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Construction of D-α-Tocopheryl polyethylene glycol succinate/PEO core-shell nanofibers on blood-contacting surface to reduce the hemolysis of preserved erythrocyte

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The hemolysis of erythrocyte is a big obstacle to the development of new non-plasticizer polymer container for erythrocyte preservation. To construct a long-term anti-hemolytic surface of plasticizer-free polymer, we coaxially electrospin core-shell structured D-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS)/poly(ethylene oxide) nanofibers on the surface of styrene-b-(ethylene-co-butylene)-b-styrene elastomer (SEBS) that is covered with the grafted poly (ethylene glycol) (PEG) chains. Our strategy is based on that the grafted layers of PEG reduce the mechanical damage to red blood cells (RBCs) while the released TPGS from the nanofibers on the blood-contacting surface can act as an antioxidant to prevent the RBCs from oxidative damage. We demonstrate that TPGS/PEO core-shell structured nanofibers have been well prepared on the surface of PEG modified SEBS; the controlled release of TPGS in the distilled water is obtained and the release can last for almost 4 days at 4 °C; during RBCs preservation, TPGS acts as the antioxidant to decrease the membrane oxidation and hemolysis of RBCs. Our work paves a new way to develop non-plasticizer polymer for RBCs preservation, which may be helpful to fabricate long-term anti-hemolytic biomaterials *in vivo*.

1. Introduction

Blood-contacting biomaterials have been widely applied in many areas, such as the platform for blood-based detection and implant devices.[1-3]Among them, polymer bags used for blood storage are necessary for modern medicine. The main polymer used for RBC preservation is polyvinylchloride (PVC), which requires the usage of high amount of plasticizers for processing.[4] However, the plasticizer has been found to leach out from the container and cause toxic effects in liver, kidney and rodents of the patients. [5] Hence, it is urgent to develop new non-plasticizer polymer containers to replace the toxic PVC blood bags for RBCs preservation. SEBS is the product of hydrogenated polystyrene-b-polybutadiene-b-polystyrene (SBS) triblock copolymer, which is inert and durable, and exhibits excellent oxidative and hydrolytic resistance. The advantage of SEBS makes it a potential candidate in fabricating non-PVC bags.[6-9] However, the long-term contact of RBCs with SEBS causes the release of intracellular hemoglobin (hemolysis), which reduces oxygen affinity and delivery, leading to an intrinsic mechanism for human disease.[10-12] So far, the hemolysis of RBCs in SEBS containers becomes a big obstacle to replacement progress.[5] It is therefore a pressing task to entitle SEBS with the anti-hemolytic capability to substitute PVC blood bags.

Oxidative injure to the membrane of RBCs is the main cause for the hemolysis of RBCs during RBC storage.[13] The oxidative damage to the RBCs induces the lipid loss from the membrane, reduces deformability and increases rigidity of RBCs. Thus, decreasing the oxidative damage to the RBC membrane is a key strategy to improve the quality of preserved RBCs fundamentally. However, the conventional methods based on surface modification have slight effects on oxidative injure to RBCs.[14-16] Very recently, we fabricate the lecithin/PEO nanofibers on SEBS surface to reduce the oxidative damage to RBC membrane.[17] During the storage of RBCs in the bags of these biomaterials, the released lecithin from the nanofibers can interact with RBCs to reduce the lipid loss from RBC membranes caused by oxidation. Although this method mitigates the lipid loss by oxidation, it cannot prevent the oxidation of RBC membrane during storage fundamentally. An effective method is to maintain the sustainable interactions of suitable antioxidants with the stored RBCs to prevent the RBC membrane from oxidation. Recently, D-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS) is reported to act as an antioxidant to decrease the oxidative damage of RBCs membrane.[18,19] TPGS is a water-soluble vitamin E formulation, which has been approved by the U.S. Food and Drug Administration (FDA).[20] Because TPGS is easily oxidized in the solution, such as in the blood plasma, controlled release of

TPGS from the blood-contacting surface to the stored RBCs and sustainable interactions between TPGS and RBC membrane are highly desired. However, precisely design is needed to prevent the oxidation of TPGS during the TPGS encapsulation and loading in the architectures on the surface. Considering the advantage of coaxial electrospinning in encapsulating multiple drugs with different solubility, eliminating the damage to bioactive agents, and preventing the premature release of the water-soluble core contents,[21-23] we encapsulate the TPGS in the core-shell structured nanofibers with coaxial electrospinning technique and fabricate the nanofibers on the grafted substrates.

Here, we coaxially electrospin core-shell structured TPGS/PEO nanofibers on the SEBS surface that has been grafted with PEG chains. Our strategy is based on that the grafted PEG layers reduce the mechanical damage to RBCs while the released TPGS from the nanofibers can act as an antioxidant to prevent the RBCs from oxidative damage. The grafted PEG chains provide a hydration layer on the SEBS surface to reduce the collusion between RBCs and SEBS surface and resist adhesion of RBCs on the SEBS surface, resulting in reduced mechanical damage to RBCs membrane. We demonstrate that TPGS/PEO core-shell structured nanofibers have been well prepared on the surface of SEBS; the controlled release of TPGS in the distilled water is obtained and the release lasts for almost 4 days at 4 °C; during RBCs preservation, TPGS acts as the antioxidant to decrease the membrane oxidation and hemolysis of RBCs. Our work thus paves a new way to develop non-plasticizer polymer for RBCs preservation, which may be helpful to fabricate long-term anti-hemolytic biomaterials *in vivo*.

2. Materials and methods

2.1 Chemicals and Materials

SEBS copolymer with 29 wt% styrene (Kraton G 1652E) was provided by Shell Chemicals. Poly (ethylene glycol) methyl ether methacrylate (PEGMA) (M_w = 300 g mol^{-1}), PEO (M_w = 5×10^6 g mol^{-1}) and D-α-tocopherol polyethylene glycol 1000 succinate (TPGS, M_w = 1513 g mol^{-1}) were purchased from Sigma-Aldrich. The chemical substances were shown in Fig. 1a. Benzophenone (BP) was purchased from 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, 0.01M phosphate buffer, PH 7.4) was prepared freshly.

2.2 Surface Modification of SEBS Films with PEGMA

SEBS was dissolved in xylene to form 15 % (w/w) solution and the solution was casted 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 PEGMA with the concentration of 15 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 monomer and dried 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 was obtained by a weight method. Data were presented as average of six replicates of each surface with standard deviation (SD).

2.3 Coaxial Electrospinning of PEO/TPGS onto PEG-Grafted SEBS

PEO was dissolved in a solvent mixture of water and ethanol in the ratio of 3:2 (w/w) to obtain the concentration of 2 wt%. Similarly, TPGS was dissolved in the distilled water with the concentration of 2 wt%. The key component in the coaxial electrospinning setup was a compound spinneret, which consists two small-diameter capillary tubes with one located inside another (Fig. 1b). The rate of flow was controlled by the syringe pump, and the feed rate of inner flow (TPGS) and outer flow (PEO) were 0.1 ml h^{-1} and 0.5 ml h^{-1}, respectively. For comparison, the neat PEO nanofibers were fabricated without the inner flow of TPGS. All electrospinnings were performed 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 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). Data were presented as average of six replicates of each surface with SD.

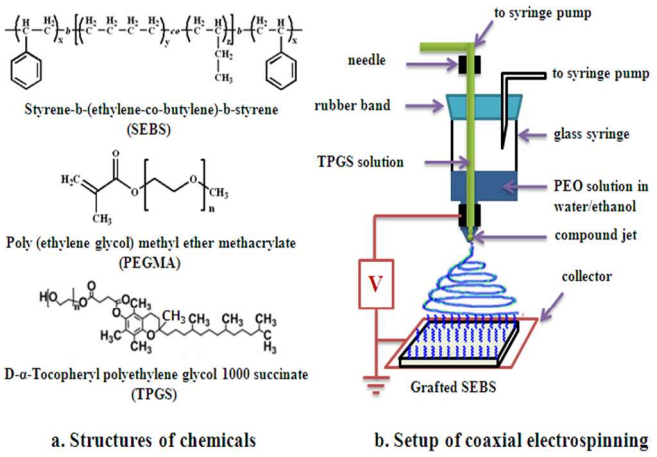

Fig. 1 Chemical structures of substances (a) and setup of coaxial electrospinning for fabrication of nanofibers on the grafted SEBS (b).

2.4 The Release of TPGS in the Distilled Water

To study the dependence of TPGS release on temperature, 1 cm × 1 cm films of electrospun SEBS with the density of 0.2±0.01 mg cm^{-2} were incubated in the distilled water at 25 °C and 4 °C, respectively. The release at 4 °C was specially investigated because hypothermic storage of RBCs was at 1-6 °C.[5,17] Then, 1 mL solution was collected at the desired time and the amount of the released TPGS were measured using a high-performance liquid chromatography (Waters 600 HPLC, evaporative light scattering detector) with the standard calibration curve. The release profile was normalized to the amount of TPGS initially loaded in PEO fibers. Data were presented as average of six replicates of each incubation time with standard deviation (SD).

2.5 Measurement of Hemolysis and Mechanical Fragility

Fresh blood was extracted from the artery in the ears of a healthy rabbit (The live-animal experiments were performed in compliance with guidelines issued by the Ethical Committee of the Chinese Academy of Sciences (CAS), and the Ethical Committee of CAS approved the experiments). The rabbits were New Zealand rabbits, 8-month old with the weight range from 1.0 to 1.5 kg. The collected blood was immediately mixed with 3.8 wt % sodium citrate solution at a dilution ratio of 9:1. Then the whole blood 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 virgin and electrospun SEBS films (4 cm × 4 cm) were then 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 and preserved at 4 °C after sealing (Fig. S2).

90 μL preserved RBCs were collected for hemolysis test after 4-day and 8-day storage, respectively. The preserved RBCs were diluted with 1 mL normal saline and centrifuged (3000 rpm, 3 min) to get the supernatant. Then, the supernatant was transferred to 96-well plates. Positive and negative controls were produced by adding 90 μL fresh RBCs to 1 mL distilled water and normal saline, respectively. After 2 h incubation, 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 TECAN absorbance reader (TECAN GENIOS, Austria) at 541 nm. The hemolysis ratio (HR) was calculated according to the following formula:

$$HR\ (\%) = \frac{OD_{test} - OD_{neg}}{OD_{pos} - OD_{neg}} \times 100 \qquad (1)$$

where OD_{test} is the absorbance value of the test samples, OD_{pos}, and OD_{neg} are the positive (water) and negative (saline) control, respectively.

The mechanical fragility (MF) test of stored RBCs was performed based on the method developed by Raval et al.[24] Briefly, 300 μL preserved RBCs were diluted with 6 mL normal saline and then averagely transfer to six tubes (1.8 mL, 8×1-mm serum blood collection tubes, Jiangsu Kangjian Biomedical Instrument Corp. China), three of which contained two ellipsoid magnetic stirrers (A-610, 6 mm ×10 mm, Beijing Rezekang Technology Co. Ltd. China) and three of which did not. The tubes with stirrers were strongly shaken on a thermostatic oscillator (Type THZ-312; Shanghai Jinghong Instrumental Corp., Shanghai, China) for 2 h, while the remaining tubes without stirrers were not shaken and served as controls to ascertain the initial concentration of free hemoglobin (Hb) in each aliquot. After shaking, all tubes were centrifuged twice, and the free Hb concentrations in the supernatants were determined by TECAN absorbance reader (TECAN GENIOS, Austria) at 541 nm. For comparison, the MF tests for fresh RBCs were performed under the same conditions. In addition, 50 μL fresh RBCs were diluted with 1 mL distilled water and incubated for 2 h. The Hb concentration was measured by the TECAN absorbance reader and used as the standard aliquots concentration for mechanical fragility index (MFI) calculation. The MFI was then calculated according to the following formula:

$$MFI = \frac{Hb_{shaken} - Hb_{control}}{Hb_{aliquots} - Hb_{control}} \times 100 \qquad (2)$$

where Hb_{shaken} is the mean free Hb concentration in the supernatants of the shaken specimens, $Hb_{control}$ is the average free Hb concentration in the supernatants of the control samples, and $Hb_{aliquot}$ is the average Hb concentration in the supernatants of 50 μL fresh RBCs in 1 mL distilled water after 2 h treatments.

2.6 RBC Morphology and Membrane Analysis after Storage

50 μL stored RBCs in the electrospun SEBS bags were carefully rinsed with PBS. Then, the fresh RBCs, preserved RBCs and rinsed RBCs were dropped onto poly-L-lysine-coated glass slides. And these RBCs were incubated at 37 °C for 60 min under static conditions to adhere firmly on the glass slides. After the incubation, the samples were carefully rinsed twice with pre-warmed PBS, followed by immersing in 2.5 vol % glutaraldehyde in PBS (3 mL) for 10 h at 4 °C to fix the adhered RBCs. Finally, the samples were freeze-dried. The morphologies of adhered RBCs on the sample surfaces were visualized by SEM (SEM, JEOL, JSM-7500F, JP).The membrane 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.

2.7 Biochemical Properties of RBC after Storage

The fresh RBCs and preserved RBCs (0.5 ml) were collected with the blood collection syringe (maximum 3 mL, Becton, Dickinson and Company). All samples were analyzed by a Blood Gas/Electrolyte Analyzer (GEM Premier 3000, Instrumentation Laboratory Company, USA) at 37 °C. The biochemical properties of RBCs, such as the concentration of K^+ and the oxygen tension were obtained.

2.8 **Statistical Analysis**

The hemolysis and MFI were given as means ± SD for the indicated number of fresh and preserved RBCs. The biochemical properties of RBCs were expressed as average with SD. Statistical analysis, one-way ANOVA with *post hoc* analysis by Bonferroni's multiple comparison test, was performed using Origin Software, At least six samples were analyzed and each sample was measured six times. Differences were considered statistically significant at $P<0.05$.

3 Result and discussion

3.1 Fabrication of core-shell nanofibers on the surface of PEG-grafted SEBS

The fabrication of core-shell structured nanofibers on SEBS surface is constructed through grafting PEGMA onto SEBS surface, followed by coaxial electrospinning of TPGS/PEO onto the grafted surface (Fig. 1b). The successful grafting reaction is confirmed by FTIR spectra (Fig. 2a and 2b). In comparison with the virgin SEBS (Fig. 2a), two new absorption peaks appear at 1720 cm^{-1} and 1110 cm^{-1} in the spectrum of PEGMA-grafted SEBS film (Fig. 2b), which correspond to the stretching vibration of C=O and C-O-C, respectively.[7] The high intensity of absorption peak (1720 cm^{-1}) indicates the high grafting density. The high grafting density (about 170±12 μg cm^{-2}) ensures the surface of SEBS is fully covered with grafted chains. When the grafted PEG chains contact the water, water molecules likely form hydrogen bonds with two adjacent oxygen of the monomer unit of PEG to stabilize the water interface with helical PEG brushes.[25]As a result, the surface of SEBS becomes hydrophilic with the water contact angle (WCA) of 58±6° (inset of Fig. 2b). And the hydration layer on the SEBS surface reduces mechanical damage of RBC membranes.[17]

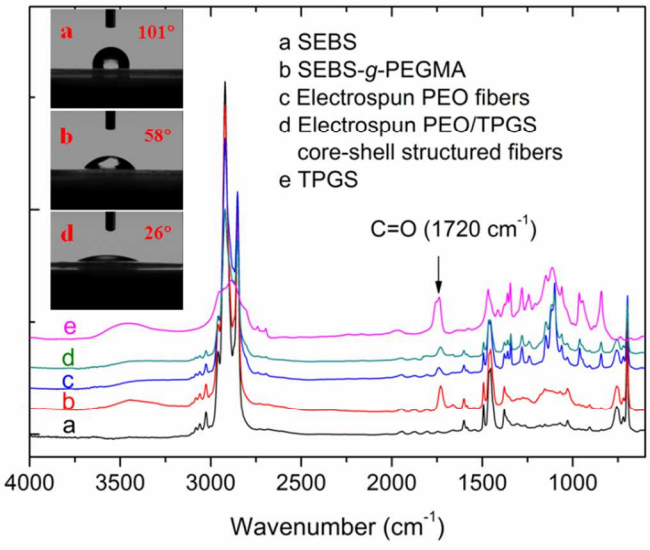

Fig. 2 ATR-FTIR spectra of SEBS, electrospun fiber and TPGS. The insets are the images of water contact angle on the surface of SEBS samples. (a) virgin SEBS, (b) SEBS-g-PEGMA, (c) electrospun PEO fibers, (d) electrospun TPGS / PEO fibers, (e) TPGS.

The core-shell structured TPGS/PEO nanofibers on the surface of grafted SEBS are fabricated by coaxial electrospinning with the density of about 0.2±0.01 mg cm^{-2}. The SEM images show that the SEBS surfaces are covered with smooth nanofibers without the formation of beads (Fig. 3). Bead structures may not only lead to aggregation of colloids to form curly fibers[26] but also prevent the release of core contents in a controlled manner.[27] Thus, obtaining core-shell structured nanofibers without bead defects is highly desired. The diameter of electrospun fibers ranges from 100 nm to 1600 nm (inset of Fig. 3a). There are little differences in the fiber morphology between virgin SEBS and grafted SEBS (Supporting Information, Fig. S1), indicating that the grafted layer has almost no influences on the morphology and structure of nanofibers. The electrospun fibers further decrease the value of WCA (26±4°, inset of Fig. 2d), which makes the surface hydrophilic to resist cell adhesion and provides the hydrogel layer to reduce the mechanical damage to the RBC membrane.[17] Typical TEM images of the bi-component nanofibers are shown in Fig. 3b. The boundaries of the core-shell structure along the length of the fiber and congregation of TPGS can be observed (inset of Fig. 3b). The scattered dispersion of TPGS may be attributed to the flow instability of the inner dope and the bending instability during the electrospinning process. [21-23]

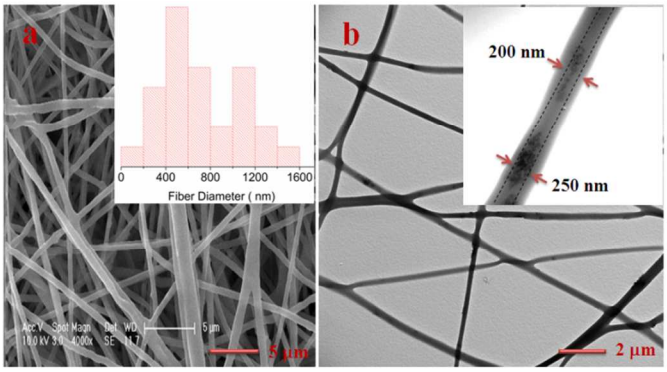

Fig. 3 SEM and TEM images of core-shell structured nanofibers. (a) SEM image of electrospun fibers; (b) TEM images of nanofibers on a copper grid. The inset is the segment of the nanofibers with scattered inner component.

The encapsulation of TPGS in PEO fibers is further confirmed by the FTIR spectra (Fig. 2c and Fig. 2d). The neat electrospun PEO fibers on SEBS show the peak at 1720 cm^{-1}, which is due to carboxyl group of grafted PEGMA.[17] The spectrum of TPGS/PEO core-shell fibers is similar with that of neat PEO fibers, and the intensity of peak at 1720 cm^{-1} is not stronger than that of neat PEO fibers after peak normalization. Because the spectrum of TPGS exhibits the strong carboxyl peak at 1720 cm^{-1} (Fig. 2e), the similar peak intensity in the spectra between PEO fibers and TPGS/PEO fibers confirms that most TPGS are dispersed in the core part and well encapsulated on the PEO shell.[22]

3.2 The Release of TPGS in the Distilled Water

The release of TPGS in the distilled water is investigated at 25 °C and 4 °C, respectively (Fig. 4). At 25 °C, the release of TPGS is completed within 48 h, but the releases of TPGS can last for 96 h at 4 °C. It has been established that polymer nanofibers are characterized by high surface area-to-volume ratio and highly active chains at surface, which render them adhesive to each other to improve the mechanical properties of electrospun meshes.[28-31] However, the adhesion between the meshes and the substrates is relatively weak and the hydrophilic meshes are easily detached from the substrate when they are immersing in water. Although chemical crosslinking of PEO nanofibers can overcome this issue, most crosslinking agents are toxic[32] and the roughness of cross-linked meshes induces RBCs hemolysis.[33] Thus, the stable adhesion of the meshes on SEBS is achieved by enhancement of fusion density between electrospun fibers and enforcement of chain entanglements between long grafted PEG chains and PEO chains in the fibers at relative high process temperature (~45°C) in this work.[31,34]

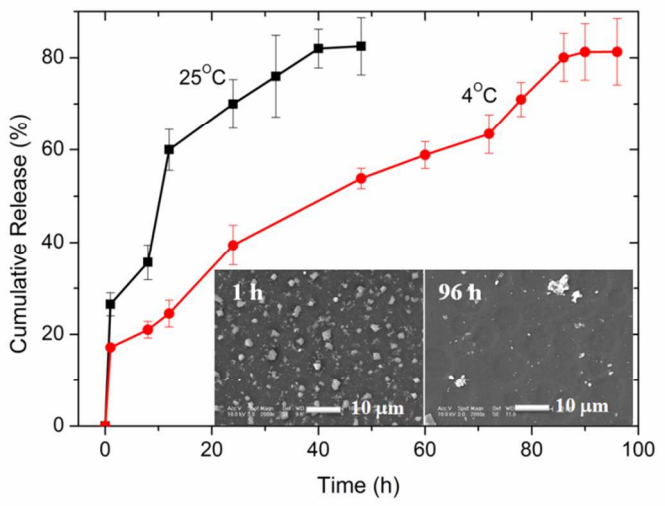

Fig. 4 Release profiles of TPGS from the constructed surface at 25 °C and 4 °C, respectively. The insets are images of electrospun fibers after immersing in the water for 1 h and 96 h, respectivley. Controlled release of TPGS for about 96 h at 4 °C is achieved. The release profile is normalized to the amount of TPGS initially loaded into PEO fibers (1/5). The density of TPGS/PEO fibers on SEBS surface is about 0.2±0.01 mg cm^{-2}.

The SEM images of nanofibers after immersing in the distilled water are shown in Fig. 4 as the insets. SEM images show that most nanofibers become physical hydrogels covering on the surface of SEBS after contacting water for 1 h. And some nanofibers with high fusion density tend to form aggregates to disperse heterogeneously on the hydrogel layer (inset for 1 h). After 96 h, most hydrogels dissolve but there are still small amount of hydrogels on the surface (inset for 96 h), confirming the interactions between nanofibers and the SEBS. Although the loading ratio of TPGS in PEO fibers has an effect on the initial release rate (Fig. S3), the release of TPGS is mainly controlled by gradually dissolution of PEO hydrogel.[31] As PEO is a Food and Drug Administration (FDA) approved polymer that has been widely applied in the food and life science,[35] the soluble PEO are expected to have slight effect on the quality of blood.

3.3 Hemolysis, Mechanical Fragility and Morphology of Stored RBCs

The virgin and electrospun SEBS are made into 0.4 mL bags and sterilized with ethanol, respectively. Then, 0.2 mL RBCs are packaged in these bags without the additive solutions and stored at 4 °C (Fig. S3). After 4-day and 8-day storage, the stored RBCs are collected for hemolysis, mechanical fragility (MF) and morphology analysis. P-values of hemolysis and MFI between virgin SEBS and electrospun SEBS are < 0.05, respectively.

No hemolysis is occurred in the fresh RBCs (Fig.5). After 4-day and 8-day storage, the hemolysis ratio of preserved RBCs in virgin SEBS bags is 1.2±0.1% and 1.8±0.3%, respectively. On the contrary, the hemolysis ratio of preserved RBCs in electrospun SEBS bags is 0.7±0.1% and 0.9±0.1%, respectively. Similarly, the MFI of fresh RBCs is about 0.5±0.05. After 4-day and 8-day preservation, MFI of RBCs in original SEBS bag is about 0.9±0.04 and 1.2±0.1, respectively, showing the serious sublethal injure of RBCs occurs due to the oxidation of membrane.[24, 36] In contrast, the MFI of the stored RBCs in the bags of electrospun SEBS is 0.6±0.03 and 0.7±0.02, respectively, which are comparable to that of fresh RBCs. These results not only confirm that the interactions between TPGS and RBCs have decreased oxidative injure to RBC membrane substantially, but also indicate that the interactions can maintain at least 8 days even the release of TPGS from the blood-contacting surface is completed in 4 days.

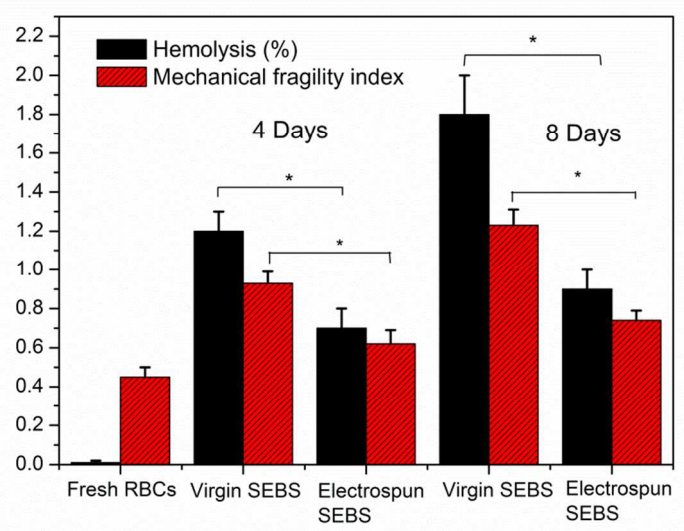

Fig. 5 Hemolysis and mechanical fragility index of fresh RBCs, preserved RBCs in virgin and electrospun SEBS bags. * P-values of hemolysis and MFI between virgin SEBS and electrospun SEBS are < 0.05, respectively.

The hemolysis and membrane fragility accompany the morphology change of RBCs. During erythrocyte preservation, erythrocytes transit from biconcave discs to poorly-deformable echinocytes with protrusions and further non-deformable spheroechinocytes, resulting in a severe decrease in membrane deformability.[37] The morphology of fresh RBCs and preserved RBCs is observed with SEM (Fig. 6). The fresh RBCs show

regular biconcave discs without any injure (Fig. 6a). The shape of preserved RBCs in the virgin SEBS bags changes significantly. Most of preserved RBCs are irregular echinocytes with many protrusions, which exhibit irreversibly deformation (Fig. 6b). In contrast, most of the preserved RBCs in the electrospun SEBS bags show regular biconcave discs (Fig. 6c), indicating the slight oxidation on the RBC membrance. The shape of regular biconcave discs is dominaint even after 8-day storage in the electrospun SEBS (Fig. 6d), confirming the anti-oxidative capability can be maintain at least for 8 days in the electrospun SEBS bags. It is well known that the changes in RBC shape is determined by the membrane deformability,[38] regular biconcave-RBCs confirm the high deformability of RBCs.

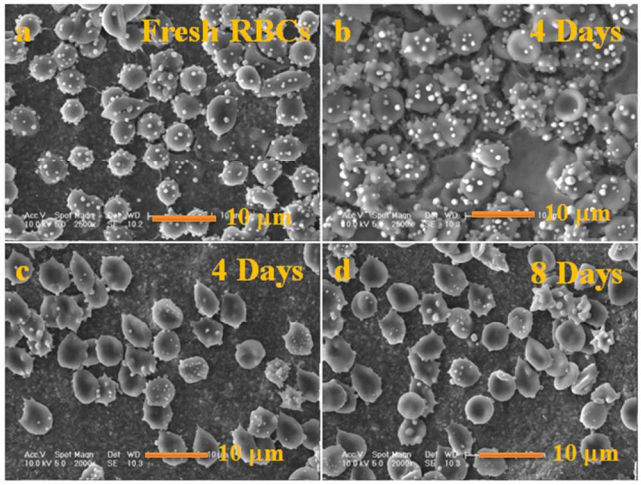

Fig. 6 The morphology of fresh RBCs (a), stored RBCs in virgin SEBS for 4 days (b), stored RBCs in electrospun SEBS bags for 4 days (c) and 8 days (d), respectively.

Table 1. Biochemical properties of fresh RBCs and 4-day stored RBCs

Samples	pH	Glucose (mmol/L)	Lactate (mmol/L)	Potassium (mmol/L)	P50[a] (mmHg)
Fresh RBCs	7.4±0.5	6.2±0.2	9.0±1.2	2.8±0.4	35.0±3.1
RBCs in virgin SEBS bags	7.1±0.6	2.9±0.3	25±2.3	20.2±0.7	43.0±6.2
RBCs in electrospun SEBS bags	7.2±0.3[b]	3.9±0.5	16±1.7	9.2±0.6	38.0±4.2[b]

Values are means ± SD. a. the oxygen tension at which oxyhaemoglobin is 50% saturated; b. $P < 0.05$ Vs. fresh RBCs and RBCs in the virgin SEBS bags.

The biochemical properties of RBCs after 4-day storage are evaluated, and the corresponding results are listed in Table 1. Data are given as means ± SD. P-values of pH and P50 between virgin SEBS and electrospun SEBS are < 0.05, respectively. Compared with biochemical properties of fresh RBCs, the preserved RBCs exhibit decreased glucose concentration and pH values, increased lactate and potassium concentration in the suspending medium. The above results can be attributed to the metabolism and apoptosis of RBCs during storage.[39,40] In addition, haemoglobin affinity for oxygen (P50, the oxygen tension at which oxyhaemoglobin is 50% saturated) decreases, resulting in low ability for haemoglobin to carry oxygen. Because the hemoglobin affinity to oxygen is known to be enhanced by increased partial pressure of oxygen, reduced temperature and acidity,[39] the reduced affinity may be due to the increased acidity. However, compared with the RBCs in the virgin SEBS, RBCs in the electrospun bags show comparable values of the potassium concentration and haemoglobin affinity to that of fresh RBCs, confirming the oxidative damage to RBCs is decreased by the action of TPGS in electrospun bags. As human and animal share some similarities in physical and biochemical properties of RBCs,[41,42] it is promising for electrospun SEBS to substitute PVC for human RBC storage.

3.4 Mechanism of Electrospun SEBS in RBCs Preservation

The fresh RBCs, preserved RBCs in the virgin SEBS bags, stored RBCs in the electrospun bags, and RBCs in the electrospun bags after rinsing are deposited onto the poly-L-lysine-coated glass slides for FTIR analysis, respectively (Fig.7). RBCs exhibit typical peaks of amide groups (1700 cm^{-1} to 1500 cm^{-1}) of membrane proteins.[34] Compared with the FTIR spectrum of fresh RBCs (Fig. 7a) and RBCs in the virgin SEBS bags (Fig. 7b), the spectrum of RBCs in the electrospun bags (Fig. 7c) shows the new peak at 1721 cm^{-1}, which is attributed to the C=O stretching vibration of TPGS.[19] As TPGS has no specific binding site to RBCs membrane, it is believed that TPGS can physically adsorb onto the RBCs membrane and prevent the membrane oxidation of RBCs through direct interactions, which has been confirmed by our previous work.[19] No TPGS signal can be detected after rinsing stored RBCs in the electrospun bags several times (Fig. 7d), indicating the physical adsorption is reversible. Thus, the release of TPGS to plasma shows no side effects on RBC quality.

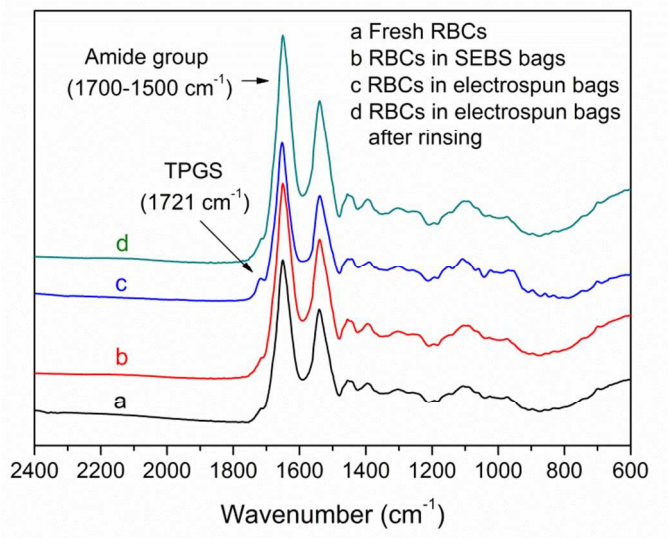

Fig. 7 FTIR spectra of fresh RBCs (a), stored RBCs in virgin SEBS for 4 days (b), stored RBCs in electrospun SEBS bags for 4 days (c), and RBCs in electrospun SEBS bags after rinsing (d), respectively.

Based on the experimental results, the mechanism of the electrospun SEBS in improving the quality of preserved RBCs is tentatively presented. The electrospun SEBS contains core-shell structured TPGS/PEO fibers, when the fibers contact the water, and these fibers become physical hydrogels covering on the SEBS surface. During the RBC storage, the TPGS is controlled released from the hydrogel layer and the released TPGS acts as an antioxidant to prevent the RBC membrane from oxidative damage, maintaining the integrity and deformability of membrane (Fig. 8). The antioxidative capability can sustain at least 8 days even the release of TPGS is completed in 4 days. Thus, low hemolysis of preserved RBCS is achieved in the bags of electrospun SEBS. Because the electrospinning is able to produce large-scale, 3D nanofibers with controllable architectures on nearly any substrate, [43,44] and TPGS can be substituted with other antioxidants, the method presented here is universal to develop new biomaterials with high anti-hemolytic capability.

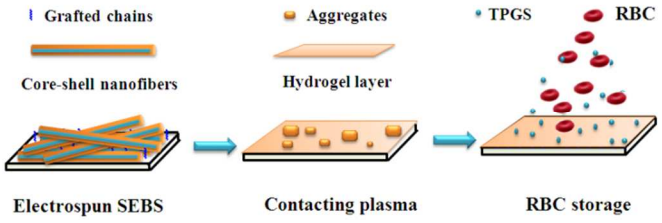

Fig. 8 Mechanism of the electrospun SEBS in improving the quality of preserved RBCs.

Conclusions

To construct a long-term anti-hemolytic surface of plasticizer-free polymer, we coaxially electrospun core-shell structured TPGS/PEO nanofibers on the surface of SEBS that was covered with the grafted PEG chains. Our strategy was based on that the grafted layers of PEG reduce the mechanical damage to the stored RBCs while the released TPGS from the blood-contacting surface could act as an antioxidant to prevent the preserved RBCs from oxidative damage. We demonstrated that TPGS/PEO core-shell structured nanofibers were well prepared on the surface of PEG modified SEBS; the controlled release of TPGS in the distilled water was obtained and the release sustained for almost 4 days at 4°C; during RBCs preservation, TPGS acted as the antioxidant to substantially decrease the membrane oxidation and hemolysis of RBCs. Our work paves a new way to develop non-plasticizer polymer for RBCs preservation, which may be helpful to fabricate long-term anti-hemolytic biomaterials *in vivo*.

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Notes and references

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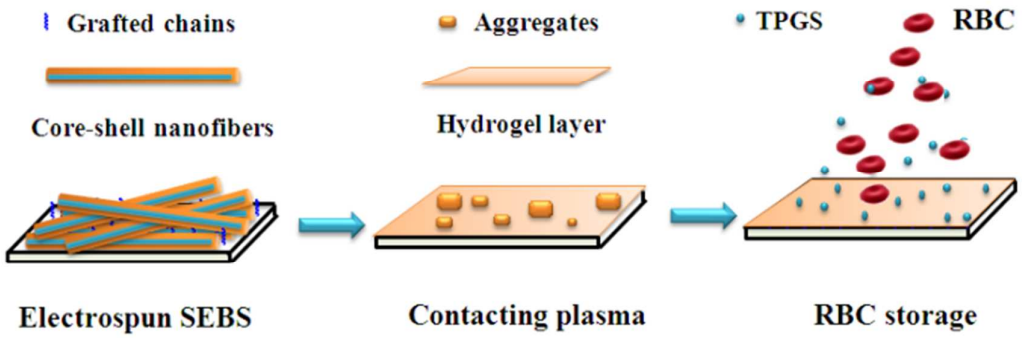
Table of Contents Entry



The released TPGS from the electrspun SEBS prevented the preserved red blood cells from the oxidation damage, resulting in low hemolysis and mechanical fragility.