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Binary release of ascorbic acid and lecithin from core–shell nanofibers on blood-contacting surface for reducing long-term hemolysis of erythrocyte

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ABSTRACT

There is an urgent need to develop blood-contacting biomaterials with long-term anti-hemolytic capability. To obtain such biomaterials, we coaxially electrospin [ascorbic acid (AA) and lecithin]/poly (ethylene oxide) (PEO) core–shell nanofibers onto the surface of styrene-b-(ethylene-co-butylene)-b-styrene elastomer (SEBS) that has been grafted with poly (ethylene glycol) (PEG) chains. Our strategy is based on that the grafted layers of PEG render the surface hydrophilic to reduce the mechanical injure to red blood cells (RBCs) while the AA and lecithin released from nanofibers on blood-contacting surface can actively interact with RBCs to decrease the oxidative damage to RBCs. We demonstrate that (AA and lecithin)/PEO core–shell structured nanofibers have been fabricated on the PEG grafted surface. The binary release of AA and lecithin in the distilled water is in a controlled manner and lasts for almost 5 days; during RBCs preservation, AA acts as an antioxidant and lecithin as a lipid supplier to the membrane of erythrocytes, resulting in low mechanical fragility and hemolysis of RBCs, as well as high deformability of stored RBCs. Our work thus makes a new approach to fabricate blood-contacting biomaterials with the capability of long-term anti-hemolysis.

1. Introduction

There is an urgent need for development of biocompatible biomaterials that need to be contacted with the blood for long time. The surfaces of these biomaterials must prevent the protein adsorption, activation of platelets and plasma proteolytic enzyme systems, as well as the hemolysis of red blood cells (RBCs) [1–5]. The release of intracellular hemoglobin from the ruptured RBCs (hemolysis) reduces oxygen affinity and delivery, resulting in an intrinsic mechanism for human disease [5–7]. Although rapid progress on antithrombotic biomaterials has been made in recent years, fabrication of blood-contacting biomaterials with the capability of long-term anti-hemolysis remains a challenge. So far, the frequent and serious intravascular hemolysis induced by implant devices threatens the patients [8,9]. And the serious hemolysis of preserved RBCs in non-plasticizer containers has been an insuperable obstacle to replace polyvinylchloride (PVC) blood bags which contain 40–60% toxic plasticizers [10,11]. Therefore, it is highly desired to develop novel anti-hemolytic biomaterials that can be applied under the condition of long-term contact of blood. Considering the complex interactions of implant devices with RBCs and difficulties in estimating the hemolysis *in vivo* [12], we fix our study on preparing anti-hemolytic biomaterials applicable for erythrocyte storage *in vitro*.

Oxidative damage to the membrane is the main cause for the hemolysis during RBCs storage [11,13]. Current methods based on surface modification [3,14,15] can reduce the mechanical damage to the membranes of RBCs, however, they have slight effects on the oxidative injure to the RBC membrane. Recently, we present a new method to construct a hydrophilic, 3D hierarchical architecture on the surface of styrene-b-(ethylene-co-butylene)-b-styrene elastomer (SEBS) with PEO/lecithin nano/microfibers [4]. When RBCs are stored in the bags of these biomaterials, the lecithin can release and interact with RBCs, which reduces both mechanical

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damage to RBC membranes and lipid loss caused by oxidation of RBC membranes, resulting in low hemolysis of preserved RBCs. One limitation of this method is that the released lecithin can mitigate the lipid loss of membrane caused by oxidation, but it cannot prevent the oxidation of membrane of stored RBCs. A practical method is to add a suitable agent to the system of stored RBCs to protect the RBC membrane from oxidation substantially. Recently, ascorbic acid (AA) is reported to act as an antioxidant to decrease the oxidative damage of RBCs membrane and improves membrane fragility during RBC storage [16]. Thus, the release of AA to the stored RBCs and active interaction between AA and RBC membrane through plasma will be an effective way to reduce oxidative damage of preserved RBCs. However, appropriate encapsulation is needed to prevent the oxidation of AA during AA loading [17].

Coaxial electrospining is a modern and robust technique for one-step encapsulation of fragile, water-soluble bioactive agents into core–shell structured nanofibers [18,19]. Compared to the conventional electrospinning [20–22], coaxial electrospining shows the advantage in eliminating the damage to bioactive agents, encapsulating multiple drugs with different solubility and preventing the premature release of the water-soluble core contents [23]. In addition, it is possible to precisely modulate the release of the encapsulated agents by varying the structure and composition of nanofibers [24]. The core–shell structured nanofibers are thus fabricated in this work to enhance the release duration and effectively decrease the oxidation of core contents.

Here, we coaxially electrospin (AA and lecithin)/PEO core–shell nanofibers onto the surface of PEG grafted SEBS to construct an anti-hemolytic surface. Our strategy is based on the grafted layers of PEG render the surface hydrophilic to reduce the mechanical injure to red blood cells (RBCs) while the AA and lecithin released from nanofibers on the blood-contacting surface can actively interact with RBCs to decrease the oxidative damage to RBCs. We demonstrate that (AA and lecithin)/PEO core–shell structured nanofibers have been developed on PEG grafted surface. The binary release of AA and lecithin in the distilled water is in a controlled manner and lasts for almost 5 days; during RBCs preservation, AA acts as an antioxidant and lecithin as a lipid supplier to the membrane of erythrocytes, resulting in low mechanical fragility and hemolysis of RBCs, as well as high deformability of stored RBCs. Our work thus paves a new way to fabricate blood-contacting biomaterials with the capability of long-term anti-hemolysis.

2. Experimental

2.1. Materials

SEBS copolymer with 29 wt.% styrene (Kraton G 1652) was provided by Shell Chemicals. Poly (ethylene glycol) methyl ether methacrylate (PEGMEMA) (M_w = 300 g mol^{-1}) and PEO (M_w = 5,000,000 g mol^{-1}) were purchased from Sigma-Aldrich. L-alpha-lecithin (M_w = 750 g mol^{-1}) was supplied by ACROS (New Jersey). L-ascorbic acid (AA) (M_w = 176.13 g mol^{-1}) was purchased from Beijing Shiji Corp, China. Benzophenone (BP) was provided by Peking Ruichen Chemical (China). Acetone and xylene were reagent grade products. Other reagents were AR grade and used without further purification. Phosphate-buffered saline (PBS 0.9 wt.% NaCl, 0.01 M phosphate buffer, pH 7.4) was prepared freshly.

2.2. Grafting of PEGMEMA onto SEBS films

SEBS was dissolved in xylene to form 15% (w/w) solutions and poured onto a clean glass to obtain a smooth SEBS film (0.2 mm thick). The SEBS films were immersed in the ethanol solution of BP (1.5 wt.%) for 30 min and dried at room temperature. Then the film was put on a quartz plate (3 mm thick) and coated with aqueous solution of PEGMEMA with the concentration ranging from 5 to 20 wt.%. The film was coved with another quartz plate, followed by exposure to UV light (high-pressure mercury lamp, 400 W, main wavelength 380 nm) for 5 min. All films were washed with deionized water and ethanol to remove the residual monomers, followed by drying in a vacuum oven for at least 24 h. The grafted SEBS was analyzed by Bruker FTIR spectrometer Vertex 70 equipped with an Attenuated Total Reflection (ATR) unit (ATR crystal 45°) at a resolution of 4 cm^{-1} for 32 scans. The grafting density (μg cm^{-2}) was obtained by a weight method [25].

2.3. Coaxial electrospinning of (AA and lecithin)/PEO core–shell structured nanofibers onto PEG-grafted SEBS

PEO was dissolved in a solvent mixture of water and ethanol (3:2 w/w) to obtain the concentration of 8 wt.%. Lecithin and AA (2:1 w/w) were dissolved in a solvent mixture of water and ethanol (4:1 w/w) to get the lecithin concentration of 2 wt.%. To prevent the oxidation of AA, AA was mixed in the solution of lecithin only for 5 min before coaxial electrospinning. The key component in the coaxial electrospinning setup was a compound spinneret, consisting of two small-diameter capillary tubes with one located inside another (Fig. 1). The rate of inner flow (AA and lecithin) was adjusted by one syringe pump with a feed rate of 0.5 ml h^{-1}, whereas the rate of PEO solution in outer tube was controlled by another syringe pump with a feed rate of 0.75 ml h^{-1}. The (AA and lecithin)/PEO core–shell structured nanofibers were fabricated onto the surface of PEG-grafted SEBS at a temperature of about 45 °C, humidity 43% with applied voltage of 10–11 kV. For simplicity, the SEBS coated with electrospun fibers was referred as 'electrospun SEBS'. The morphology of electrospun SEBS was then characterized by a field-emission scanning electron microscopy (SEM, Sirion-100, FEI, USA) and a transmission electron microscopy (TEM, JEM1011, Japan). The surface wettability of SEBS was evaluated by the sessile drop method with a pure water droplet (*ca.* 3 μL) using a contact angle goniometer (DSA, KRUSS GMBH, Germany).

2.4. Binary release of ascorbic acid and lecithin

1 cm × 1 cm films of electrospun SEBS with density of 0.2 mg cm^{-2} were incubated in PBS at 4 °C. Then, at the desired time, 1 mL micelles solution was collected and amount of the released ascorbic acid and lecithin were measured using high-performance liquid chromatography (Waters 600 HPLC, evaporative light scattering detector) with a standard calibration curve. The release profile was normalized to the amount of ascorbic acid and lecithin initially loaded in PEO fibers.

2.5. Hemolysis and mechanical fragility measurements

Fresh blood extracted from a healthy rabbit was immediately mixed with 3.8 wt.% sodium citrate solution at a dilution ratio of 9:1. Then the whole blood sample was centrifuged at 1000 rpm for 15 min to separate RBCs, white blood cells, and platelet rich plasma. The plasma and buffy coat layers (platelets and white cells) were carefully removed to obtain concentrated RBCs (100% hematocrit). The original and electrospun SEBS films with size of 4 cm × 4 cm were made into 0.4 mL bags, respectively. After sterilization with ethanol for 24 h and drying, 0.2 mL RBCs were transferred to the bags. For each kind of bags, three bags were used to preserve the RBCs (total 0.6 mL RBCs), and these bags were sealed and preserved at 4 °C for 5 days.

90 μL preserved RBCs were collected for hemolysis test after 5-day storage. The preserved RBCs were diluted with 1 mL normal saline and centrifuged (3000 rpm, 3 min) to get the supernatant,