Supplementary Information (SI) for Soft Matter.

Multifunctional hybrid poly(ester-urethane)urea/resveratrol electrospun nanofibers for potential vascularizing matrix

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1 Supplementary experimental section

1.1 Materials

Polycaprolactone diol (PCL diol, Mn= 2000) was purchased from Sigma-Aldrich Trading Co., Ltd. (Shanghai, China). 1,6-hexamethylene diisocyanate (HDI), 1,4-butanediamine (BDA), stannous octanoate ($Sn(Oct)_2$), dimethyl sulfoxide (DMSO), and resveratrol (Res) were purchased from Shanghai Titan Technology Co., Ltd. (Shanghai, China). Dimethylformamide (DMF) was purchased from Jiangsu Qiangsheng Functional Chemical Co., Ltd. (Suzhou, China), and 1, 1, 1, 3, 3, 3-hexafluoro-2-propanol (HFIP) was purchased from Shanghai Dari Fine Chemical Co., Ltd (Shanghai, China). Human umbilical vein endothelial cells (HUVECs) used for in vitro experiments were obtained from the Shanghai Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Cell counting kit (CCK-8), Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), and antibiotics-antimicrobial agents (penicillin/streptomycin) were purchased from Hyclon Trading Co., Ltd. Reagents and materials described above are used in the manner in which they are received, unless otherwise stated.

1.2 Synthesis of PEUU elastomer and preparation of hybrid P/R fiber

PCL diol and HDI were pre-polymerized under nitrogen protection at 80 ℃ for 2 h, the system temperature was cooled to 40 ℃, and putrescine was added for chain extension reaction for 18 h. After the molecular chain was extended, the PEUU elastomer was repeatedly washed with deionized water, then washed and purified for 3 days, during which the deionized water was changed once a day, and then freeze-dried to obtain dry and pure PEUU elastomer. During the reaction, the molar

ratio of PCL diol, HDI, and BDA was 1:2:1.

According to the literature (Wang et al., Biochem. Pharmacol., 2010, 80(9): 1386-1395.) that showed the best vascularization ability in culture of HUVECs when resveratrol concentration was 5μM. We took the membrane with an area of $2cm^2$ as the weight of the fiber membrane to release Res concentration of 5μM (the bottom area of a 24-well plate and the volume of working fluid) in 1mL medium and calculated that the Res load in it was about 1wt%.and there were fewer previous studies of this kind, so in order to further investigate the effects of resveratrol-loaded PEUU nanofibers on vascular regeneration, we set gradient concentrations of 0 wt%, 0.5 wt%, 1.0 wt% and 1.5 wt% as experimental groups for the study.

To prepare hybrid PEUU/Res nanofibers, 0, 5, 10, and 15 mg Res powder were dissolved in 0.83 mL of DMF to form a mixture with stirring until clarified, respectively. Dissolve 1 g PEUU in 5 mL HFIP and stir until clear. Then the clarified Res/DMF mixture was added and strongly stirred at room temperature for 72 h to form electrospinning solutions with relative mass fraction W_{Res}/W_{PEUU} of 0%, 0.5%, 1.0% and 1.5%, respectively. Then the prepared mixed solution was absorbed with a 10 mL syringe to generate the fiber through electrospinning, where the voltage applied to the needle was 9kV and the injection speed was 0.8 mL/h. The distance between the needle and the aluminum foil (receiving device) of the ground wire was 20 cm. The electrospinning process is controlled at about 25 °C and 50%±2% relative humidity.

1.3 Characterization and testing

The prepared P/R nanofibers were first sputter plated with gold at 4 mA for 30 s, and subsequently the morphology and structure of the surface were observed using a scanning electron microscope (SEM, Phenom XL, the Netherlands). The image analysis software (Image J) was used

to take 100 random fibers from each group of P/R images for measurement, and the average fiber diameter and distribution of each group of P/R nanofibers were obtained. Successful loading of Res in fibers was confirmed using Fourier transform infrared (FTIR) (Nicolet AVATAR 380, Thermo Fisher), and spectra of four groups of P/R nanofibers were recorded.

The porosity of P/R nanofibers were measured by the liquid displacement method. 5 mL (V_1) anhydrous ethanol was first loaded into the measuring cylinder, and then the nanofibers (n= 3) were immersed in anhydrous ethanol for 10 min. The total volume of ethanol and fiber was observed and recorded as V_2. During this process, sealing film was used to seal to avoid the volatilization of ethanol. The nanofibers were then removed and the remaining volume of ethanol V_3 in the cylinder was measured. Finally, the porosity (P) of the P/R nanofibers were calculated by the following equation (1):

$$P(\%) = \frac{V_1 - V_2}{V_2 - V_3} \times 100\% \quad (1)$$

The wettability of P/R nanofibers were evaluated by using a contact angle measuring instrument (JC 2000D2A, Shanghai Zhongshan Digital Technology Equipment Co., Ltd.). During the test, 0.02 mL of deionized water was slowly added through a capillary sampler, and three different positions on the material were randomly selected for the test. Finally, the water contact angle was measured by the Contact Angle plug-in of Image J, and its average value was calculated. The mechanical properties of the P/R nanofibers at room temperature and different humidity were tested by a high-precision tensile testing machine (HY-025CS, Shanghai Hengyu Instrument Co., Ltd.) with a load range of 0~ 200N sensor. According to the national standard GB/T 1040.3-2006, the samples were prepared with dimensions of 50 mm × 10 mm × 0.4 mm (length × width × thickness). These strips were precisely cut to the specified size, and their tensile strength was measured at room

temperature using a crosshead speed of 10 mm/min. To simulate a wet environment *in vivo*, the samples were soaked in PBS phosphate buffer (pH = 7.4) for 24 h and the same test were performed immediately after removal.

1.4 Cytotoxicity test by CCK-8

For each experiment, three independent experiments were performed at each time point, and each experiment was repeated three times. Dulbecco's modified eagle's culture-medium (DMEM) containing 10% fetal bovine serum and 1% antibiotic antifungals was prepared and added. HUVECs were cultured at 5% CO_2 and 37 ℃, and the medium was replenished every 2 days. The electrospun nanofiber membranes were sterilized under ultraviolet light for 24 hours, then placed at the bottom of 24-well plates. HUVECs were subsequently seeded onto the membranes at a density of 5×10^4 cells per well. HUVECs were cultured in a cell incubator at 37 °C and 5% CO_2 for a maximum of 5 days, during the medium need to be replaced every other day at a time. Cell adhesion was observed after 12 h of inoculation whereas, cell proliferation was observed at various time points such as 1, 3, and 5 days. The culture plate was removed at a preset time point, the medium was removed, and the cells were gently washed with PBS, and washed with PBS, and 360 μL DMEM medium and 40 μL CCK-8 solution were added to each well. After incubating for 4 h, the liquid in the culture plate was removed and 400 μL dimethyl sulfoxide (DMSO) was added. Then the culture plate was placed in an incubator at 37℃ for 30 min, the wavelength was set to 490 nm, and the OD values were measured with a microplate reader.

The cell culture plate containing the cell nanofibers membranes cultured for 5 days was removed, and the medium was gently sucked out, and an appropriate amount of PBS was added twice and sucked out for washing cells. Calcein/PI (beyotime, China) staining solution was

prepared, and the staining solution was added to the cell culture plate and gently shook to make the staining even. Then incubate in the incubator for 15 minutes. Gently wash the cells with PBS 3 times to remove excess stain. The stained cell culture plates were observed under a confocal laser microscope (ZEISS, Germany). The microscope parameters were adjusted, the cells were observed at 494 nm and 535 nm respectively, and reconstructed. Images of living cells (green) and dead cells (red) are recorded, the captured images are processed and analyzed using ZEISS companion software (ZEISS ZEN 3.8).

2. Results

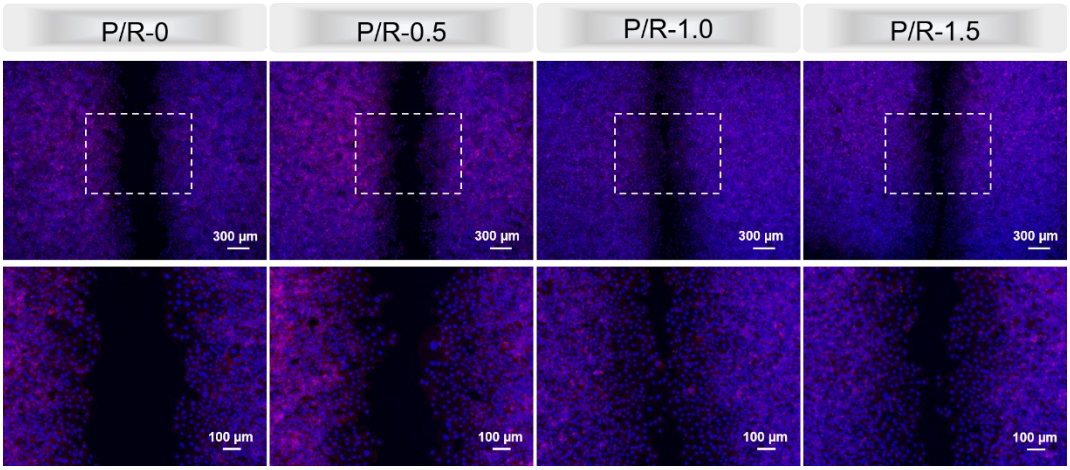

Fig. S1. High-magnification images of HUVECs migration experiments on P/R-0, P/R-0.5, P/R-1.0, and P/R-1.5 after incubation for 48 h.