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Controlled Lecithin Release from a Hierarchical Architecture on Blood-Contacting Surface to Reduce Hemolysis of Stored Red Blood Cells

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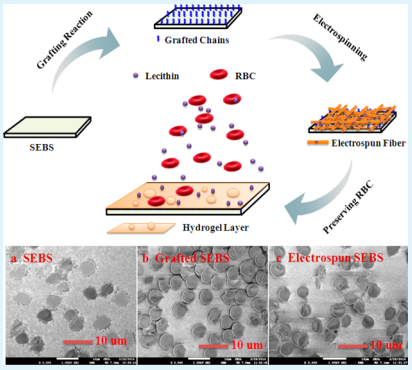
Supporting Information

ABSTRACT: Hemolysis of red blood cells (RBCs) caused by implant devices in vivo and nonpolyvinyl chloride containers for RBC preservation in vitro has recently gained much attention. To develop blood-contacting biomaterials with long-term antihemolysis capability, we present a facile method to construct a hydrophilic, 3D hierarchical architecture on the surface of styrene-*b*-(ethylene-*co*-butylene)-*b*-styrene elastomer (SEBS) with poly(ethylene oxide) (PEO)/lecithin nano/microfibers. The strategy is based on electrospinning of PEO/lecithin fibers onto the surface of poly [poly(ethylene glycol) methyl ether methacrylate] [P(PEGMEMA)]-modified SEBS, which renders SEBS suitable for RBC storage in vitro. We demonstrate that the constructed 3D architecture is composed of hydrophilic micro- and nanofibers, which transforms to hydrogel networks immediately in blood; the controlled release of lecithin is achieved by gradual dissolution of PEO/lecithin hydrogels, and the interaction of lecithin with RBCs maintains the membrane flexibility and normal RBC shape. Thus, the blood-contacting surface reduces both mechanical and oxidative damage to RBC membranes, resulting in low hemolysis of preserved RBCs. This work not only paves new way to fabricate high hemocompatible biomaterials for RBC storage in vitro, but provides basic principles to design and develop antihemolysis biomaterials for implantation in vivo.



KEYWORDS: *blood-contacting surface, hemolysis, electrospinning, lecithin, controlled release*

1. INTRODUCTION

A wide range of biomedical devices has been applied clinically in contact with blood. The contact often leads to activation of blood cells and plasma proteolytic enzyme systems such as complement, coagulation, fibrinolysis, and hemolysis.[1,2] Although numerous attempts have been made to prevent thrombus formation on the surface of materials, hemolysis of red blood cells (RBCs) is equally important to address.[1−6] Hemolysis is the dissolution of RBCs with the release of intracellular hemoglobin, which reduces oxygen affinity and delivery, leading to an intrinsic mechanism for human disease.[4−6] So far, decreasing the hemolysis caused by long contact of RBCs with biomedical devices remains a challenge. It has been reported that implant devices inevitably induce frequent and serious intravascular hemolysis in patients.[7,8] In addition, the toxic effects of plasticizer in polyvinyl chloride (PVC) blood bags for RBCs preservation, such as damage to liver and kidney, and hints of carcinogenicity, makes it urgent to develop new nonplasticizer polymer containers.[9,10] However, the hemolysis of RBCs in nonplasticizer containers becomes a big obstacle to replacement progress.[10] It is therefore a pressing task to design and develop blood-contacting biomaterials with long-term, antihemolysis capability. Because of the complex interactions between implant devices and RBCs, and difficulties in evaluating the hemolysis in vivo,[11] this work focuses on designing and fabricating antihemolysis biomaterials applicable for RBCs preservation in vitro.

Hemolysis mainly results from RBC membrane injury during storage. RBC membrane is a composite structure in which a plasma membrane envelope is composed of amphiphilic lipid molecules.[12] The lipid loss from the membrane leads to reductive deformability of RBCs and a decrease in critical hemolytic volume of RBCs.[4,7,10] Many approaches based on surface modification, such as passivation of the surface, surface

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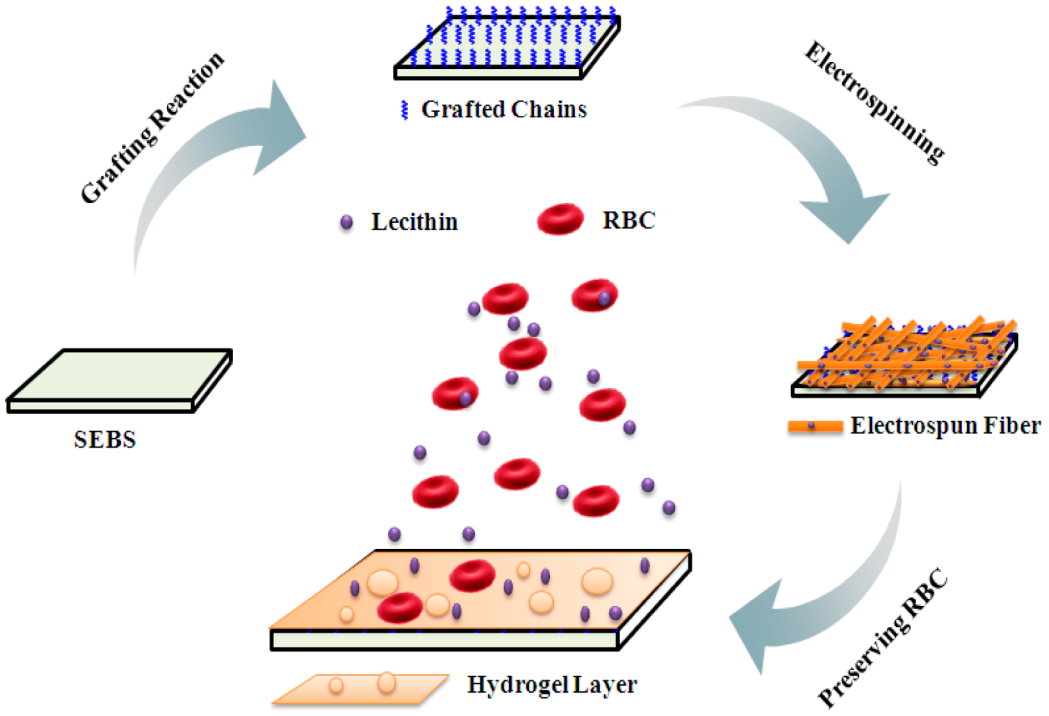

Figure 1. Schematic of constructing 3D architecture on SEBS surface by combined grafting reaction and electrospinning technique. The architecture transforms to hydrogel layer after contacting blood. The hydrophilic layer and interaction between RBCs and released lecithin from PEO/lecithin hydrogels reduce the mechanical damage and oxidative damage to RBC membranes.

immobilization of active molecules, and fabrication of biomimic membrane, have been developed to enhance the hemocopatiblility of biomaterials.[13−15] However, these methods only can reduce the mechanical damage to the membranes of RBCs, but have slight effects on prevention of lipid loss from the membrane and improvement of structural status of the RBC membrane. Recently, liposomes are reported to interact with RBCs and transfer lipid to RBC membrane.[16,17] Because of their biodegradability, nontoxicity, and nonoimmunogenicity, liposomes have been used as drug delivery vehicles over the past decades.[18] Controlled interaction between liposomes and RBC membrane through aqueous media will be an effective way to reduce the hemolysis of stored RBCs.

Here, we present a facile method to construct a hydrophilic, 3D hierarchical architecture on the surface of SEBS with PEO/lecithin nano/microfibers. The strategy is based on electrospinning of PEO/lecithin fibers onto the surface of P(PEGMEMA)-modified SEBS, rendering SEBS suitable for RBC storage in vitro. SEBS possesses good biocompatibility, thermal stability, and outstanding mechanical performance, which makes it a promising nonplasticizer candidate for blood preservation.[19,20] We demonstrate that the constructed 3D architecture is composed of hydrophilic micro- and nanofibers, which transforms to hydrogel networks immediately in aqueous media; the gradual dissolution of PEO/lecithin hydrogels controls the lecithin release and interaction between lecithin with the membrane of RBCs, which effectively maintains membrane flexibility and normal RBC shape. Thus, the blood-contacting surface reduces both mechanical and oxidative damage to RBC membranes, resulting in low hemolysis of preserved RBCs. This work not only makes a novel approach to fabricate high hemocompatible biomaterials for RBCs storage in vitro, but provides basic principles on the design and development of antihemolysis biomaterials for implantation in vivo.

2. EXPERIMENTAL SECTION

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). 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% NaCl, 0.01 M phosphate buffer, PH 7.4) was prepared fresh.

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. The film was then put on a quartz plate (3 mm thick) and coated with aqueous solution of PEGMEMA with the concentration ranging from 5 to 15 wt %. The film was covered 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 residual monomer, followed by drying in a vacuum oven for at least 24 h. The grafted SEBS was analyzed by a 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 weighing after grafting reactions.[19]

2.3. Electrospinning of PEO/Lecithin onto PEG-Grafted SEBS. PEO and L-alpha-lecithin (lecithin) (6:1 and 6:2 w/w) were dissolved in a solvent mixture of water and ethanol in the ratio of 3:2 (w/w). The PEO/lecithin micro/nanofibers were then electrospun onto the surface of P(PEGMEMA)-grafted SEBS at a temperature of about 45 °C, humidity 43%, and solution feed rate ~0.5 mL/h with applied voltage of 10−11 kV. The smooth PEO/lecithin fibers were

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easily fabricated at the above ratios of PEO to lecithin, and further increase of lecithin loading led to large defects on the fibers. For simplicity, the SEBS coated with electrospun fibers was referred as "electrospun SEBS". The morphology of electrospun SEBS was then characterized by field-emission scanning electron microscopy (SEM, Sirion-100, FEI, USA). 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. Lecithin Release. The films of electrospun SEBS with size of 1 cm × 1 cm were incubated in PBS at 4 and 12 °C, respectively. Then, at the desired time, 1 mL of micelles solution was collected and the amount of released lecithin was 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 lecithin initially loaded in PEO fibers.

2.5. Hemolysis Assay. Fresh blood extracted from a healthy rabbit was immediately mixed with 3.8 wt % sodium citrate solution at a dilution ratio of 9:1. The whole blood sample was then centrifuged at 1000 rpm for 15 min to separate RBCs, white blood cells, and platelet-rich plasma.[20] The plasma and buffy coat layers (platelets and white cells) were then carefully removed to obtain concentrated RBCs. The as-prepared SEBS films with size of 4 cm × 4 cm were made into 0.4-mL bags. After sterilization with ethanol for 24 h and drying, 0.2 mL of RBCs was transferred to SEBS bags, which were sealed and preserved at 4 °C. Then 100 μL of preserved RBCs was collected for hemolysis test after storage for 1 day and 3 days, respectively. For comparison, the hemolysis ratio of preserved RBCs in the bags of virgin SEBS, grafted SEBS, and commercial PVC bags were performed under the same conditions.

Preserved RBCs were collected and centrifuged (3000 rpm, 3 min) to get the supernatant, which then was transferred to 96-well plates. Positive and negative controls were produced by adding 0.1 mL of fresh RBCs to 2 mL of distilled water and normal saline, respectively. After incubation for 2 h, the RBCs were removed by centrifugation (3000 rpm, 3 min) and the supernatant was transferred to 96-well plates. Optical density (OD) of the supernatant was measured with a TECAN absorbance reader (TECAN GENIOS, Austria) at 541 nm. The hemolysis ratio (HR) was calculated according to the following formula:

$$\mathrm{HR}(\%) = \frac{\mathrm{OD}_{\mathrm{test}} - \mathrm{OD}_{\mathrm{neg}}}{\mathrm{OD}_{\mathrm{pos}} - \mathrm{OD}_{\mathrm{neg}}} \times 100 \quad (1)$$

where $\mathrm{OD}_{\mathrm{test}}$ is test sample absorbance value, and $\mathrm{OD}_{\mathrm{pos}}$ and $\mathrm{OD}_{\mathrm{neg}}$ are the positive (water) and negative (saline) control, respectively. Results are given as the mean of triplicate experiments and standard deviation.

2.6. RBC Morphology. The preserved RBCs were dropped onto poly-L-lysine-coated glass slides and incubated at 37 °C for 60 min under static conditions to provide enough time to adhere RBCs. After the incubation, the samples were carefully rinsed twice with prewarmed PBS, followed by immersing in 3 mL of 2.5 vol % glutaraldehyde in PBS for 10 h at 4 °C to fix the adhered RBCs. Finally, the samples were freeze-dried and the morphologies of adhered RBCs on the sample surfaces were visualized by FESEM (SEM, JEOL, JSM-7500F, JP).

3. RESULTS AND DISCUSSION

3.1. Fabrication of 3D Hierarchy Architecture on SEBS Surface. The 3D hierarchy architecture on SEBS surface is constructed via grafting PEGMEMA onto SEBS surface, followed by electrospinning of PEO/lecithin onto the grafted surface (Figure 1). The architecture transforms to hydrogel layer after contacting blood during RBC storage. The hydorphilic layer and interaction between RBCs and released lecithin from PEO/lecithin hydrogels are expected to reduce the mechanical damage and lipid loss to RBC membranes. For grafting reaction, the SEBS film is coated with the aqueous solution of PEGMEMA and sandwiched with quartz plates, followed by exposure to UV irradiation at room temperature. Grafting PEGMEMA onto SEBS is initiated by the free radicals on the SEBS surface generated through UV-induced decomposition of BP initiator (Supporting Information (SI), Figure S1).

The successful grafting of PEGMEMA onto SEBS surface is confirmed by FTIR spectra (Figure 2). In comparison with

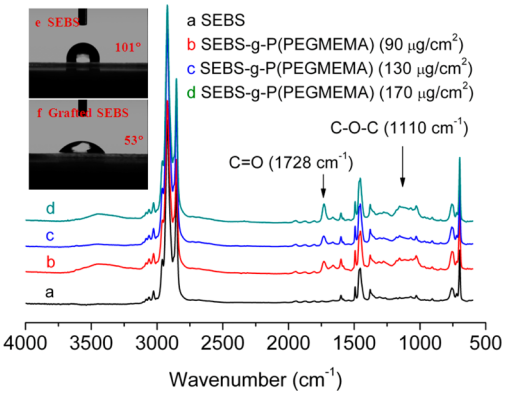

Figure 2. ATR-FTIR spectra of SEBS films and images of water contact angle on the SEBS films: (a) virgin SEBS, (b–d) P(PEGMEMA)-grafted SEBS with grafting densities of 90, 130, and 170 μg/cm^2, respectively, (e, f) images of water contact angle on the films of virgin SEBS and P(PEGMEMA)-grafted SEBS with grafting density of 170 μg/cm^2, respectively.

virgin SEBS (Figure 2a), two new absorption peaks at 1728 and 1110 cm^{-1} corresponding to the stretching vibration of C═O and C–O–C, respectively, appear in the spectra of P-(PEGMEMA)-grafted SEBS films (Figure 2b–d).[20] The grafting density of P(PEGMEMA) increases with the increment of feeding monomer concentration, reaching maximal density (about 170 μg/cm^2) at 15 wt % PEGMEMA concentration. The grafting density is much higher than that reported in our previous works,[19,20] rendering the surface of SEBS hydrophilic with the water contact angle less than 56° (Figure 2e, f). The grafted P(PEGMEMA) not only provides the hydration layer to reduce mechanical damage of RBC membranes,[20] but also enhances the adhesion of electrospun fibers with substrate by chain entanglements at the interface.[21]

The 3D architectures composed of PEO/lecithin microfibers and nanofibers are constructed via electrospinning of PEO/lecithin fibers to the surface of grafted SEBS with density of about 0.2 mg/cm^2. The SEM images show that the SEBS surfaces are coated with smooth fibers without the formation of beads (Figure 3). The diameter of electrospun fibers ranges from 400 to 3500 nm (inset of Figure 3a), and the electrospun PEO fibers further increase the hydrophilicity of SEBS surface, with equilibrium CA of 22° (inset of Figure 3b). One advantage of the electrospinning technique is that it can be adaptable to any substrate with a large area, which is especially important for fabricating blood-storage containers. In addition, the electrospinning process creates architectures with micro- to nanoscale topography and high porosity.[21−24] The inherently high surface-to-volume ratio of electrospun meshes enhances lecithin loading. The loading of lecithin in electrospun fibers is evidenced by FTIR spectra, and the load amount of lecithin is nearly the feed ratio of lecithin to PEO in the solution (SI Figure S2). No obvious differences can be observed on the

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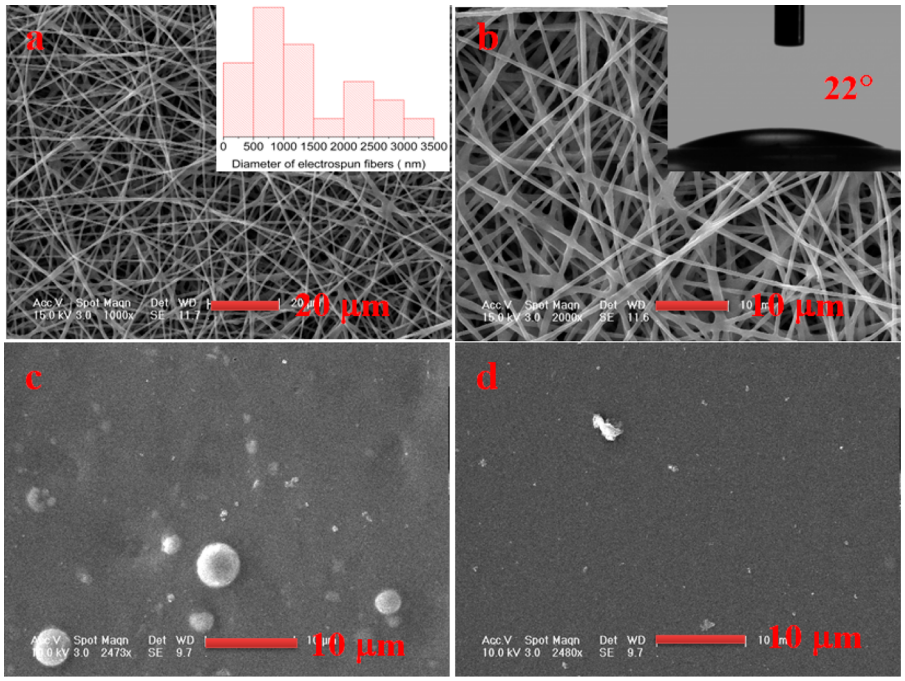

Figure 3. SEM images of SEBS surface after electrospinning and incubation in distilled water at 4 °C: (a) electrospun nano/microfibers on SEBS, (b) enlarged nano/microfibers on SEBS, (c, d) SEBS surfaces after incubation in distilled water for 1 and 72 h, respectively.

morphology of electrospun fibers with varied lecithin loading (SI Figure S3).

3.2. Controlled Release of Lecithin. Generally speaking, electrospun PEO/lecithin fibers instantly dissolve in water with fast lecithin release, resulting in failure of sustainable interactions between lecithin and RBC membrane. Therefore, cross-linking is necessary to stabilize the electrospun meshes for controlled-release of lecithin.[24] However, most cross-linking agents are toxic to RBCs[24,25] and stable electrospun meshes increase the surface roughness, leading to mechanical damage to the membrane of RBCs.[26] To overcome these problems, the stability of PEO/lecithin fibers is achieved by physical methods in this work: (1) fabricating the electrospun fibers with high molecular weight of PEO (5×10^6 g mol^{-1}),[27] (2) enhancing interfiber adhesion and fusion with increased electrospinning temperature to enlarge the physical cross-linking density of meshes,[21,28] and (3) enforcing the entanglement between grafted chains and electrospun fibers.[29] As a result, a large amount of fused junctions have been formed in the electrospun meshes (Figure 3b). In addition, the adhesion between electrospun fibers and grafted SEBS is strong enough to prevent the detachment of electrospun meshes from the surface of grafted SEBS (SI Figure S4). After contacting water, some electrospun fibers dissolve immediately, but most electrospun micro/nanofibers become a physical hydrogel covering on the surface of SEBS.[30] The hydrogels with high cross-linking density tend to form beads, and heterogeneous beads on hydrogel layer can be clearly observed on the SEBS surface after the film is incubated in distilled water for 1 h (Figure 3c). After 72 h, small amount of hydrogels remain on the surface, which may result from the chain entanglement between hydrogels and grafted chains of P(PEGMEMA). SEM images confirm that it is gradual dissolution of PEO/lecithin hydrogels that controls the release of lecithin.[27]

Controlled lecithin release in distilled water is shown in Figure 4. Lecithin release is initial loading and temerature-dependent. At 4 °C, lower lecithin loading releases faster than higher, but the higher lasts longer (b vs. c). With the same lecithin loading, lecithin release reaches the equilibrium after 75 h at 4 °C, while the release is completed after 24 h at 12 °C (a vs. c). Figure 4 shows the dissolution rate of PEO hydrogels, as lecithin loading and temperature control the lecithin release in aqueous media. The long release of lecithin at 4 °C is very important, because hypothermic storage of RBCs is at 1−6 °C.[10,16]

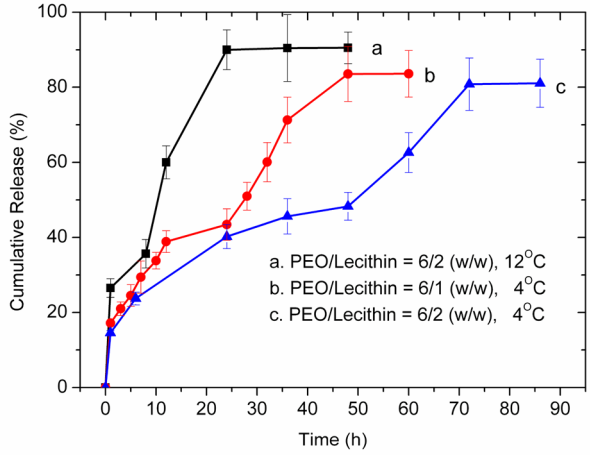

Figure 4. Release profiles of lecithin from PEO/lecithin hydrogel networks. Controlled release of lecithin for long time is achieved with higher lecithin loading (PEO/lecithin = 6/2 w/w) at 4 °C. The release profile is normalized to the amount of lecithin initially loaded into PEO fibers.

3.3. Hemolysis and Morphology of Stored RBCs. The electrospun SEBS is then made into 0.4-mL bags for RBC preservation. After sterilization with ethanol and drying, 0.2 mL of concentrated RBCs is transferred to SEBS bags, which are then sealed and preserved at 4 °C. To clarify the interactions

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between SEBS and RBCs, no additive solutions such as saline adenosine glucose mannitol are added.[11]

For comparison, the bags made by virgin SEBS, grafted SEBS, and commercial PVC bag are used under the same conditions. Figure 5 shows the grafted SEBS bag (a), electrospun SEBS bag (b), and stored RBCs in electrospun SEBS bag (c). After storage for 1 day and 3 days, 100 μL of preserved RBCs are collected for hemolysis test and morphology observation, respectively. The hemolysis ratio of RBCs in different bags is shown in Figure 6. After 1-day storage, the hemolysis ratio of preserved RBCs decreases in the order of SEBS bag, PVC bag, bag of electrospun SEBS, and grafted SEBS with grafting density of 170 μg/cm^2. The slightly higher hemolysis ratio in electrospun SEBS bag than that in grafted SEBS bag may be due to the damage of RBCs caused by rough surface of PEO/lecithin hydrogels networks.[26] After 3-day preservation, RBCs in the bag of electrospun SEBS show the lowest hemolysis ratio, indicating interaction of lecithin with RBCs has substantial effects on the quality of RBCs.

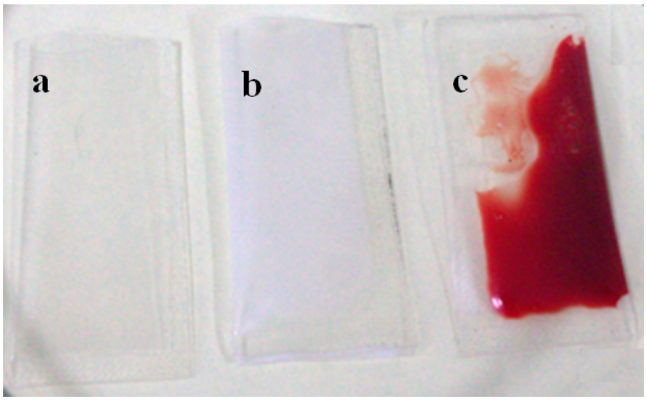

Figure 5. Polymer bags for RBCs storage. (a) Polymer bag made of grafted SEBS; (b) polymer bag made of electrospun SEBS; (c) preserved RBCs in the polymer bag with electrospun SEBS. The volume of polymer bags is about 0.4 mL, and 0.2 mL of fresh concentrated RBCs is preserved in bags at 4 °C.

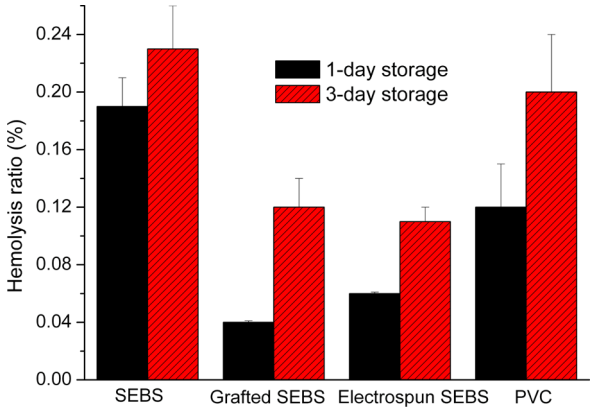

Figure 6. Hemolysis ratio of RBCs in different bags. After 3-day preservation, RBCs in the bag of electrospun SEBS show the lowest hemolysis ratio, indicating interaction of lecithin with RBCs has substantial effects on the quality of RBCs.

SEM studies show a significant RBC shape change after 3-day storage in different bags (Figure 7). Changes in RBC morphology during storage include a transition from a deformable biconcave disc to poorly deformable echinocytes with protrusions, and ultimately nondeformable spheroechinocytes.[31] These irreversible changes result in a severe decrease in membrane deformability. The RBCs in SEBS bags are irregular and flat, and some irreversibly changed RBCs can be observed (Figure 7a). The shape of RBCs in grafted SEBS bags exhibits regular flat discs, as well as a few biconcave discs, indicating only grafted layers cannot prevent membrane of RBCs from oxidative damage (Figure 7b). In contrast, most RBCs in the bags of elestrospun SEBS exhibit biconcave shape (Figure 7c). Both biconcave and flat disks of RBCs are observed in the PVC bag, showing the advantage of commerical PVC bag in RBC storage (Figure 7d).[10] As the shape changes of RBCs are controlled by the deformability of the membrane, which arises from the underlying cytoskeleton, a prototypical assembly of structural proteins,[32] the dominant bioconcave shape of RBCs in the bag of electrospun SEBS confirms the slight oxidation on the membranes of preserved RBCs.

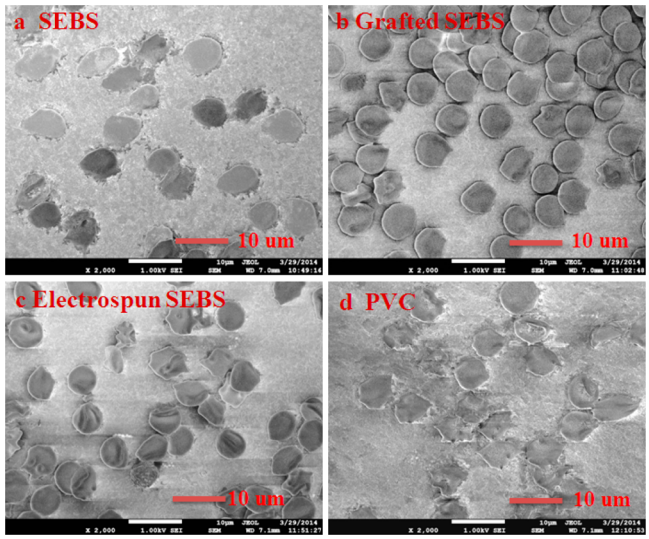

Figure 7. SEM images of RBCs on the third day of storage: (a) in neat SEBS bags, (b) in bags of grafted SEBS with grafting density of 170 μg/cm^2, (c) in electrospun SEBS bags, and (d) in commercial PVC bags.

3.4. Stabilization of RBC Membrane with Released Lecithin. It has been established that an unexpected benefit of storing blood in the PVC bags is the presence of leachable plasticizers that incorporate themselves into the RBCs membrane, reducing membrane loss and bridging asymmetric bilayer of RBCs membrane.[10] Lecithin is a kind of liposome that can form bilayer membranes in aqueous media and alter the thermal phase behavior and composition of RBC membranes through the interaction of liposome with RBC membrane.[16,17] Therefore, released lecithin is expected to play a role similar to that of plasticizers in the PVC bag,[10] which can incorporate into the RBC membrane to stabilize the cellular membranes, maintaining the integrity and deformability of membrane (Figure 1). The role of lecithin in membrane stability is evidenced by low hemolysis ratio and dominant discocyte of RBCs in the bag of electrospun SEBS.

As electrospining is versatile and applicable to any substrates, lecithin can be substituted with other membrane stabilizers and the release can be elongated by precisely controlling the cross-linked networks; the method presented here is universal to construct antihemolysis surface of biomaterials. As a result, our

work not only paves a new way to prepare the antihemolysis container for RBCs preservation in vitro, it also establishes the basic principle to develop antihemolysis implant biomaterials in vivo.

CONCLUSIONS

We presented a facile and universal method to develop blood-contacting biomaterials with long-term, antihemolysis capability by constructing a hydrophilic, 3D hierarchical architecture on the surface of SEBS with PEO/lecithin nano/microfibers. The strategy was based on electrospinning of PEO/lecithin fibers onto the surface of PEG-grafted SEBS, which rendered SEBS suitable for RBCs storage in vitro. The constructed 3D architecture was composed of hydrophilic micro- and nanofibers, which transformed to a physical hydrogel layer immediately with water; and the gradual dissolution of PEO/lecithin hydrogels controlled the release of lecithin that interacted with the membrane of RBCs to maintain the normal RBC shape and membrane flexibility. Thus, the blood-contacting surface reduced both mechanical and oxidative damage to RBC membranes, resulting in low hemolysis of preserved RBCs. This work not only made a novel approach to fabricate high hemocompatible biomaterials for RBCs storage in vitro, but provided basic principles to design and develop antihemolysis biomaterials for implantation in vivo.

ASSOCIATED CONTENT

Supporting Information

Mechanism of grafting PEGMEMA from SEBS surface via BP initiator; ATR-FTIR spectra of PEO and PEO/lecithin electrospun fibers; SEM images of electrospun meshes with different lecithin loading; adhesion measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Ratner, B. D. The Catastrophe Revisited: Blood Compatibility in the 21st Century. *Biomaterials* **2007**, *28*, 5144−5147.
(2) Chen, H.; Yuan, L.; Song, W.; Wu, Z.; Li, D. Biocompatible Polymer Materials: Role of Protein-Surface Interactions. *Prog. Polym. Sci.* **2008**, *33*, 1059−1087.
(3) Xu, F. J.; Neoh, K. G.; Kang, E. T. Bioactive Surfaces and Biomaterials via Atom Transfer Radical Polymerization. *Prog. Polym. Sci.* **2009**, *34*, 719−761.
(4) Gladwin, M. T.; Kanias, T.; Kim-Shapiro, D. B. Hemolysis and Cell-free Hemoglobin Drive an Intrinsic Mechanism for Human Disease. *J. Clin. Invest.* **2012**, *122*, 1205−1208.
(5) Medzhitov, R.; Schneider, D. S.; Soares, M. P. Disease Tolerance as a Defense Strategy. *Science* **2012**, *335*, 936−941.
(6) Schaer, D. J.; Buehler, P. W.; Alayash, A. I.; Belcher, J. D.; Vercellotti, G. M. Hemolysis and Free Hemoglobin Revisited: Exploring Hemoglobin and Hemin Scavengers as a Novel Class of Therapeutic Proteins. *Blood* **2013**, *121*, 1276−1284.
(7) Mecozzi, G.; Milano, A. D.; Carlo, M. D.; Sorrentino, F.; Pratali, S.; Nardi, C.; Bortolotti, U. Intravascular Hemolysis in Patients with New-generation Prosthetic Heart Valves: A Prospective Study. *J. Thorac. Cardiovasc. Surg.* **2002**, *123*, 550−556.
(8) Birschmann, I.; Dittrich, M.; Eller, T.; Wiegmann, B.; Reininger, A. J.; Budde, U.; Strüber, M. Ambient Hemolysis and Activation of Coagulation is Different Between Heartmate II and Heartware Left Ventricular Assist Devices. *J. Heart Lung Trans.* **2014**, *33*, 80−87.
(9) Ishihara, K.; Nishiuchi, D.; Watanabe, J.; Iwasaki, Y. Poly-ethylene/phospholipid Polymer Alloy as an Alternative to Poly-(vinylchloride)-based Materials. *Biomaterials* **2004**, *25*, 1115−1122.
(10) Simmchen, J.; Ventura, R.; Segura, J. Progress in the Removal of Di-[2-ethylhexyl]-phthalate as Plasticizer in Blood Bags. *Trans. Med. Rev.* **2012**, *26*, 27−37.
(11) Henkelman, S.; Rakhorst, G.; Blanton, J.; van Oeveren, W. Standardization of Incubation Conditions for Hemolysis Testing of Biomaterials. *Mater. Sci. Eng., C* **2009**, *29*, 1650−1654.
(12) Mohandas, N.; Gallagher, P. G. Red Cell Membrane: Past, Present, and Future. *Blood* **2008**, *112*, 3939−3947.
(13) Yang, W.; Neoh, K.-G.; Kang, E. T.; Teo, S.L-M.; Rittschof, D. Polymer Brush Coatings for Combating Marine Biofouling. *Prog. Polym. Sci.* **2014**, *39*, 1017−1042.
(14) Li, M.; Neoh, K.-G.; Kang, E. T.; Lau, T.; Chiong, E. Surface Modification of Silicone with Covalently Immobilized and Crosslinked Agarose for Potential Application in the Inhibition of Infection and Omental Wrapping. *Adv. Funct. Mater.* **2014**, *24*, 1631−1643.
(15) Ye, W.; Shi, Q.; Wong, S.-C.; Hou, J.; Xu, X.; Yin, J. Precise Patterning of the SEBS Surface by UV Lithography to Evaluate the Platelet Function through Single Platelet Adhesion. *Biomater. Sci.* **2014**, DOI: 10.1039/C4BM00072B.
(16) Stoll, C.; Stadnick, H.; Kollas, O.; Holovati, J. L.; Glasmacher, B.; Acker, J. P.; Wolker, W. F. Liposomes Alter Thermal Phase Behavior and Composition of Red Blood Cell Membranes. *Biochim. Biophys. Acta* **2011**, *1808*, 474−481.
(17) Holovati, J. L.; Acker, J. P. Emerging Role for Use of Liposomes in the Biopreservation of Red Blood Cells. *Transfus. Med. Hemother.* **2011**, *38*, 99−106.
(18) Lynge, M. E.; Laursen, M. B.; Hosta-Rigau, L.; Jensen, B. E. B.; Ogaki, R.; Smith, A. A. A.; Zelikin, A. N.; Städler, B. Liposomes as Drug Deposits in Multilayered Polymer Films. *ACS Appl. Mater. Interfaces* **2013**, *5*, 2967−2975.
(19) Ye, W.; Shi, Q.; Wong, S. C.; Hou, J.; Shi, H.; Yin, J. Patterning Surfaces for Controlled Platelet Adhesion and Detection of Dysfunctional Platelets. *Macromol. Biosci.* **2013**, *13*, 676−681.
(20) Hou, J.; Shi, Q.; Stagnaro, P.; Ye, W.; Jin, J.; Conzatti, L.; Yin, J. Aqueous-based Immobilization of Initiator and Surface-initiated ATRP to Construct Hemocompatible Surface of Poly (styrene-*b*-(ethylene-*co*-butylene)-*b*-styrene) Elastomer. *Colloids Surf., B* **2013**, *111*, 333−341.
(21) Shi, Q.; Wong, S.-C.; Ye, W.; Hou, J.; Zhao, J.; Yin, J. Mechanism of Adhesion between Polymer Fibers at Nanoscale Contacts. *Langmuir* **2012**, *28*, 4663−4671.
(22) Reneker, D. H.; Chun, I. Nanometre Diameter Fibres of Polymer, Produced by Electrospinning. *Nanotechnology* **1996**, *7*, 216−223.
(23) Shi, Q.; Wan, K.-T.; Wong, S.-C.; Chen, P.; Blackledge, T. A. Do Electrospun Fibers Stick? *Langmuir* **2010**, *26*, 14188−14193.
(24) Zhou, C.; Shi, Q.; Guo, W.; Terrell, L.; Qureshi, A. T.; Hayes, D. J.; Wu, Q. Electrospun Bio-Nanocomposite Scaffolds for Bone Tissue Engineering by Cellulose Nanocrystals Reinforcing Maleic Anhydride Grafted PLA. *ACS Appl. Mater. Interfaces* **2013**, *5*, 3847−3854.
(25) Casper, C. L.; Yang, W.; Farach-Carson, M. C.; Rabolt, J. F. Coating Electrospun Collagen and Gelatin Fibers with Perlecan Domain I for Increased Growth Factor Binding. *Biomacromolecules* **2007**, *8*, 1116−1123.

(26) Umezu, M.; Yamada, T.; Fujimasu, H.; Fujimoto, T.; Ranawake, M.; Nogawa, A.; Kijima, T. Effects of Surface Roughness on Mechanical Hemolysis. *Artif. Org.* **1996**, *20*, 575−578.
(27) Maggi, L.; Bruni, R.; Conte, U. High Molecular Weight Polyethylene Oxides (PEOs) as an Alternative to HPMC in Controlled Release Dosage Forms. *Int. J. Pharm.* **2000**, *195*, 229−238.
(28) Yoon, K.; Hsiao, B. S.; Chu, B. Formation of Functional Polyethersulfone Electrospun Membrane for Water Purification by Mixed Solvent and Oxidation Processes. *Polymer* **2009**, *50*, 2893−2899.
(29) Geoghegan, M.; Clarke, C. J.; Boué, F.; Menelle, A.; Russ, T.; Bucknall, D. G. The Kinetics of Penetration of Grafted Polymers into a Network. *Macromolecules* **1999**, *32*, 5106−5114.
(30) Hoffman, A. S. Hydrogels for Biomedical Applications. *Adv. Drug Delivery Rev.* **2002**, *54*, 3−12.
(31) Berezina, T. L.; Zaets, S. B.; Morgan, C.; Spiller, C. R. Influence of Storage on Red Blood Cell Rheological Properties. *J. Surg. Res.* **2002**, *102*, 6−12.
(32) Discher, D. E.; Mohanda, N.; Evans, E. A. Molecular Maps of Red Cell Deformation: Hidden Elasticity and in situ Connectivity. *Science* **1994**, *266*, 1032−1035.