were immobilized via self-polymerization and deposition of polydopamine (PDA) on polyurethane (PU) nanofibrous scaffolds to improve endothelialization. Polyurethane nanofibrous scaffold (PUNF) that mimics vascular extracellular matrix (ECM) was chosen owing to its biocompatibility, biodegradability. Scanning electron microscopy (SEM), water contact angle (CA) measurement and Raman spectroscopy were used to characterize the surface, and tensile test was used to analyze mechanical properties before and after surface modification of the scaffolds. It was found that tensile strength and young's modulus were significantly increased after PDA coating on PUNF membranes. The hemocompatibility tests revealed that surface heparinization significantly inhibited the adhesion of platelet on the scaffolds. Immobilization of VEGF on the scaffolds significantly enhanced the proliferation of human umbilical vein endothelial cells (HUVECs) through enhanced cells adhesion and improved cell-scaffold interactions. The results suggest that dual-factor immobilization resulted in not only confluent monolayer of endothelial cells but also conferred excellent antithrombotic properties to the surface. This method of surface modification (immobilization of Heparin, VEGF by PDA layer) is suggested as a promising modification technique to increase hemocompatibility of small-diameter vascular grafts.

1. Introduction

Many prosthetic vascular grafts made from biocompatible polymers such as polyurethane (PU) [1], expanded poly (tetrafluoroethylene) (ePTFE) [2], poly(ethylene terephthalate) (PET) [3], and polycaprolactone (PCL) [4] have been developed. However, they frequently suffer from low patency rate when applied as small diameter (<6 mm) bypass grafts [5].

Efforts to improve a small caliber vascular graft have been challenging over the past decade. A fully functional endothelial layer is essential for a small diameter vascular graft to be patent in long-term [6], owing to its role in the prohibition of excessive tissue ingrowth (intimal hyperplasia) and thrombogenesis that are two main reasons of graft failure. Therefore, both seeding of endothelial cells onto the luminal surface of the graft or in situ endothelialization improves long-term permanence of cardiovascular implants [7] and small-diameter prostheses. Since endothelial cells attach on the surface of an extracellular matrix (ECM) in nature, production of biomaterials equivalent to ECM can help the attachment, proliferation, and phenotypic maintenance of endothelial cells on vascular scaffolds [8].

Recently, electrospinning technology has been extremely investigated as a method to make ECM-mimicking structures which can be noted as artificial ECM or scaffolds [9–11]. Electrospinning is a potent technique, which can generate fibrous structures from diverse materials such as polymers, ceramics, and composites [12]. Electrospun fibers are similar to the natural ECM components with many advantages such as the simplicity of the production process and scale-up, and also, capability to produce fibers with various diameters ranging from nanometer to micrometer sizes [13].

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Therewith, the high surface area to volume ratio and interconnected pores of these fibrous meshes result in desirable cell attachment and oxygen/nutrient transport, respectively. Also, multiple polymers entailing blood compatible ones (i.e. PTFE, PET, PCL and PU) can be electrospun into ultrafine fibers. All the mentioned benefits make electrospinning an ideal method for production of nanofibrous mats [14]. In comparison, PU-based materials have been vastly investigated for preparation of bio- and blood-compatible products. Recently, Adipurnama et al. have reviewed a variety of different surface modifications of PU and their effects on endothelialization for vascular graft applications [15].

Several surface modification methods have been applied to improve blood-compatibility of vascular grafts especially polyurethane based ones [16]. Functional groups or bioactive macromolecules can modify the surface of nanofibrous vascular grafts in order to accelerate endothelialization of electrospun mats [17]. For immobilization of biomolecules on polymeric biomaterials, various physical and chemical techniques are attainable. An appropriate functionalization method must be selected because most vascular grafts are produced from inert hydrophobic polymers lacking functional moieties for chemical conjugation. In our earlier attempts, collagen type I was grafted onto plasma-activated and acrylic acid coated PU nanocomposite [17]. In irradiation techniques such as plasma treatment high energy sources are used to introduce reactive groups for subsequent immobilization of desired biomolecules [18]. For versatile solid materials from metal to synthetic polymers, another facile surface modification method is simple dip-coating with dopamine solution [19]. Under slightly basic conditions, a stable layer that is adherent to the surface of materials, is created by oxidative polymerization of dopamine. The reaction environment in post-modification with polydopamine layer is simple and clean compared to other chemical immobilization methods and allows functionalization with bioactive molecules containing thiols or primary amines via imine formation and/or Micheal addition [18].

It is known that rapid endothelialization is a prerequisite for artificial vascular grafts, and having non-thrombogenic surface before composition of a fully endothelial layer can make it ideal blood contacting surface. Vascular endothelial growth factor (VEGF) promotes proliferation and migration of endothelial cells (EC), results in angiogenic vascular growth, and induces differentiation of pluripotent stem cells to blood progenitor cells [20]. Heparin, one of the most commonly used clinical anticoagulant reagents, has positive effects on ECs growth and proliferation by binding and stabilizing cell growth factors (GFs) [21]. Binding of heparin and GFs principally happens via electrostatic interactions between N- and O-sulfated groups with negative charge of heparin and the basic lysine and arginine residues of FGF-2 or VEGF [22]. Diffusion of GFs is decelerated by binding to heparin [23], and interaction of growth factors with heparin is observed to be necessary for storage, release, and protection from pH, heat, and enzymatic degradation [24]. Recently, heparin modified biomaterials demonstrated excellent exploits in ECs growth and proliferation [25].

In the present investigation, nanofibrous electrospun vascular scaffolds based on elastic biodegradable PU substrates were fabricated to mimic the native vascular ECM. For surface modification, a poly dopamine-mediated immobilization platform together with a cell adhesive growth factor, VEGF, and an anti thrombogenic factor, heparin was developed. The effects of PDA-coating and VEGF and heparin immobilization on the morphology, hydrophilicity, hemocompatibility and mechanical properties of nanofibrous PU membranes were evaluated in details. Finally, the effect of PDA, VEGF, heparin and dual factors on regulation of HUVEC attachment and proliferation was evaluated.

2. Materials and methods

2.1. Materials

Thermoplastic Polyurethane (PU, Tecoflex, SG-80A) was purchased from Lubrizol, USA. 3-Hydroxytyraminium chloride was purchased from Merck Co., Schuchardt OHG. *Tris* (hydroxymethyl) amino methane was obtained from Merck KGaA. Chloroform and Methanol were purchased from Merck Co., Germany. Heparin sodium salt and VEGF were obtained from Sigma-Aldrich (St. Louis, MO, USA). HUVEC cells were obtained from the National Cell Bank of Iran (Pasteur Institute of Iran). Other unspecified chemicals were purchased from Sigma. All the chemicals were of analytical grade and used without further purification. Electrospinning machine was provided by Fanavarn Nanomeghyas Co. Ltd, Tehran, Iran.

2.2. Preparation of polyurethane nanofibers (PUNF)

Nanofibrous scaffolds were produced utilizing a standard electrospinning setup. PU was dissolved in a mixture of chloroform and methanol with a ratio of 1:1 (v/v) as solvent system to prepare electro-spinning solution at 3/5% concentration (w/v), according to our previous study [26]. Solutions were pumped at a flow rate of 1 mL/h using a syringe pump while a potential of 20 kV was applied between the spinneret and a grounded collector located 10 cm apart from the spinneret.

2.3. Fabrication of nanofibrous vascular grafts

A one-step electrospinning method was used in order to fabricate nanofibrous vascular graft. For this, a cylindrical mandrel with diameter of 4 mm was fabricated using stainless steel alloy. Then it was used in the electrospinnig machine as a rotatory collector, in order to fabricate the tubal scaffolds in one-step process. Previously, optimized set up was used to fabricate nanofibrous scaffolds in the shape of small diameter vessel.

2.4. Surface modification of PUNF

2.4.1. Poly dopamine coating (D-PUNF)

For polydopamine (PDA) coating, the PUNF mats were cut into a circular shape (area: 1.99 cm^2) which was submerged in 3,4-dihydroxyphenylamine (2 mg/ml) dissolved in Tris-HCl buffer (10 mM, pH 8.5) and shaken on a rocker for 1 h. After this process, the samples were washed three times in deionized water to remove unbound PDA. The resultant membranes were used for characterization and further surface modifications. Then, polydopamine-coated PUNF (D-PUNF) were modified with either heparin and VEGF or both of them.

2.4.2. Heparin immobilization (H-D-PUNF)

The prepared D-PUNF was immersed into the heparin solution (PBS buffer solution as solvent, pH 7.4) at 4 °C overnight. The heparin concentration (C) in solution was 2.0 g/L. Then the membrane was taken out and rinsed with deionized water entirely in order to remove physically adsorbed heparin. Thereafter, the resultant modified membranes (H-D-PUNF) were used for characterization.

2.4.3. VEGF immobilization (V-D-PUNF)

For immobilization of VEGF, the PDA deposited mats were immersed in 1000 ng/ml VEGF (dissolved in 10mMTris-HCl buffer, pH 8.5) overnight at 4 °C. The volume of VEGF solution was fixed to 400 μL for the reaction.

2.4.4. VEGF and Heparin immobilization (VH-D-PUNF)

For concurrent immobilization of VEGF and heparin, equal volumes of heparin and VEGF solutions (200 μl) were added on the PDA-deposited mats (D-PUNF) for overnight at 4 °C.