Supporting Information

A Smart Core-sheath Nanofiber that Captures and Releases Red Blood Cells from the Blood

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Contents:

1. Electrospinning of Polycaprolactone (PCL), Poly (N-isopropylacrylamide) (PNIPAAm) and PCL/ PNIPAAm nanofibers.

PNIPAAm and PCL were dissolved in DMF/chloroform (2:3) at room temperature and stirring for 2 h. The ratio of PNIPAAm to PCL was set as 1/9, 3/7 and 5/5 with the total polymer concentration of 20 w/v%. Pure solutions of PNIPAAm (30 w/v%) and PCL (12 w/v %) were prepared for comparison. The polymer solutions were placed in a l mL syringe fitted with a metallic needle of 0.4 mm inner diameter. The syringe was fixed horizontally on the syringe pump (Model: OPON mini). And an electrode of high voltage power supply (Tianjin High-voltage Co.) was clamped to the metal needle tip. The flow rate of polymer solution was 0.7 mL h^{-1}, and the applied voltage was 18 kV. The tip-to-collector distance was set to 12 cm, and a grounded stationary rectangular metal collector (15 cm × 15 cm) covered by a piece of clean aluminum foil was used for the fiber collection. The electrospun nanofibers were characterized by field emission scanning electron microscopy (FESEM) and X-ray photoelectron spectroscopy (XPS).

2. FTIR spectra of PCL, PNIPAAm and PCL/PNIPAAm nanofibers.

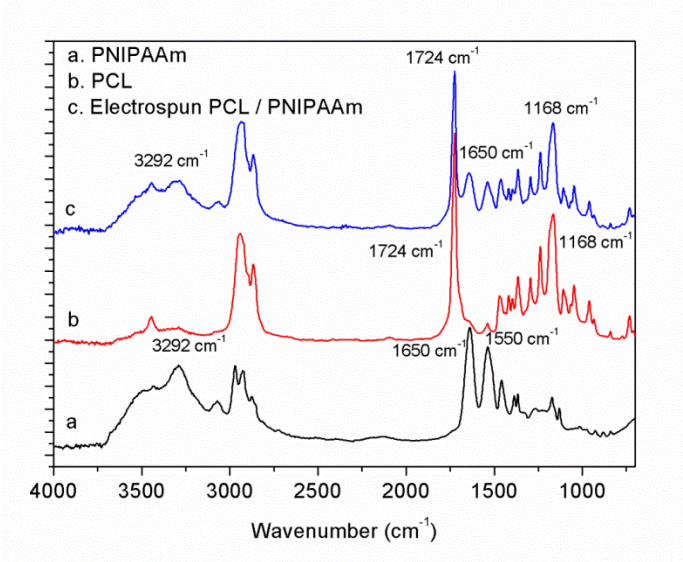

Figure S1. FTIR spectra of PCL, PNIPAAm and PCL/PNIPAAm nanofibers

The FTIR spectra of PCL, PNIPAAm and PCL/PNIPAAm nanofibers are shown

in Figure S1. PNIPAAm spectrum exhibits the typical peak at 1650 cm^{-1}, which are assigned to the carboxyl groups connected to amino groups of PNIPAAm.[1] PCL spectrum appears the typical peak at 1724 cm^{-1}, which is attributed to the carboxyl groups of PCL.[2] For the spectrum of PCL/PNIPAAm nanofibers, both signals at 1724 and 1650 cm^{-1} are observed, indicating the PCL/PNIPAAm nanofibers have been successfully fabricated.

3. XPS spectra of PCL and PNIPAAm nanofibers.

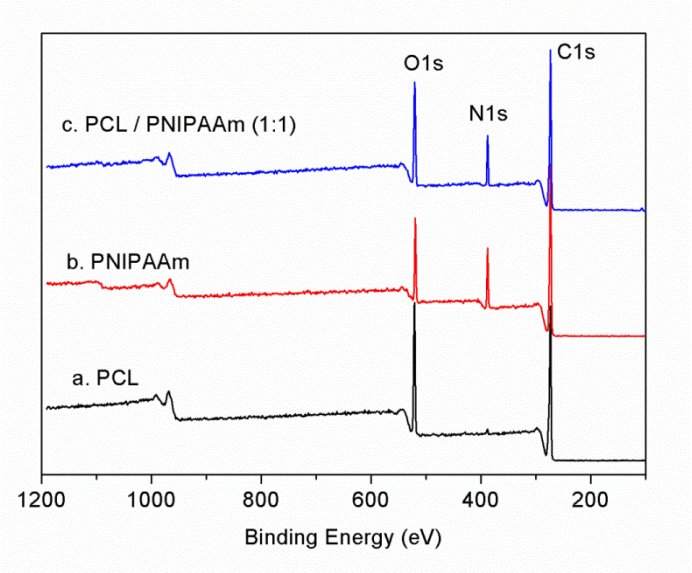

Figure S2. XPS spectra of PCL, PNIPAAm and PCL/PNIPAAm nanofibers

The wide energy survey scan of nanofibers is shown in Figure S2. The binding energy (BE) at about 531 eV, 399 eV and 285 eV are attributed to O1s, N1s and C1s, respectively.[3] N content is relatively high on the spectrum of PCL/PNIPAAm nanofiber. Based on the method provided by Chen et al., the surface coverage of PNIPAAm (% PNIPAAm) is estimated to be 6[N]/[C] from the wide energy survey scans.[4]

4. SEM images of PCL/PNIPAAm nanofibers with varied composition.

SEM images of PCL/PNIPAAm nanofibers with varied composition are shown in Figure S3. The nanofibers change from the smooth fiber to the beaded fiber with the

increasing PNIPAAm content in the solution due to decreased solution viscosity. [5] When the ratio of PNIPAAm to PCL is 5/5, the core-sheath structure of nanofibers is readily formed. Thus, the ratio of PNIPAAm to PCL (5/5) is employed in this work.

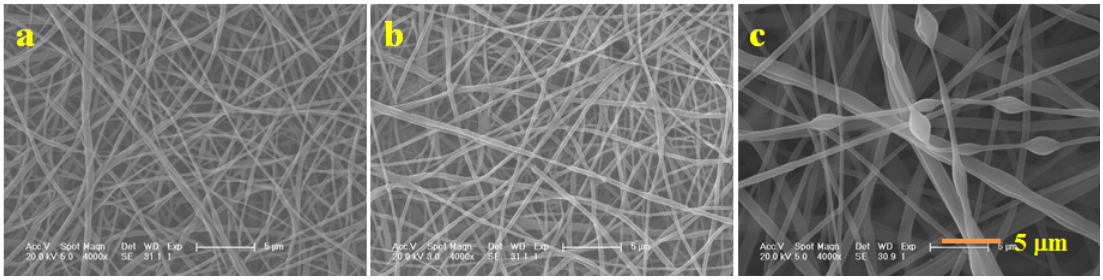

Figure S3. SEM images of PCL/PNIPAAm nanofibers.

a) PCL/PNIPAAm=9/1; b) PCL/PNIPAAm=7/3; c) PCL/PNIPAAm=5/5

5. PNIPAAm content on the sheath of nanofibers.

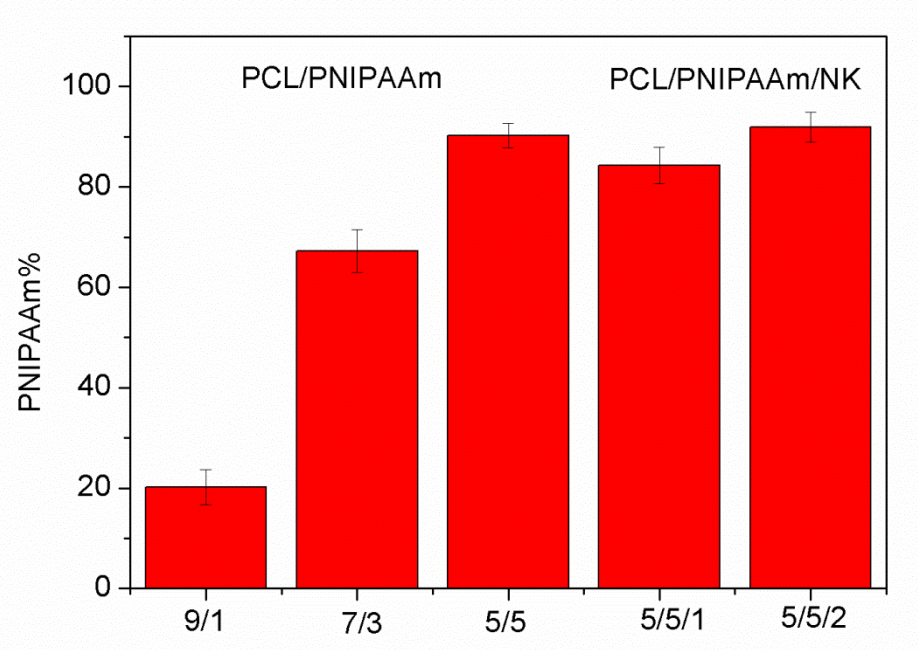

Figure S4. PNIPAAm content on the sheath of nanofibers.

PNIPAAm content on the sheath of nanofibers is calculated based on the method provided by Chen et al. [4] and the results are shown in Figure S4. Figure S4 shows that the PNIPAAm content on the out layer of PCL/PNIPAAm nanofibers is 90% when the ratio of PNIPAAm to PCL is 5/5, indicating the formation of core-sheath structure of nanofibers.[4] In addition, PNIPAAm content on the sheath part of

PCL/PNIPAAm/NK nanofiber is over 85%, suggesting the load with nattokinase (NK) has slight effects on the core-sheath structure generation.

6. Stability of core-sheath PCL/PNIPAAm nanofibers.

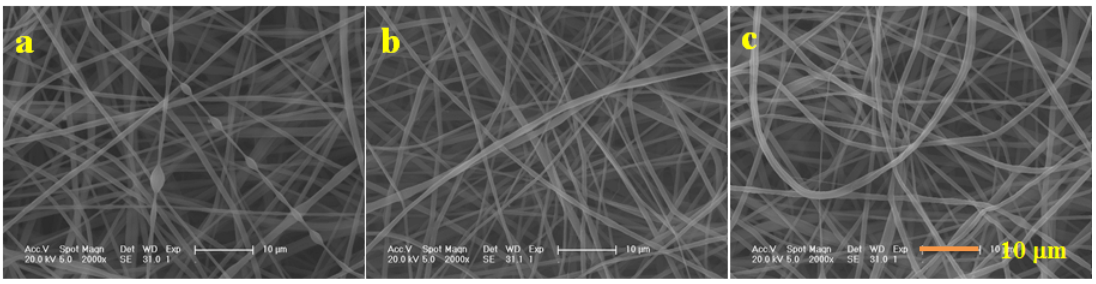

Figure S5. SEM images of PCL/PNIPAAm (5/5) nanofibers after immersion in water. a) 0 h; b)1 h; c) 2 h.

Table S1. PNIPAAm content on the sheath of PCL/PNIPAAm nanofibers

	In water	PNIPAAm%
PCL/PNIPAAm (5/5)	0h	90±2
	1h	85±3
	2h	68±5
PCL/PNIPAAm/NK (5/5/1)	0h	85±4
	1h	78±3
	2h	62±2
PCL/PNIPAAm/NK (5/5/2)	0h	92±3
	1h	73±2
	2h	60±5

The stability of core-sheath PCL/PNIPAAm nanofibers is tested by immersing the nanofibers in water for 1-2 h. The SEM images of PCL/PNIPAAm (5/5) nanofibers after immersion in water are shown in Figure S5. Contrast to the fast dissolution of PNIPAAm nanofibers in water, the nanofibers structure remain after 1-2 h immersion

in water, demonstrating the blending of PNIPAAm with other high molecular weight polymer enhances the stability of PNIPAAm-based nanofibers. The PNIPAAm content on the sheath of nanofibers is over 60% (Table S1), confirming that the PNIPAAm-based nanofibers possess enhanced stability in water.

7. Reversible hydrophobic-hydrophilic transition of PCL/PNIPAAm/NK nanofibers

Surface wettability of nanofiber platform was evaluated by the sessile drop method with a pure water droplet (ca. 3 μL) using a contact angle goniometer (DSA, KRUSS GMBH, Germany). The temperature-controlled experiments were performed using a custom-made heating plate with a PID-controller (Panasonic). The samples after measurement were dried in vacuum at 37°C for 2 days to make the nanofiber completely dry without damage to the topographical structure of nanofibers by heating. The nanofiber (PCL/PNIPAAm/NK) showed the reversible hydrophobic-hydrophilic transition under the alternation of temperature (Fig.S6). But the nanofibers tended to be less hydrophobic after one-circle transition, which might due to the damage of topographical structure on the nanofibers caused by loss the PNIPAAm component.

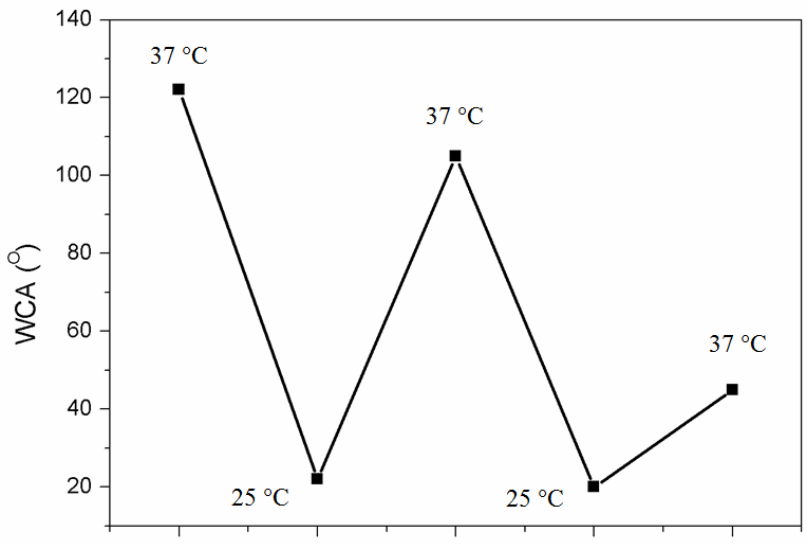

Fig. S6. The reversible hydrophobic-hydrophilic transition of PCL/PNIPAAm/NK nanofibers

8. Platelet adhesion on the PCL/PNIPAAm nanofibers in the presence of NK

The nanofibers were immersed in PBS (pH 7.4) at 37°C for 20 min to equilibrate the surfaces. Then platelet rich plasma (PRP) and PRP with nattokinase (NK, 100 μL PRP+10 μLNK) were deposited onto PCL/PNIPAAm nanofibers, respectively. The samples were cultured at 37°C for 30 min. Then, the sample was rinsed away by PBS for several times and fixed with a fresh solution of 2.5 wt% glutaraldehyde in PBS at 37 °C for 2 h. All samples were freeze-dried and finally sputter coated by gold. Platelet adhesion was characterized by a field-emission SEM (SEM, Sirion-100, FEI, USA).

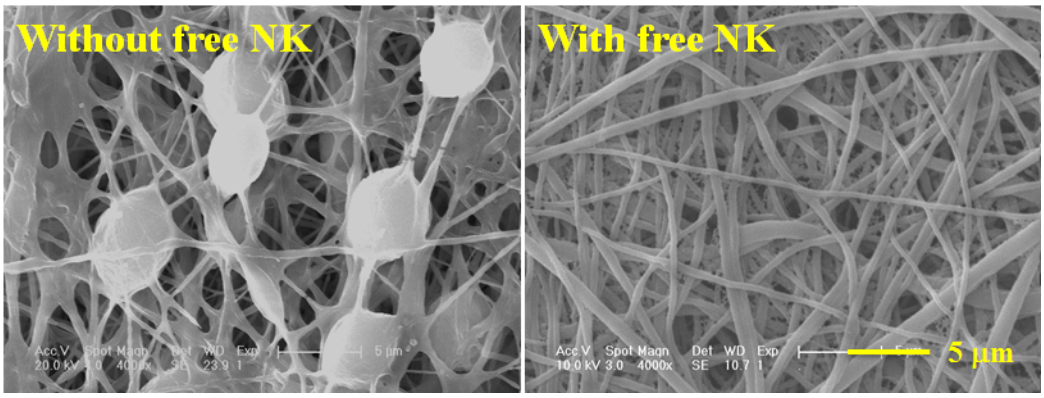

Fig. S7. Platelet adhesion on PCL/PNIPAAm nanofibers with and without free NK

The results were shown in Fig.S7. Fig.S7 showed that there were amount of platelets adhered on the surface of PCL/PNIPAAm nanofibers at 37°C without free NK in the PRP. In contrast, no platelet was observed on the nanofibers surface at 37 °C with free NK in the PRP. This result confirmed that it was NK released from the nanofibers that resisted the platelet adhesion. Although most NK was released from the nanofibers within 3 hours, the released NK remained in the plasma and maintained the anti-thrombus ability for long time.

9. Protein adsorption on nanofibers

Bovine serum albumin (BSA) was used as model protein to evaluate the protein adsorption on nanofibers at different temperatures. After being equilibrated with PBS

overnight, the web specimens (1×1×0.01 cm^3) were moved into PBS solution containing BSA (0.1 mg/mL) for 2 h at 37 ℃ and 25 ℃, respectively. Then each sample was rinsed five times by gentle shaking in the fresh PBS solution. Subsequently, the samples were immersed in 1 mL of PBS solution containing 1 wt% of sodium dodecyl sulfate (SDS), and the protein adsorbed on the surface was completely desorbed by sonication for 20 min. A micro BCATM protein assay reagent kit based on the bicinchoninic acid (BCA) method was used to determine the concentration of the protein in the SDS solution. The concentrations were determined using a TECAN (TECAN GENIOS, Austria) operating at 562 nm. The results for protein adsorption were listed in **Table S2**. **Table S2** shows that the amount of protein adsorbed on the PNIPAAm-based nanofibers at 37 ℃ is nearly four-fold increase compared with that adsorbed on the nanofibers at 25 ℃, and the presence of NK has slight effect on protein adsorption.

Table S2. BSA adsorption on nanofibers at different temperatures

	Temperature (°C)	Protein Adsorption (μg/mg)
PCL/PNIPAAm (5/5)	25	0.5±0.03
	37	2.4±0.3
PCL/PNIPAAm/NK (5/5/1)	25	0.4±0.02
	37	2.5±0.1
PCL/PNIPAAm/NK (5/5/2)	25	0.6±0.05
	37	2.3±0.2

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