Electronic Supplementary Information

Annealing regulates performances of electrospun poly(ε-caprolactone) membrane to accommodate potential tissue engineering

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Materials and methods

Materials

Poly(ε-caprolactone) (PCL) with viscosity-average molecular weight (M_η) of 8.4 × 10^4 g mol^{-1} was provided by Changchun SinoBiomaterials Co., Ltd. (Changchun, P. R. China). Chloroform (analytical grade) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, P. R. China) and used without any further purification.

Preparation and annealing treatment of electrospun PCL membranes

The nonwoven PCL membranes were fabricated by the electrospinning apparatus consisting of an infusion pump, a high voltage power supply, and a grounded target. Firstly, PCL was dissolved in chloroform with a concentration of 10 wt.%. Then, PCL solution was loaded in a 10 mL syringe with an injection rate of 0.1 mm min^{-1}. An electrical field of 15 kV was applied by a high voltage power supply. Finally, the PCL fabrics were collected on a grounded aluminium sheet kept at a distance of 14 cm from the needle tip. Before characterization, the electrospun membranes were annealed at different temperatures of 35, 42, or 50 °C for 12 h. The long annealing time is to ensure that all rearrangements of polymer chains were complete.

Morphology observation of electrospun PCL membranes

A field emission scanning electron microscopy (SEM; Inspect-F, FEI, Finland) was employed to observe the surface morphologies of all the electrospun PCL membranes without and with annealing at various temperatures, operating in high vacuum and with an accelerating voltage of 20 kV. Samples were dried under vacuum and sputter-coated with gold-palladium for 60 s.

Contact angles of electrospun PCL membranes

The contact angles of electrospun PCL membranes without and with annealing at different temperatures were measured by KRUSS drop shape analyser (DSA 100). The films were fixed into the sample holder, and the distilled water was taken in a 2 mL Leur lock syringe. The volume of a single droplet poured on the membranes was about ~ 2 μL. The drop shape on the surface was automatically taken using the camera matched with system. From these images, the contact angles were calculated by the inbuilt software of the instrument *via* the Young equation " $0 = \gamma_{SG} - \gamma_{SL} - \gamma_{LG}\cos\theta_C$".[1] At least five samples were tested for each type, and the results were represented as mean ± standard deviation.

Mechanical properties of electrospun PCL membranes

All electrospun fibrous membranes without and with annealing at various temperatures were cut into the rectangular shape with 0.5 × 4 cm^2 in size and approximately 0.2 mm in thickness before examination. Uniaxial tensile tests were performed using an Instron-4502 machine at ambient conditions (25 °C and 50% relative humidity), with a cross-head speed of 5.0 mm min^{-1}. From the stress-strain curves, Young's modulus, tensile strength, and elongation at break of membranes were obtained. At least five samples were tested for each type, and the results were averaged.

Thermal behaviors of electrospun PCL membranes

The thermal behaviors of electrospun PCL membranes without and with annealing at different temperatures were characterized by differential scanning calorimeter (DSC; TA Q2000 V7.3) in a temperature range from –80 to 100 °C at a scanning rate of 10 °C min^{-1}. The calibration was performed with indium, and all tests were carried out in ultrapure nitrogen. The melting points were determined from the DSC curves as peak temperatures. The crystallinity (X_c) was calculated by measuring the ΔH values of the samples and the melting enthalpies of perfect PCL crystal, which was taken as 136 J g^{-1} in the present study.[2]

In vitro degradation of electrospun PCL membranes

The degradability of the electrospun mats without and with annealing at various temperatures was carried out in a closed vial containing a small piece of fabric film (*ca.* 3 mg) and 10.0 mL of phosphate-buffered saline (PBS; pH 7.4, 0.1 M) with 2.0 mg α-chymotrypsin. The vial was incubated at 37 °C with a constant reciprocal shaking (75 rpm). The incubation medium was replaced daily to maintain the enzymatic activity, and the degradation test was lasted for a period of 6 days. At predetermined time intervals, triplicate specimens for each condition were carefully rinsed with distilled water, lyophilized, and weighted. The weight loss was illustrated gravimetrically by comparing the remaining dry weight of membranes at a specific time against their initial mass. In addition, the morphology of the degraded fibers was observed with SEM as described above.

Cellular adhesion and proliferation on electrospun PCL membranes

The UV-treated electrospun membranes without and with annealing at different temperatures were transferred into the 24-well tissue culture plates, seeded with 50,000 osteoblast cells (*i.e.*, MC3T3-E1 cells) for cellular adhesion test. Cells were cultured in complete Dulbecco's modified Eagle's medium (DMEM) containing 10% (V/V) fetal bovine serum (FBS; Gibco BRL, 30 Gaithersburg, MD, USA) at 37 °C. After 24 h incubation, the cells were gently rinsed with PBS twice, and the cellular spreading situation on membranes was observed by optical microscope.

The proliferation of MC3T3-E1 cells on electrospun PCL membranes without and with annealing at various temperatures was qualitatively and quantitatively evaluated by the live/dead and Cell Counting Kit-8 (CCK-8) assays.

LIVE/DEAD ASSAYS: The electrospun PCL membranes seeded with MC3T3-E1 cells with a density of 2.0×10^4 cells per well were incubated for 1, 3, 5, or 7 days. At each time point, 20.0 μL of PBS containing calcein-acetoxymethyl ester (calcein AM; 2.0 μg mL^{-1}) and propidium iodine (PI; 3.0 μg mL^{-1}) was added into each well, and then the stained cell constructs were examined using fluorescence microscopy (Nikon Eclipse *Ti*, Optical Apparatus Co., Ardmore, PA).

CCK-8 ASSAYS: The proliferation of MC3T3-E1 cells on the electrospun membranes was quantitatively evaluated by adding CCK-8 (Dojindo, Japan) solution at desired time intervals. After incubation for 4 h, the absorbance at 450 nm was measured by a Bio-Rad microplate reader (Model 550, Hercules, CA, USA). The absorbance at 600 nm was used for baseline correction. The relative cellular proliferation was calculated with respect to the result of the 1st day.

Statistical analyses

All data were expressed as mean ± standard deviation. Statistical software of SPSS 13.0 was used to analyze the data by one-way analysis of variance. $*p < 0.05$ was considered significantly different, and $**p < 0.01$ and $***p < 0.001$ were considered highly significantly different.

Notes and references

1. J. T. Chen, W. L. Chen, P. W. Fan and I. Yao, *Macromol. Rapid. Comm.*, 2014, 35, 360–366.

2. P. Sarazin, X. Roy and B. D. Favis, *Biomaterials,* 2004, 25, 5965–5978.

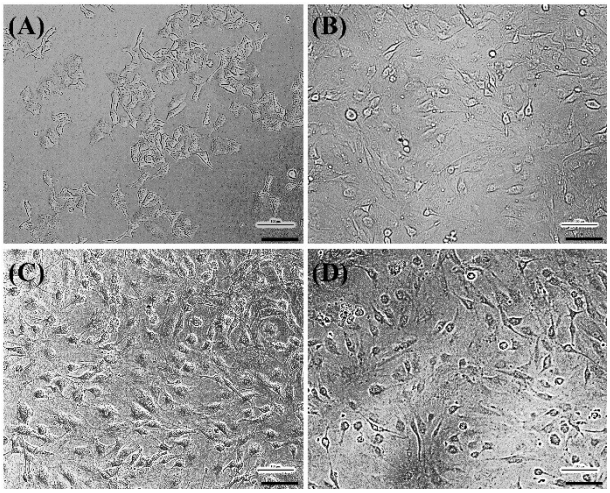

Fig. S1 Cellular adhesion of MC3T3-E1 cells on electrospun PCL membranes without annealing (A) and with annealing at different temperatures: T_a = 35 °C (B), T_a = 42 °C (C), and T_a = 50 °C (D). Scale bar represented 100 μm in the micrographs.