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Full Length Article

Surface immobilization of heparin on functional polyisobutylene-based thermoplastic elastomer as a potential artificial vascular graft

Yi-Bo Wu [a,b], Kang Li [c], Dong Xiang [a,b], Min Zhang [a,b], Dan Yang [a,b], Jin-Han Zhang [a,b], Jing Mao [d,*], Hao Wang [a,b], Wen-Li Guo [b,*]

[a] Department of Material Science and Engineering, Beijing Institute of Petrochemical Technology, Beijing 102617, China
[b] Beijing Key Laboratory of Special Elastomeric Composite Materials, Beijing 102617, China
[c] Department of Cardiology, Beijing Hospital, Beijing 100730, China
[d] Beijing Key Laboratory of Functional Materials for Building Structure and Environment Remediation, Beijing University of Civil Engineering and Architecture, Beijing 102612, China

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ABSTRACT

Polyisobutylene-based thermoplastic elastomer (TPE) is a new soft biomaterial. Hydroxyl functional dendritic polyisobutylene-based TPEs (*arb*-SIBS-OH), which satisfy the design requirements for small-diameter vascular substitutes, were synthesized by controlled carbocationic polymerization. Creep property, which is the destructive weakness of polyisobutylene-based TPEs, was significantly improved with the formation of a "double network" promoted by branched structure and microphase separation. Compatibility of *arb*-SIBS-OH with rabbit blood was markedly enhanced by modifying heparin grafted from these hydroxyl functional groups. Application of "click chemistry" to immobilize heparin on *arb*-SIBS-OH surface was apparently effective in enhancing the bioactivity of heparin. Immobilized heparin, which directly bonded by ester bonds, was more likely to form multi-point binding on *arb*-SIBS-OH surface. This process hindered the accessibility of the heparin active sequence to antithrombin.

1. Introduction

Vessel transplantation is the main treatment for vascular diseases, which are among the most common diseases that pose considerable threat to human health [1–3]. Although a certain degree of success has been attained using synthetic grafts with large diameters, clinically applicable vascular grafts with diameters of less than 6 mm are still elusive. Autologous grafts remain as the gold standard for repairing small vessels. However, the availability of these grafts may be limited, and their harvest increases patient morbidity. Thus, the development of new, biocompatible vascular substitutes with small diameters is an ongoing concern for further advancement of vascular surgery.

The key characteristics of an ideal vessel substitute should be considered in designing artificial vascular grafts. These attributes include excellent mechanical and fatigue properties for long-term tolerance to blood flow, compliance or elasticity to prevent restenosis and hyperplasia, and antithrombogenicity for clot inhibition. Certain synthetic biodegradable polymers have been used to fabricate candidate vascular grafts with small diameters, and encouraging results have been obtained [4,5]. However, these polymers degrade in vivo. That is, they will lose an adequate percentage of their mechanical structure, which will render these polymers unsuitable for long-term use. Currently used synthetic grafts made of Dacron or expanded polytetrafluoroethylene have acceptable patency rates when used in large- and intermediate-sized vessel reconstructions [6]. However, the application of these grafts is limited under high-flow/low-resistance conditions, particularly to vessels with small diameters, because of their relatively poor elasticity, low compliance, and tendency to stimulate thrombosis and neointima formation in vascular anastomoses. By contrast, polyurethane biomaterials are characterized by excellent elasticity and fatigue properties. However, they are subject to biodegradation. In particular, the in vivo hydrolysis of polyurethanes may lead to potentially toxic diamines [7]. Although silicone rubbers are classified as "thermoset rubber," reinforced medical-grade silicone rubber is weaker than other types of reinforced rubber.

Poly(styrene-*b*-isobutylene-*b*-styrene) triblock copolymer (SIBS) is a new soft biomaterial with mechanical properties that could bridge the gap between polyurethanes and silicone rubber [8]. Moreover, SIBS exhibits superior oxidative, chemical, and thermal stabilities compared with the two aforementioned materials because of the saturated structure of SIBS. A

* Corresponding authors.
E-mail addresses: maojing@bucea.edu.cn (J. Mao), gwenli@bipt.edu.cn (W.-L. Guo).

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microphase-separated morphology provides physical strength to a material without requiring chemical cross-linking. SIBS has been demonstrated to be biocompatible and biostable in vivo and in vitro [9]. This polymer is also approved by the Food and Drug Administration as a coating for drug-eluting cardiovascular stents [10]. Various medical devices (e.g., ophthalmic implants and heart valves) are currently being developed using SIBS, because this material leads to minimal platelet activation and polymorphonuclear leukocyte formation, as well as clinically nonsignificant scarring and encapsulation around the implant in the eye [11]. To date, the degradation of SIBS has not yet been observed in any living system [12]. SIBS-based biomaterials exhibit lower permeability, higher tensile strength, and higher elongation at break than silicone analogs [13,14]. These characteristics have led us to consider the use of SIBS as an alternative material for artificial vascular grafts. However, SIBS may suffer from creep which could be an issue in applications for artificial vascular grafts where the implant should be able to withstand considerable stress and deformation during and after implantation.

Herein, we report hydroxyl functional dendritic polyisobutylene (PIB)-based thermoplastic Elastomer TPE (*arb*-SIBS-OH). Creep property was significantly improved with the formation of "double network" promoted by its branched structure and microphase separation. Meanwhile, blood compatibility was greatly enhanced by modifying heparin grafted from functional groups.

Blood compatibility is of primary importance in artificial vascular grafts. Surface modification is an attractive and efficient approach to improve blood compatibility with tailored surface properties in a defined, selective manner while preserving the original bulk structure of the grafts. Heparin is a classic anticoagulant that can effectively reduce the incidence of thrombosis during the early and immediate stages after bypass grafting [15–17]. Two common approaches for the surface immobilization of heparin are ionic binding and covalent immobilization. A critical disadvantage of ionically bound heparin is its easy removal from the surface through ion exchange in physiologic media [18–20]. Therefore, such surfaces are unsuitable for long-term use. By contrast, polymer surfaces with covalently immobilized heparin are more stable and can potentially maintain their anticoagulant property for a longer period [21–24]. However, this approach is limited by the fact that the activity of heparin should not be affected by chemical reactions involved in the process. The anticoagulant activity of covalently immobilized heparin is frequently less than that of free heparin in a solution because of reduced mobility. A previous report suggested that heparin was more active when attached to chain ends or when poly(ethylene oxide) was used as a spacer, because such heparin could better conserve the activity of the pentasaccharide sequence [25,26]. In the current work, we propose "click chemistry" as an alternative method to enhance the mobility and bioactivity of immobilized heparin based on highly selective and quantitative coupling reactions [27–29]. To our knowledge, this work is the first to report on the immobilization of heparin on the surface of PIB-based TPE to improve the anticoagulant activity of heparin.

2. Experimental section

2.1. Materials

Commercially available titanium tetrachloride ($TiCl_4$, 99.9%; J&K Scientific Ltd.), azidobenzoic acid (purity > 99%; Beijing Chemical Co.), 1-ethyl-3-(dimethyl-aminopropyl) carbodiimide hydrochloride (EDAC, purity > 98%; Beijing Chemical Co.), N-hydroxysuccinimide (NHS, purity > 98%; Beijing Chemical Co.), propiolic acid (purity > 98%; Beijing chemical Co.), heparin (unfractionated heparin, average molecular weight (MW) = ~18000, activity: 178 units/mg; Sigma-Aldrich), and methylcyclohexane (MeCHx, anhydrous grade; Aldrich) were used as received. Tetrahydrofuran (THF; Beijing Chemical Reagent Co.) was dried over potassium and distilled under argon atmosphere. Methyl chloride (CH_3Cl) and IB were dried in gaseous state by passing through in-line gas-purifier columns packed with $CaSO_4$/drierite. These compounds were condensed in a cold bath in a glovebox prior to polymerization. Styrene (purity > 97.0%; J&K Scientific Ltd.) and dimethylacetamide (DMA, purity > 98.0%; J&K Scientific Ltd.) were used with further purification by double distillation from CaH_2 under reduced pressure. 4-(2-Methoxyisopropyl) styrene [30,31] and 4-[2-(*tert*-butyldimethylsiloxy)ethyl]styrene [32,33] (TBDMES) were synthesized according to the literature. Human Antithrombin (ATIII) (lyophilized vial containing about 5 IU/mL, contains BSA, 2 vials of 1 mL), purified bovine Factor Xa (lyophilized vial containing about 40 μg, 2 vials of 1 mL) were supported by CoaChrom Diagnostica GmbH. CH_3SO_2-D-Leu-Gly-Arg-pNA (CBS 31.39, vial of about 4 mg, 2 vials of 1 mL) was purchased from Diagnostica Stago (Asnières, France). Reconstitute these contents of each vial with exactly 1 mL of distilled water, shake vigorously until fully dissolved. Allow to stabilize for 30 min at room temperature (18–25 °C), just before use.

2.2. Synthesis of precursor of functional dendritic PIB-based TPE

Cationic polymerizations were conducted under a dry nitrogen atmosphere in a stainless steel glovebox using CH_3Cl/MeCHx (40/60 v/v) solvent mixtures. The first PIB-based inimer-type polymerization was performed by Paulo and Puskas using the 4-(2-methoxyisopropyl)styrene/$TiCl_4$/DtBP/−80 °C initiating system [30,31]. After the polymerization of *arb*-IB for 1–2 h, DMA was added, and the solution was stirred for 5 min. Next, a pre-chilled mixture of TBDMES and styrene with 50% MeCHx was added. Blocking was allowed to proceed for 25–30 min. Polymerizations were terminated by prechilled methanol. After evaporation of the volatiles, the polymer was dried in a vacuum oven at 40 °C to a constant weight. This method resulted in the controlled polymerization of precursor of functional *arb*-SIBS. Afterward, deprotection of the precursor triblock copolymer with tetrabutylammonium fluoride in anhydrous THF at room temperature yielded hydroxyl functional PIB-based TPE (*arb*-SIBS-OH). For stress-strain measurements sheets and surface modification membranes were made by compression molding the *arb*-SIBS-OH at 180 °C for 10 min.

2.3. Surface modification with heparin by esterification reaction

The *arb*-SIBS-OH membrane (2 cm × 2 cm) was put in 30 mL of buffer solution (pH 5, molar ratio of sodium hydroxide/citric acid = 1/1, 0.1 M in water) containing 0.03 g of EDAC. 0.25 mL of heparin solution in above-mentioned buffer solution (1.0 mol/L) was subsequently added to the solution where immobilization reaction proceeded for 48 h. The formed heparin/*arb*-SIBS-OH composite membrane was washed in a buffer solution containing 0.1% Triton X-100 for 1 h to remove residual unreacted heparin and by-product. The composite membrane was then rinsed in water and freeze dried. The amount of heparin immobilized was measured by employing toluidine blue dye binding assay. The difference in the absorbance at 631 nm of the heparin solution before and after the immobilization reaction can be used to calculate the amount of heparin immobilized.

2.4. Surface modification with heparin by click chemistry

Azide-modified *arb*-SIBS-OH membrane: Azidobenzoic acid (0.1 g) was dissolved in 30 mL of deionized (DI) water, followed