Supporting Information for

Micropatterned Conductive Electrospun Nanofiber Mesh Combined with Electrical Stimulation for Synergistically Enhancing Differentiation of Rat Neural Stem Cells

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Contents

1. Materials and methods

1.1. Chemicals and materials

Methacrylic anhydride was purchased from Aladdin and distilled prior to use. N, N-Dimethylformamide (DMF), tetrahydrofuran (THF) and $CHCl_3$ were purchased from Beijing Chemical Reagents Company. Ammonium persulfate (APS) was obtained from Solarbio. 1,1,1,3,3,3-Hexafluoro-2-propanol and CH_2Cl_2 were purchased from Energy Chemical. THF, DMF and CH_2Cl_2 were dried and distilled prior to use. Sodium tetraborate decahydrate was purchased from Aladdin. All the other chemical reagents were used without further purification.

Fetal bovine serum (FBS) and horse serum were obtained from Kang Yuan Biology. Dulbecco's modified Eagle's medium (DMEM), RPMI 1640 medium Modified and DME/F-12 1:1 (1X) was purchased from Hyclone. Penicillin-streptomycin was obtained from Beijing Solarbio Science & Technology Co., Ltd. B-27 Supplement, N2 and Cell Dissociation Reagent were purchased from Gibco (New York, USA). β-NGF solution, EGF solution and bFGF solution were obtained from Peprotech. Poly-L-lysine solution was obtained from SIGMA. Cell Counting Kit-8 (CCK-8) was purchased from 7sea biotech. Inc. Calcein-AM and Propidium Iodide (PI) were purchased from Aladdin. Phalloidin was obtained from Cytoskeleton, Inc. (USA). Primary antibodies include mouse anti-Tuj-1 (abcam, ab78078) for neuronal cell staining and rabbit antiglial fibrillary acidic protein (abcam, ab33922) for astrocytes staining. The second antibody include goat antimouse lgG (alexa 488, abcam, ab150113) and goat antirabbit IgG (Alexa 594, abcam, ab150080). Mouse embryonic fibroblast NIH 3T3 cells and Rat pheochromocytoma PC12 cells were obtained from Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences. Rat neural stem cells (NSCs) were extracted by researchers of our laboratory.

1.2. Synthesis of poly(ε-caprolactone) (PCL)

Briefly, ε-CL, BDO and stannous octoate were weighed and added to a dried round-bottomed flask in a glovebox purged with nitrogen. Then mixture was reacted at 120 ℃

for 24 h in an oil bath under a nitrogen atmosphere. Then the mixture was cooled down to room temperature and chloroform (50 ml) was added to the flask to dissolve the mixture, then precipitated into 800 mL of petroleum ether/methanol solution (v/v 95:5). The polymer was then dissolved in chloroform and reprecipitated for two additional times and dried under vacuum overnight to give a white solid. ^{1}H NMR (500 MHz, *CDCl*$_3$, ppm) of PCL: 4.06 (t, 2H, –CH_2O–), 3.66 (m, 2H, –CH_2OH), 2.31 (t, 2H, –CH_2–), 1.65-1.38 (m, 6H, –CH_2–) (**Figure S1a**). The molecular weight of product was determined at a concentration of 2 mg/mL in N, N-Dimethylformamide referenced to polystyrene standards via gel permeation chromatography (GPC), M_w=95 kDa, PDI=1.40.

1.3. Characterizations

Nuclear magnetic resonance (^{1}H NMR) spectra of PAT and PCL were recorded on a Bruker AV 500 MHz spectrometer in DMSO-d_6 and $CDCl_3$ with tetramethylsilane (Me_4Si) as internal reference. The electrospun meshes dissolved in $CHCl_3$ solution were characterized by Fourier transform infrared (FT-IR, Bio-Rad Win-IR spectrometer, UK) spectroscopy using KBr slice method. Molecular weights were conducted on GPC at 80 °C using a PL-GPC50 Integrated GPC System with eluent DMF at a flow rate of 1 mL/min. The narrow polystyrene standards were used for calibrate the standard curve of molecular weight. X-ray photoelectron spectroscopy (XPS) was performed using an ESCALAB 250 system (Thermo, USA) equipped with a monochromated Al Kα X-ray source operated at 1486.6 eV. Data were transmission function corrected and analyzed using Thermo ESCALAB 250 Software. The static water contact angle were measured by the standard sessile drop method with ultrapure water on a Rame-Hart Goniometer (Model 250 Rame-Hart, New Jersey, USA). At least five droplets were dropped onto one same electrospun meshes. The morphology of the electrospun meshes were observed by a field emission scanning electron microscope (FESEM) (Philips XL30 ESEM FEG, Japan).

2. Supplementary characterization

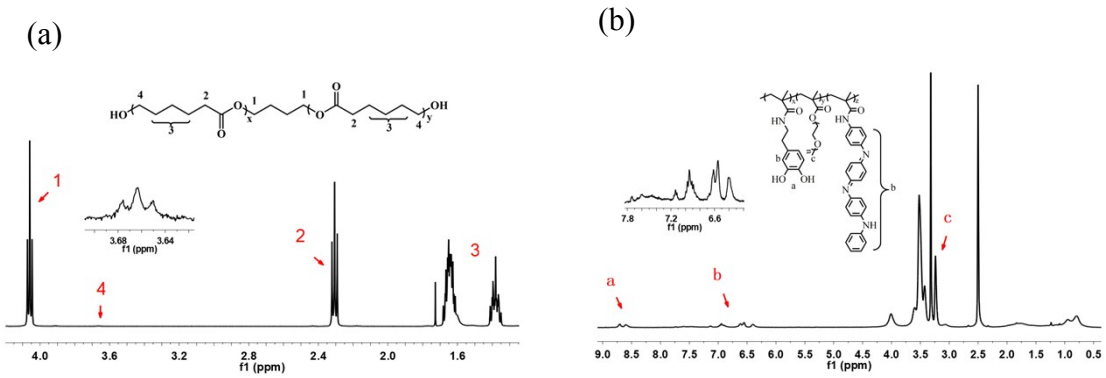

Figure S1. (a) ^{1}H NMR spectrum of PCL in $CDCl_3$. Assignments are: δ = 4.06 ppm (t, 2H,-CH_2O-), 3.66 (m, 2H,-CH_2OH), 2.31(t, 2H, -CH_2-). **(b)** ^{1}H NMR spectrum of PAT terpolymer in DMSO-d_6. Assignments are: δ: 0-2.3 ppm (broad, polymer backbone), 3.2 ppm (broad, -OCH_3 from PEGMA), 3.4-3.7 (broad, -OCH_2CH_2 from PEGMA), 3.8-4.2 ppm (-OCH_2 from PEGMA), 6.2-6.7 ppm (broad, aromatic), and 8.5-8.8 ppm (broad, hydroxyl).

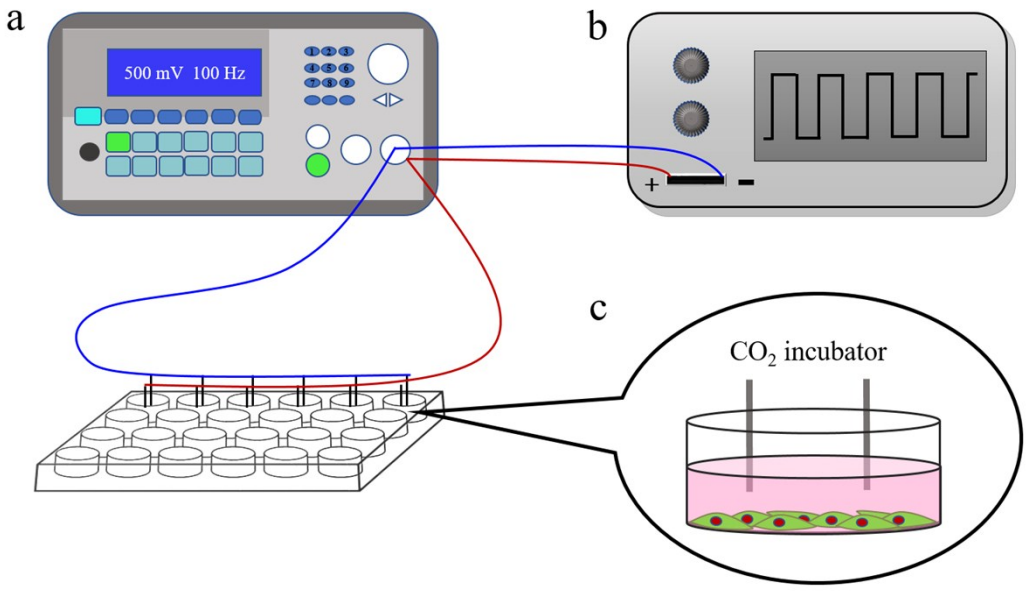

Figure S2. Electrical stimulus (ES) apparatus. **(a)** ES generator (pulse electric signal) for power supply, **(b)** Oscilloscope for ES verification, **(c)** Cell culture unit of platinum electrode chamber design.

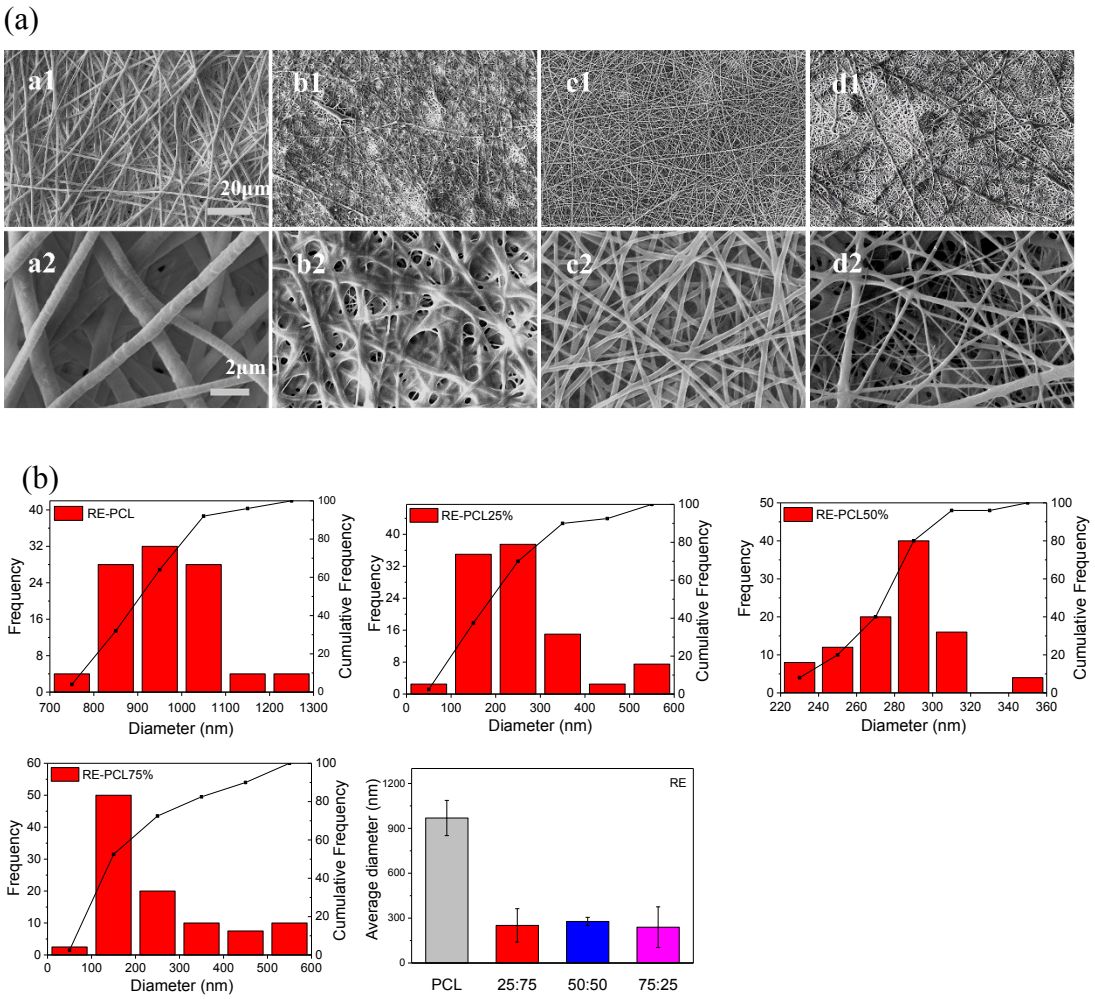

Figure S3. Scanning electron micrographs (SEM) **(a)**, and statistical analysis of size distribution and average diameter **(b)** of random electrospun nanofibers of blend of PCL and PAT at various weight ratios. a1 & a2 (100: 0), b1 & b2 (25: 75), c1 & c2 (50: 50) and d1 & d2 (75: 25). Suffix '1' and '2' stand for magnification at 1 K and 8 K, respectively.

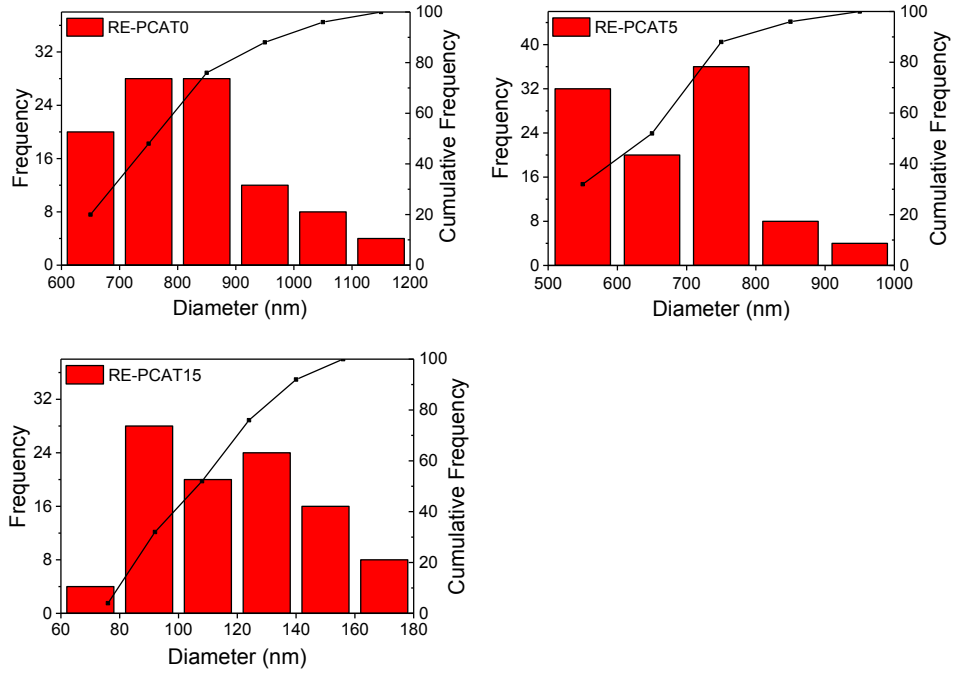

Figure S4. Statistical analysis of the average diameter and size distribution of random (PCAT0, PCAT5 and PCAT15) nanofibers.

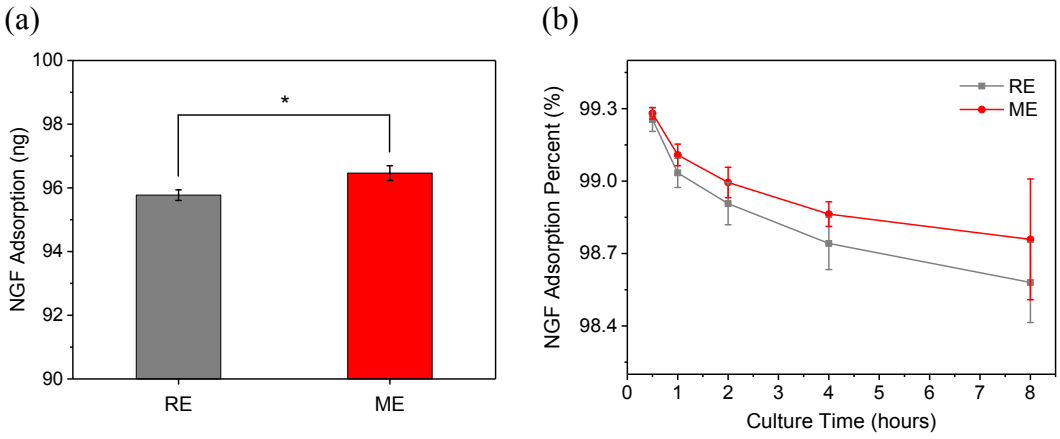

Figure S5. (**a**) The NGF adsorption amount of the random (RE) and micropatterned electrospun (ME) meshes. (*) represents statistical significance with $p < 0.05$. (**b**) The NGF adsorption percent change (remaining adsorption amount/initial adsorption amount) of RE and ME meshes with the increase of immersion time in PBS.

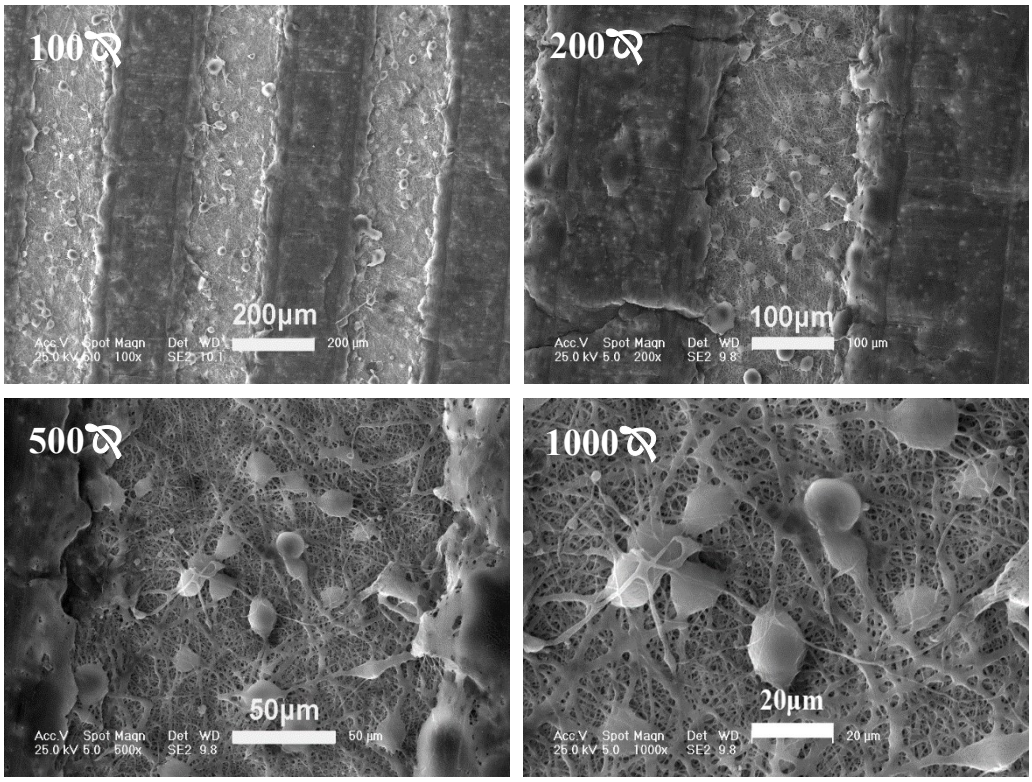

Figure S6. Scanning electron micrographs of NSCs after 7 days cultured on micropatterned nanofibers of PCAT10/NGF with ES at different magnification. The magnification were 100, 200, 500 and 1 K.