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Synthesis of polycarbonate urethanes with functional poly(ethylene glycol) side chains intended for bioconjugates

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ABSTRACT

Traditional poly(ethylene glycol) (PEG)-modified polyurethanes usually exhibit high biocompatibility, but still lack reactivity with biological molecules to induce appropriate cell and tissue responses. In this study, PEG diglycidyl ether (M_n = 526 Da) and PEG bis(amine) (M_n = 1000 Da) were respectively grafted onto carboxyl-group-containing poly(carbonate urethane) backbones that chain-extended with lysine, to generate reactivity while maintaining biocompatibility. The PEG chains disordered and plasticized the hard segments where they attached, reducing H-bonded urea groups and lowering glass transition temperatures. The M_n ranged from 33,000 to 70,000 Da for the precursor polyurethanes, which largely decreased by 24–75% following PEG grafting. Hemocompatibility was enhanced due to the flexibility and hydrophilicity of the PEG chains. Solutions of the PEG-grafted polyurethanes were transformed into hydrocolloids when dropped into water. Reactivity was proved by immobilization of albumin onto the colloidal particles. These new functional PEG-grafted polyurethanes can potentially form multifunctional bioconjugates for applications as biomaterials and in pharmaceutics.

1. Introduction

The alternating arrangement of hard and soft segments along the macromolecular backbones confers polyurethanes their unique mechanical properties and relatively good biocompatibility [1], resulting in their wide applications in cardiovascular devices such as pacemaker insulating leads [2], vascular access grafts [3] and various intravenous catheters [4]. Polycarbonate urethanes (PCUs) in particular have been considered as promising candidates to fabricate small-diameter (<6 mm) vascular prostheses because of their superior biostability [5] and elastic compliance similar to those of natural arteries [6]. However, acute thrombogenicity and chronic intimal hyperplasia hinder the success of small-diameter PCU vascular grafts [7]. Therefore, polyurethanes, like most synthetic materials, are still insufficient in terms of biocompatibility and biofunctionality.

Poly(ethylene glycol) (PEG) is generally known as a biocompatible material, with unique properties of hydrophilicity, nontoxicity and nonimmunogenicity. As a result, it has been grafted onto polyurethane surfaces to reduce protein adsorption and platelet activation [8–10]. Moreover, various functional groups or molecules, such as sulfonic group [11], lysine [12], RGD peptide [13] and heparin [14,15], have been attached to the PEG free ends to further improve biocompatibility. Although promising, these surface modifications are seldom used clinically because they usually require very complicated surface activation processes, either physically [13,15] or chemically [8–12,14], which are hardly suitable in some cases, like treating the lumen of vascular prostheses.

In addition to surface modifications, bulk grafting with PEG has also been explored, using raw materials like soft segment diols [16] and chain extenders [17,18] that had PEG side chains, or adopting chemical reactions between polyurethane backbones and PEG oligomers [19–24]. All these bulk modifications have enhanced hemocompatibility to some extent. However, the free ends of the PEG side chains are either methoxy or sulfonic groups, without reactivity with biological molecules. To the best of our knowledge, only one study has involved to graft double-bond-containing polyurethane backbones with PEG diamine molecules [25], leaving an amino group at each PEG free end.

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Recently, anti-CD34 monoclonal antibody [26] and vascular endothelial growth factor [27] were immobilized onto the luminal surfaces of vascular prostheses to facilitate *in situ* endothelialization. This highlights the importance of the reactivity of synthetic polymers with biological molecules. We hereby report the synthesis of new polyurethanes with pendant carboxyl groups to which bifunctional PEG oligomers were attached. Biological molecules, like albumin, can react with the functional groups at the PEG ends, forming bioconjugates for various applications.

2. Materials and methods

2.1. Materials

Poly(hexamethylene carbonate) diol (PCD, M_n = 860 Da), PEG diglycidyl ether (M_n = 526 Da) and *L*-lysine were all purchased from Sigma–Aldrich (St. Louis, MO, USA). 4,4′-Diphenylmethane diisocyanate (MDI) was obtained from Acros Organics (Geel, Belgium). Dicyclohexylcarbodiimide (DCC) was from Jiangsu Tianhua Reagent Co. (Xuzhou, Jiangsu, China), and N-hydroxysuccinimide (NHS) from the Kelong Chemical Reagent Plant (Chengdu, Sichuan, China). PEG bis(amine) (M_n = 1000 Da) was bought from Aladdin Chemistry Co. (Shanghai, China). Tetrahydrofuran (THF) and dimethylformamide (DMF) were from Tianjin Bodi Chemical Holding Co. (Tianjin, China). All the materials were used as received without further purification.

2.2. Synthesis

2.2.1. Synthesis of precursor polycarbonate urethanes (PCULx)

PCULx (x = 1, 2 and 3, Table 1 and Fig. 1) with pendant carboxyl groups, using PCD as the soft segment and MDI/lysine as the hard segment, were synthesized through a conventional two-step method [28]. The feed amounts of raw materials are shown in Table 1. In a three-necked round-bottomed flask, PCD was added and degassed at 100 °C for 2 h. The flask was cooled to about 60 °C and MDI added to begin the first step. The reaction mixture was stirred for 3 h in a N_2 atmosphere, resulting in an isocyanate-terminated prepolymer. In the second step, the prepolymer was chain-extended with *L*-lysine that was dissolved in a water/THF mixture (for PCUL1 and PCUL2: 15 mL H_2O + 100 mL THF; PCUL3: 20 mL H_2O + 100 mL THF). Once the lysine solution was added, the temperature dropped to about 40 °C and quickly increased by 5–7 °C due to the exothermal reaction. The resulting mixture was cooled to room temperature, kept overnight and poured into water to precipitate the polymer, which was thoroughly washed with water and desiccated at room temperature for 2 d, followed by vacuum drying at 100 °C for 6 h.

2.2.2. Synthesis of polyurethanes with glycidyl ether-terminated PEG side chains (PCULx-PO)

One gram of PCULx (x = 1, 2 or 3, Table 1) and PEG diglycidyl ether were dissolved in 50 mL of DMF (the molar ratio of carboxyl groups in each PCULx to PEG molecules was 1:4), and heated to 130–140 °C (Fig. 1). After a 30-h reaction in a N_2 atmosphere, the resulting mixture was slowly dropped into 200 mL of distilled water, where a colloid-like suspension was formed under stirring (Fig. 2A). The colloid was purified through filtration prior to dialysis against 1000 mL of water for 3 d, with 3 water changes per day, using cellulose tubing dialysis membrane (Sigma) with a molecular weight cutoff of 14000 Da. One half of the purified colloid was concentrated to about 2% (w/v). Another half was centrifuged at 12,000 rpm and freeze-dried for 30 h to obtain the polymer (PCULx-PO, Fig. 1).

2.2.3. Synthesis of polyurethanes with amino-terminated PEG side chains (PCULx-PN)

One gram of PCULx (x = 1, 2 or 3, Table 1) was dissolved in 40 mL of DMF, to which DCC, NHS and PEG bis(amine) were added. The molar ratio of the pendant carboxyl groups to molecules of DCC, NHS and PEG bis(amine) was 1:1.5:1.6:2. After 30 h of reaction at room temperature, 3 drops of hydrochloric acid (37 wt.%) were added to transform the non-reacted DCC into 1,3-dicyclohexylurea (DCU). Three hours later, the DCU crystals were filtered out and the filtrate was dropped into 200 mL of distilled water to form a colloidal suspension. The ~2% colloid (w/v) and the corresponding amine-PEG-grafted polymer (PCULx-PN, Fig. 1) were obtained in the same way as described in Section 2.2.2.

2.3. Blood compatibility tests

Blood was harvested from the hearts of healthy New Zealand white rabbits using 5-mL heparinized vacuum tubes (Heparin Tube®, Jiangsu Kangjian Medical Apparatus Co., Taizhou, Jiangsu, China). The Chinese standard for Animal Welfare Requirements (GB/T 16886.2) was observed. The 100-μm thick polyurethane films were cut into 5 mm × 10 mm rectangles and served as the test specimens for both hemolysis and platelet adhesion assays.

2.3.1. Hemolysis analysis

Four milliliters of fresh blood was diluted with 5 mL of saline solution as the test blood. Each film was flushed with distilled water for 5 min, rinsed in saline solution 2 times, and placed in a silanized tube to which 10 mL of saline solution were added. The tubes were capped and conditioned at 37 °C for 30 min, followed by the addition of 0.2 mL of test blood. Ten milliliters of distilled water and saline solution, each mixed with 0.2 mL of the test blood, served as the positive and negative control, respectively. Three replicates were used for each sample and control (n = 3). After incubation in a water bath at 37 °C for 1 h, all the tubes were centrifuged at 570 *g* for 10 min. The supernatants were collected, and the optical density (OD) was determined at 545 nm, using a UV-1800 PC spectrophotometer (Mapada instruments, Shanghai, China). Hemolytic index (HI) was defined as the percentage of the OD value in the test sample relative to that in the positive control. The HI of distilled water was 100%.

2.3.2. Platelet adhesion

The harvested blood was centrifuged at 100 *g* for 10 min to obtain the upper clear platelet-rich plasma (PRP). The polyurethane

Table 1
Compositions of the precursor polyurethanes PCULx.

Sample	Feed amounts of raw materials (g or mol)			Found molar ratio[a] (PCD : MDI : Lysine)	Hard segment (wt %)	Theoretical carboxyl equivalent (g/mol)
	PCD	MDI	*L*-lysine			
PCUL1	25.8 (0.03)	11.0 (0.044)	1.46 (0.01)	2.75 : 4.17 : 1.00	32.6	3826
PCUL2	8.60 (0.01)	5.50 (0.022)	1.46 (0.01)	1.07 : 2.28 : 1.00	44.8	1556
PCUL3	17.20 (0.02)	16.50 (0.066)	5.84 (0.04)	1.00 : 3.53 : 1.84	56.5	989

[a] From ^{1}H NMR analyses. The found molar ratios are consistent with the feed ones.