Supplementary Information

Fabrication of PCL/keratin composite scaffold for vascular tissue engineering with Catalytic generation of nitric oxide potential

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1. Characterization

The morphologies of nanofibrous mats were observed by scanning electron microscopy (SEM) (JEOL, 6500) with an accelerating voltage of 10 kV. Prior to imaging, the specimens were sputtered with gold for 40 s. The average fiber diameter was measured based on SEM images. For each sample, five SEM images were analyzed, and at least 50 fibers were manually measured on each image and analyzed using Image J software (NIH USA, 2008). Results were expressed as mean±standard deviation. ATR-FTIR measurements were performed on a Nicolet 170sx Fourier transform infrared spectrometer (USA), coupled with ATR accessory. The spectra were performed with a resolution of 4 cm^{-1}, an Omni sampler over 32 scans and recorded at a 45^{o} incident angle using a Ge crystal. XPS spectrums were obtained on ESCALab MK II (V. G. Scientific Co. Ltd. UK) spectrometer using AlK_{α} radiation. The binding energy was referenced by setting the C_{1s} hydrocarbon peak to 285 eV. To investigate the surface wettability of scaffolds, the water contact angle of the scaffolds were measured by a video contact angle instrument (SL200B, KNO, USA) using the sessile drop method. Briefly, a drop of water (4 uL) was dropped onto the surface of the scaffolds. The change of the water bead shape was recorded and the surface contact angles were measured from 5, to 30 s and recorded interval 5s. The WCA of each scaffold was the average value of three different spots[1].

2. Synthesis of S-nitrosoglutathione (GSNO)

S-nitrosoglutathione (GSNO) was synthesized as described in the literature[2]. Briefly, reduced glutathione (GSH, 1.53 g, 5 mmol) was first dissolved in ice water (8 mL), and then mixed with HCl (2 mL, 1 M). An equimolar amount of $NaNO_2$ was added and the mixture was stirred at 0 °C for 40 min. After the addition of acetone (20 mL) stirring was continued for another 10 min, followed by filtration of the precipitate. GSNO was washed thrice with ice water (1 mL), acetone (10 mL), and diethylether(10 mL) and finally dried under vacuum.

3. In vitro clotting time tests

To evaluate the antithrombogenicity of the nanofibrous mats, activated partial thromboplastin time (APTT), prothrombin time(PT), and thrombin time (TT) were measured by an automated blood coagulation analyzer CA-50 (Sysmex Corporation, Kobe, Japan). Rabbit fresh blood was collected using vacuum tubes, containing

sodium citrate as an anticoagulant (anticoagulant to blood ratio, 1:9, v/v). The platelet-poor plasma (PPP) was obtained after centrifuging at 3000 rpm for 15 min. Synchronously, the round mats (0.5 cm diameter, three pieces) were immersed in PBS (0.2 mL, pH = 7.4) for 1 h. Then the PBS was removed and 0.1 mL of fresh PPP was introduced. After incubating at 37 ℃ for 30 min, 50 μL of the incubated PPP was added into the test cup, followed by the addition of 50 μL of APTT agent (incubated 10 min before use) and incubation at 37 ℃ for 3 min. Thereafter, 50 μL of 0.025 M $CaCl_2$ solution was added, and then the APTT was measured. PT test was similar to the APTT test except the addition of PT agent. For the TT test, 50 μL of TT agent was added into the test cup (containing 50 μL of the incubated PPP) after 10 min incubating, and then the TT was measured. At least three measurements were averaged to get a reliable value, and the results were analyzed by the statistical method. Platelet poor plasma (PPP) acted as the control.

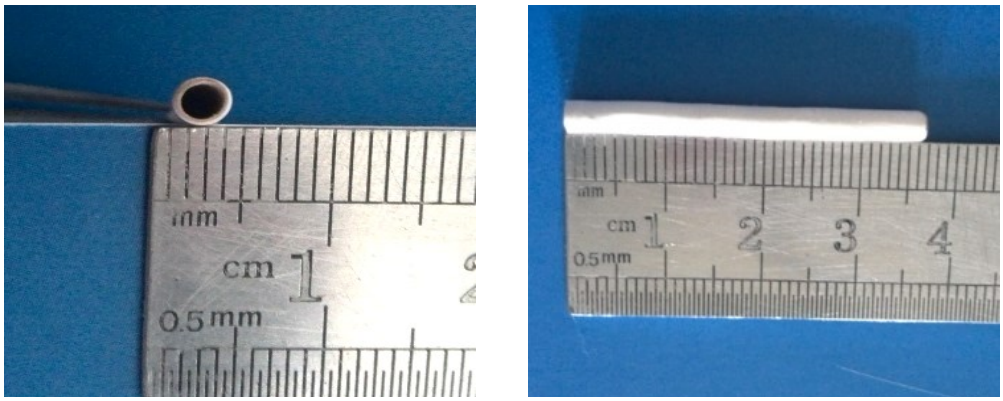
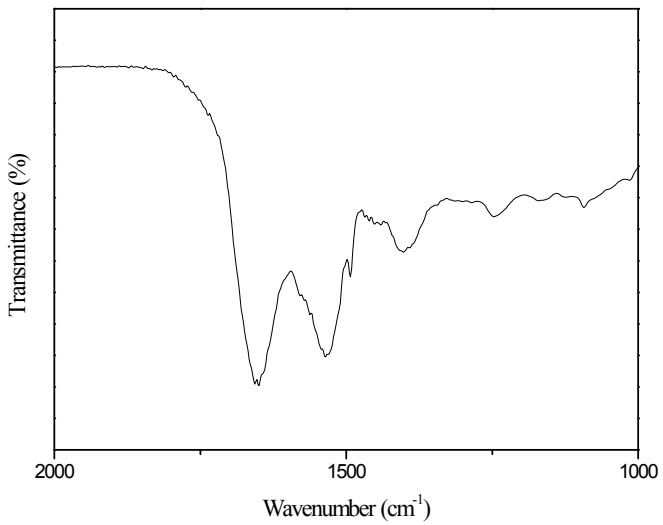
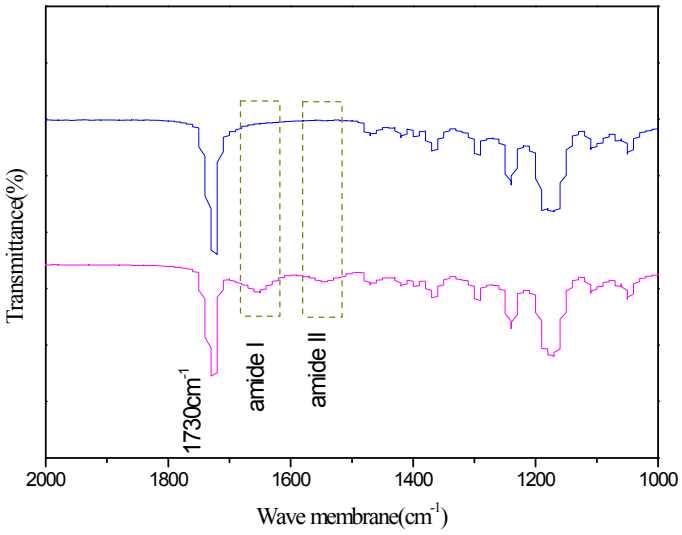


Fig. S1 Digital optical image of PCL/keratin mats(7/3), which were electrospun into tube shape.



a



b

Fig.S2 (a) FT-IR spectrum of keratin; (b) ATR-IR spectra of PCL and PCL/keratin=7/3.

Table S1 Elemental surface composition of PCL/keratin mats determined from XPS

Samples	Element (atom %)			
	C_{1s}	O_{1s}	N_{1s}	S_{2p}
PCL	75.19	24.81	0	0
PCL/keratin=7/3	74.50	18.50	6.26	0.74

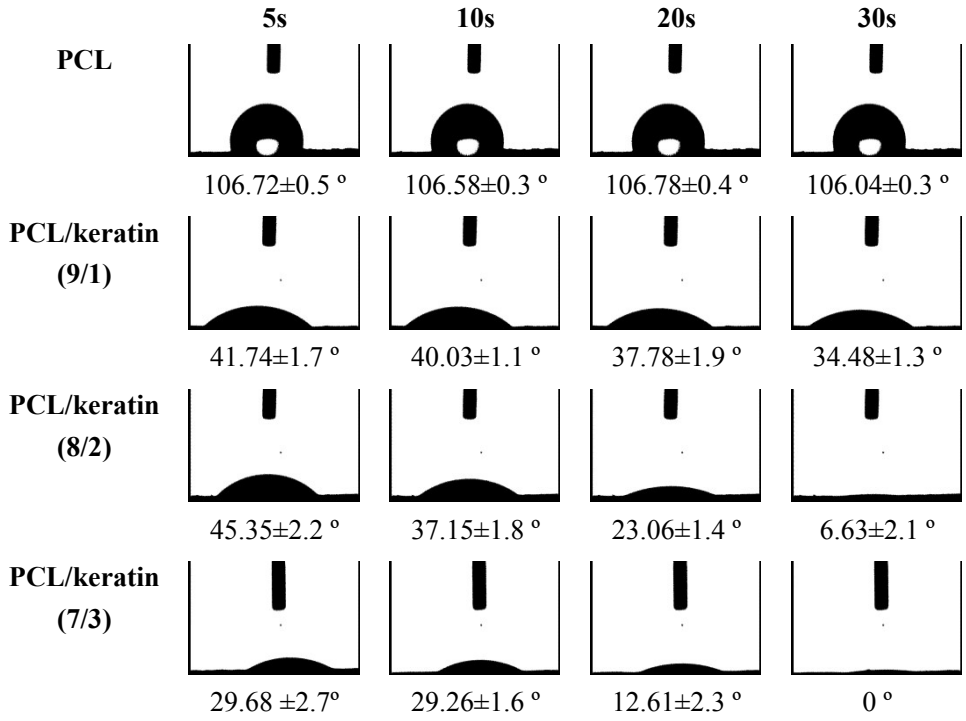


Fig.S3 Water contact angles of PCL/keratin mats.

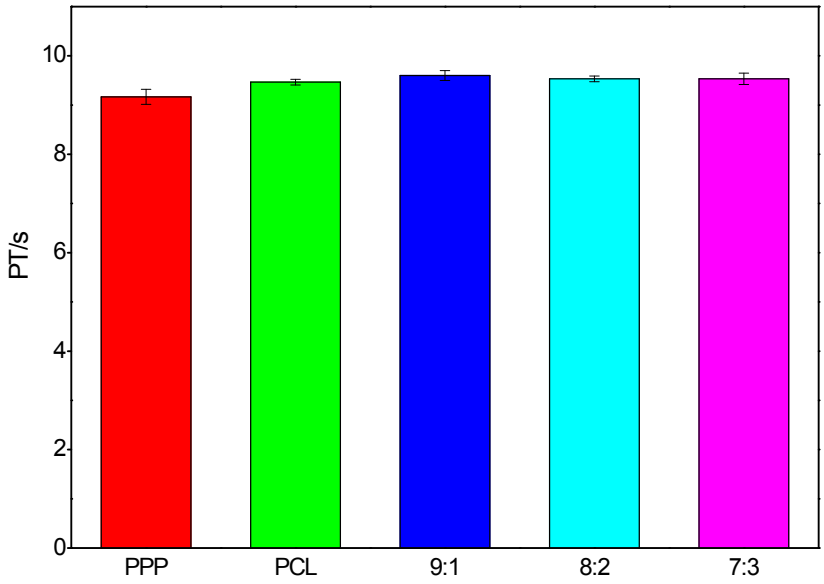


Fig.S4 Prothrombin time (PT of PCL and PCL/PK mats. Data are presented as mean ± standard deviation of three different experiments.

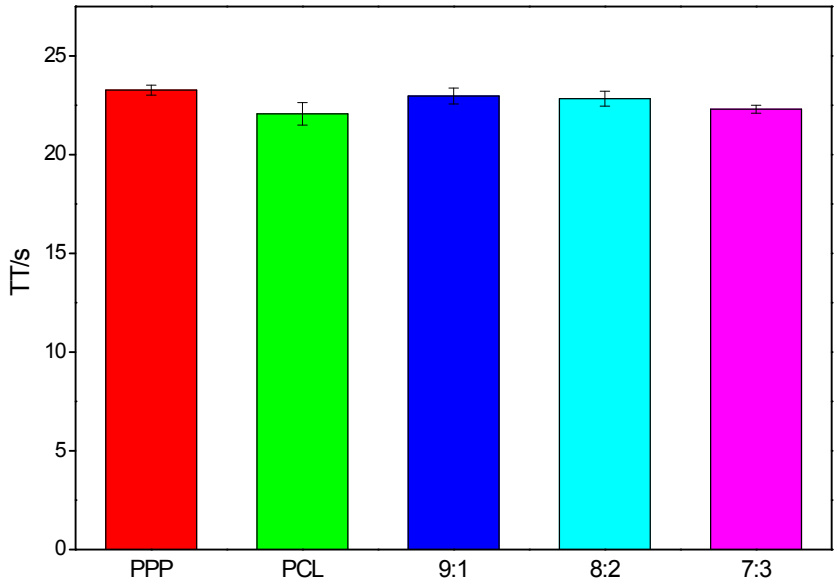


Fig.S5 Thrombin time(TT) of PCL and PCL/PK mats. Data are presented as mean ± standard deviation of three different experiments.

References

[1] Y.M. Li, Y.F. Wang, J.J. Ye, J. Yuan, Y.H. Xiao. Fabrication of poly(ε-caprolactone)/keratin nanofibrous mats as a potential scaffold for vascular tissue engineering. Materials Science and Engineering C 68 (2016) 177–183

[2] L. Heikal, G.P. Martin, L.A. Dailey. Characterisation of the decomposition behaviour of S-nitrosoglutathione and a new class of analogues: S-nitrosophytochelatins, Nitric Oxide 20 (2009) 157–165. Hart, T. W. Tetrahedron Lett. 26(1985) 2013-2016