Acta Biomaterialia 51 (2017) 138–147

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

End-point immobilization of heparin on plasma-treated surface of electrospun polycarbonate-urethane vascular graft

Xuefeng Qiu [a,b,c,1], Benjamin Li-Ping Lee [a,1], Xinghai Ning [a], Niren Murthy [a], Nianguo Dong [b], Song Li [a,c,d,*]

[a] Department of Bioengineering, University of California, Berkeley, CA 94720, USA
[b] Department of Cardiovascular Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, People's Republic of China
[c] Department of Bioengineering, University of California, Los Angeles (UCLA), Los Angeles, CA 90095, USA
[d] Department of Medicine, University of California, Los Angeles (UCLA), Los Angeles, CA 90095, USA

ARTICLE INFO

Article history:
Received 5 September 2016
Received in revised form 9 December 2016
Accepted 5 January 2017
Available online 6 January 2017

Keywords:
Vascular grafts
Electrospinning
Polycarbonate-urethane
Surface modification
Plasma treatment
Heparin

ABSTRACT

Small-diameter synthetic vascular grafts have high failure rate due to primarily surface thrombogenicity, and effective surface chemical modification is critical to maintain the patency of the grafts. In this study, we engineered a small-diameter, elastic synthetic vascular graft with off-the-shelf availability and anti-thrombogenic activity. Polycarbonate-urethane (PCU), was electrospun to produce nanofibrous grafts that closely mimicked a native blood vessel in terms of structural and mechanical strength. To overcome the difficulty of adding functional groups to PCU, we explored various surface modification methods, and determined that plasma treatment was the most effective method to modify the graft surface with functional amine groups, which were subsequently employed to conjugate heparin via end-point immobilization. In addition, we confirmed *in vitro* that the combination of plasma treatment and end-point immobilization of heparin exhibited the highest surface density and correspondingly the highest anti-thrombogenic activity of heparin molecules. Furthermore, from an *in vivo* study using a rat common carotid artery anastomosis model, we showed that plasma-heparin grafts had higher patency rate at 2 weeks and 4 weeks compared to plasma-control (untreated) grafts. More importantly, we observed a more complete endothelialization of the luminal surface with an aligned, well-organized monolayer of endothelial cells, as well as more extensive graft integration in terms of vascularization and cell infiltration from the surrounding tissue. This work demonstrates the feasibility of electrospinning PCU as synthetic elastic material to fabricate nanofibrous vascular grafts, as well as the potential to endow desired functionalization to the graft surface via plasma treatment for the conjugation of heparin or other bioactive molecules.

Statement of Significance

Vascular occlusion remains the leading cause of death all over the world, despite advances made in balloon angioplasty and conventional surgical intervention. Currently, autografts are the gold-standard grafts used to treat vascular occlusive disease. However, many patients with vascular occlusive disease do not have autologous vascular graft available. Therefore, there is a widely recognized need for a readily available, functional, small-diameter vascular graft (inner diameter of <6 mm). This work addresses this critical need by developing a method of antithrombogenic modification of synthetic grafts.

1. Introduction

Vascular diseases, specifically coronary and peripheral arterial diseases, affect millions of people and remain a worldwide problem as the prevalence continues to rise due to continued growth of the aging of the population [1]. Thus, there is a major unmet need for small-diameter (<6 mm) vascular grafts as bypass and blood vessel replacement, since autologous vessels, which represent the gold standard and have been shown to demonstrate superior clinical performance, are not always available [2–4]. However, the success of synthetic grafts, such as ones made of Dacron (polyethylene terephthalate; PET) and Teflon (expanded polytetrafluoroethylene; ePTFE), is limited primarily to large-caliber vessels with high blood flow because of their surface thrombogenicity as well as poor elasticity and low compliance that

* Corresponding author at: Department of Bioengineering and Medicine, University of California, Los Angeles, 410 Westwood Plaza, 5121 Engineering V, Los Angeles, CA 90095, USA.
E-mail address: songli@ucla.edu (S. Li).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.actbio.2017.01.012
1742-7061/

cause acute thrombus formation and intimal hyperplasia, respectively [5,6]. Similarly, despite the availability of commercially available heparin-coated synthetic vascular grafts, such as Propaten, that offer significantly better long-term patency over the standard ePTFE grafts but still has 63.5% failure rate after 48 months, the unmet need for small-diameter vascular grafts with low thrombogenicity still exists [7].

In order to address the shortcoming and develop an ideal, small-diameter vascular graft with mechanically compliant and anti-thrombogenic properties similar to those of native vessels, different combinations that integrated unique *in vitro* endothelialization techniques or novel surface modifications to endow non-thrombogenic properties with synthetic (i.e. polyesters) as well as natural (i.e. decellularized) surfaces have been investigated [8–13]. From a synthetic material perspective, many research groups have employed electrospinning to fabricate fibrous scaffolds for vascular regeneration, since fibrous structures obtained through electrospinning can be tailored to closely resemble the structure and function of the native extracellular matrix (ECM) in order to facilitate cell-material interactions [14–17]. More importantly, such synthetic vascular grafts for small-diameter vessel applications are advantageous because they not only offer off-the-shelf availability but also reduce complications associated with donor-site morbidity and *in vitro* cell source and compatibility.

Recently, one particular polymer that has been electrospun to produce vascular grafts is polyurethane [18–20]. Polyurethanes (PUs) possess excellent biocompatibility and more importantly mechanical properties, which make them ideal for vascular graft applications [21]. However, despite their long-term biostability, PUs eventually degrade *in vivo*; polyester-based PU is susceptible to hydrolytic degradation in the body, whereas polyether-based PU is prone to oxidative degradation [22–24]. As a result, polycarbonate-urethanes (PCUs) have gained more attention and have been used recently for their improved stability and resistance to both hydrolytic and oxidative degradation [25–27]. Although PU grafts have been reported to have variable patency rates, which in some studies exhibited lower patency rates compared to ePTFE grafts [28,29], the newer PCUs with and without modifications have reduced thrombogenicity and better *in vivo* performance [30,31]. More importantly, unlike ePTFE and Dacron, which are much stiffer and less compliant than native vessels, PCU better matches the mechanical properties of native vessels in terms of stiffness and compliance [32,33].

Numerous strategies of surface modification have been explored to further improve the blood compatibility (i.e. conjugation of heparin) of these PCU surfaces, including chemical immobilization, physical adsorption, and plasma treatment. Heparin, a commonly used anticoagulant agent, has been utilized extensively in vascular therapies because of its ability to interact with anti-thrombin-III (AT-III) in preventing thrombus formation [34,35]. For example, we have previously shown that heparin-modified nanofibrous vascular grafts fabricated using biodegradable poly (L-lactide) (PLLA) exhibited higher patency and greater cell infiltration, suggesting that heparin may play multiple roles in maintaining function and promoting remodeling [36]. Other chemical approaches to enable covalent conjugation of heparin using EDC chemistry range from bulk carboxylation of PU via bromoalkylation to the synthesis of PCU with pendant carboxyl groups or with PEG [20,37,38]. In addition, recent findings on facile surface modification using mussel-inspired dopamine indicated that such passively adsorbed coating could not only enhance endothelial cell adhesion and viability but also immobilize biomolecules such as VEGF on the surface of vascular graft for accelerated endothelialization [39,40]. Because this adhesive polydopamine coating serves as a primer for further biofunctionalization, it can be easily applied to different polymeric surfaces for various applications [41,42]. Furthermore, plasma treatment has been utilized to modify the surface properties of PU as well. For instance, an early study by Kawamoto et al. demonstrated that plasma treatment altered the wettability of the surface of segmented-polyurethane, making it more favorable for the adhesion and proliferation of bovine aortic endothelial cells [43]. Similarly, Bae et al. used oxygen plasma glow discharge to prepare carboxyl group-introduced PU for coupling of polyethylene oxide to immobilize heparin [44]. Although one disadvantage of plasma treatment is its limited penetration depth, the porous electrospun fibers can enhance the penetration, and it is a powerful surface modification technique useful for the development of small-diameter vascular graft in that surface features can be manipulated to facilitate subsequent biofunctionalization as well as desired endothelialization.

In this study, we electrospun and fabricated small-diameter nanofibrous vascular grafts using Carbosil®, a commercially available thermoplastic PCU. We then explored and selected three common surface modification techniques from an array of options, and investigated which of them would provide the most effective modification as a primer for subsequent immobilization of heparin on the surface of our PCU grafts. Specifically, we utilized aminolysis with 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) chemistry as a chemical immobilization, polydopamine coating as a passive adsorption, and plasma treatment paired with end-point immobilization to initially introduce amine functional groups and ultimately conjugate heparin on the graft surface. After comparing the three methods, we determined the most effective modification with respect to the surface amine density as well as the anti-thrombogenic activity of the immobilized heparin. Lastly, we proceeded with these optimized PCU grafts immobilized with heparin for short-term *in vivo* studies, focusing on the performance of heparin-modified electrospun PCU grafts on graft patency as well as endothelialization and overall biocompatibility.

2. Materials and methods

2.1. Fabrication and characterization of polycarbonate-urethane (PCU) nanofibrous vascular graft

Electrospinning was performed as previously described with minor modification to produce polycarbonate-urethane (PCU) vascular graft [36,45]. Briefly, polycarbonate-urethane (Carbosil® 90A, DSM Biomedical, Berkeley, CA) was dissolved via sonication in dimethylformamide (DMF) at 16.5% (w/v) concentration. To deliver the polymer solution, a programmable pump along with a 5 mL syringe, which was fitted with flexible silicon tubing connected to 1.5-inch long stainless steel 23G dispensing needles, was used. Two high-voltage generators were utilized to apply approximately +9.7 kV voltage to the needle and −9.2 kV voltage to the collecting mandrel. In addition, the humidity was controlled to be 50–52% during the electrospinning process. PCU solution was delivered at a flow rate of 1.05 mL/hr and gap distance (distance between the positively charged needle tip and the negatively charged collecting mandrel) of 16.5 cm, with a spinneret that traversed in the longitudinal direction to achieve a uniform thickness of the graft longitudinally. PCU fibers with random orientation were obtained by using a low rotation speed (100 rpm) for the collecting mandrel. Electrospinning was allowed to proceed until the wall of the vascular graft reached a desired thickness based on measurements with a thickness gauge (Mitutoyo America, Aurora, IL). The finished graft was placed in a chemical hood overnight to remove any residual DMF.

The overall fibrous structure and integrity of the PCU graft were inspected and imaged using scanning electron microscopy (SEM; TM-1000, Hitachi, Pleasanton, CA). In addition, to determine the