Electronic Supplementary Material (ESI) for Polymer Chemistry.

Facile Fabrication of 3D Electrospun Fibrous Mat by Ice-Templating for Tumor Spheroid Culture

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Materials. Polystyrene (average Mw~350000) was obtained from Sigma-Aldrich. 3-（4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) was purchased from Aladdin.

Fabrication of cubic and triangle 3D electrospun mat. 0.10 g/mL PCL solution was prepared in HFIP after 12 hours stirring at room temperature. The polymer solution was dispensed *via* 21G blunt stainless steel needle at a constant flow rate of 1 mL/h in a cubic chamber. Humidity of the chamber was controlled under 15% relative humidity by dry air flow. The temperature of collector was controlled under -3 °C by cooling system. Deionized (DI) water droplets were patterned on the aluminum foil surface to form a cubic or triangle shape by pipetting. Then aluminum foil was kept at freezer for 3-5 min to form ices. Finally, the aluminum foil was transferred on the cool water block as used as grounded collector. The grounded aluminum foil collector was placed under the tip of the needle with a distance of 10.5 cm. Electrospinning of the PCL solution was carried out by applying a positive voltage of 5-6 kV between the needle tip and the collector. The resulting PCL fibrous mats were kept overnight on a vacuum-dry oven in order to remove residual solvents.

Fabrication of polystyrene electrospun mat. 0.2 g/mL polystyrene solution was prepared in DMF after 12 hours stirring at room temperature. The polymer solution was dispensed *via* 21G blunt stainless steel needle at a constant flow rate of 1.2 mL/h in a cubic chamber. Humidity of the chamber was controlled under 15% relative humidity by dry air flow. The temperature of collector was controlled under -3 °C by cooling system. Deionized (DI) water droplets were patterned on the aluminum foil surface to form a spherical shape by pipetting. Then aluminum foil was kept at freezer for 3-5 min to form ices. Finally, the aluminum foil was transferred on the cool water block as used as grounded collector. The grounded aluminum foil collector was placed under the tip of the needle with a distance of 10.5 cm. Electrospinning of the PS solution was carried out by applying a positive voltage of 10 kV

between the needle tip and the collector for around 30 min. The resulting PS fibrous mats were kept overnight on a vacuum-dry oven in order to remove residual solvents.

Cell Culture and MTT assay. Breast cancer cell line MCF-7, obtained from Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China, was used for spheroids formation and drug screening studies. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) and supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. Cells were grown in 60 mm cell culture dish at 37 °C in a humidified air atmosphere of 5 % CO_2. For MTT assay of monolayer MCF-7 cells: Suspension MCF-7 cells was harvested by centrifugation at 25000 per mL and 200 μl of suspension cells were planted into wells of a 96-well plates in triplicate. Then, after incubation for 12 hours, the cells were treated with a serious of Dox concentrations (0, 0.1, 1, and 10 μg/ml) for 24 hours in triplicate. 20 μl of MTT solution (5 mg/mL in PBS) was added to each well for 2 h. Afterwards, the culture medium was removed carefully. All wells were added 150μl of Dimethyl sulfoxide (DMSO) reagent and oscillated for 10 min on shaking table to make the crystal fully dissolved. Finally, absorbance of each well were measured by using a Biotek Epoch plate reader at 490 nm.

Viability assay of the tumor spheroids: Confocal microscopy images were used to determine cell viability of the tumor spheoids. The images from different cross section of the tumor spheroid were analyzed by using the particle analysis application of ImageJ software. The viability is presented as the percentage of live cell number *vs* total cells number.

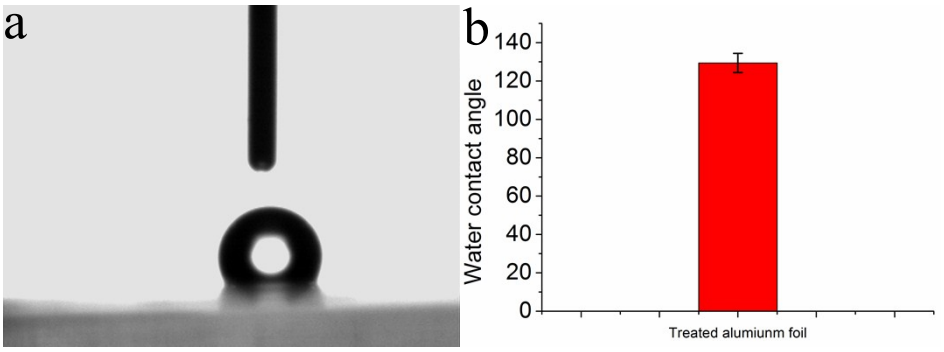

Figure S1 a) Optical image of the water droplet on the surface of treated aluminum foil. b) water contact angle calculated from images. The error bar represents standard deviation (n=3).

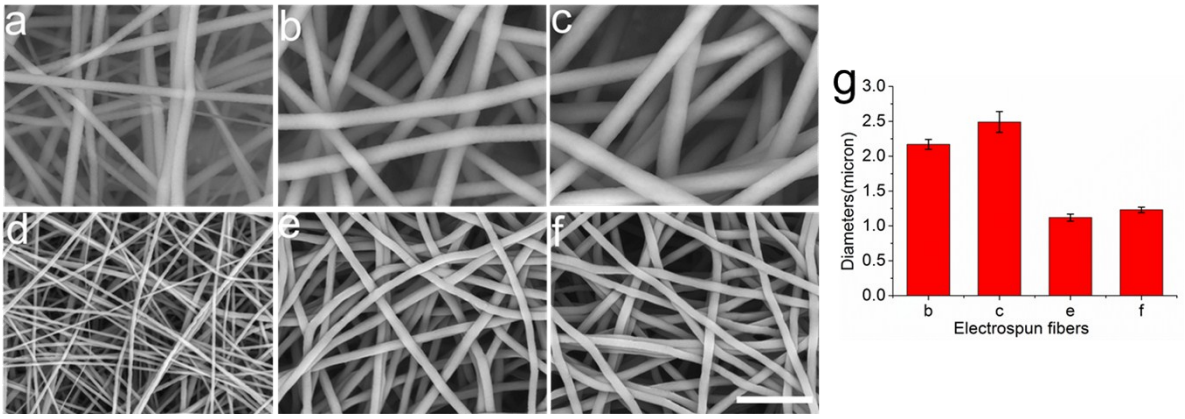

Figure S2 SEM images of PCL electrospun fibers produced from different solvents and concentrations of PCL solution. a), 0.12; b), 0.15 and c), 0.18 g/mL PCL in the mixed chloroform: methanol = 7:1. d), 0.8; e), 0.10 and f), 0.12 g/mL PCL in HFIP. All images have the same magnification (×5.0 k) and the scale bar is 10 microns. g) the diameters of fibers from b, c, e and f. Diameters were quantitatively measured by ImageJ software from their high-magnification SEM images. The diameters of fibers from a) were range from 0.44 to 1.84 μm; the diameter of fibers from d varied from 0.28 to 0.71 μm.

Figure S3 Images of polystyrene electrospun mat collected on our cooled collector with array of icy balls. The diameter of the coin (ten cent CNY) is 19.5 mm and the thickness is 16.7 mm.

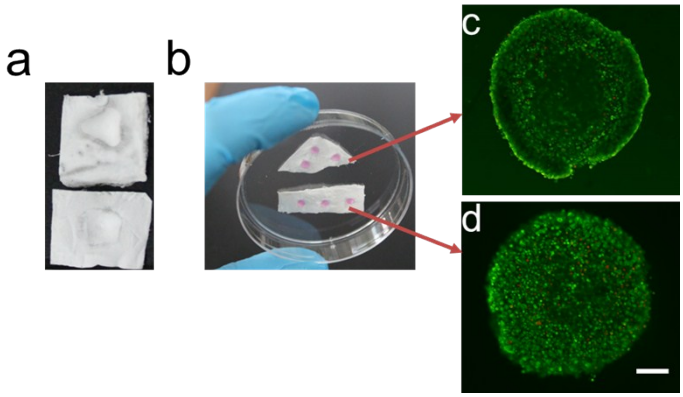

Figure S4 a) Image of cubic and triangle 3D electrospun mat; b) Image of cubic and triangle mat with drops of cell culture medium mounted on a 30 mm cell culture dish; c,d) Fluorescence images of spheroids formed in triangle and cubic shaped electrospun mats. Cells are stained with Calcein-AM (live cells) and PI (dead cells). Scale bar is 100 microns.

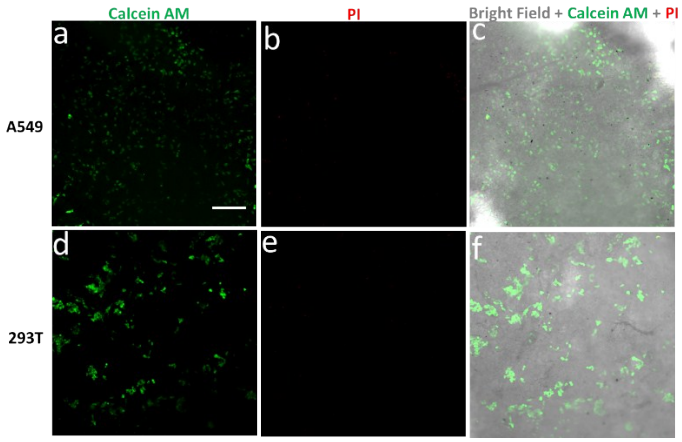

Figure S5. Live-dead staining of tumor and normal cells on fibers. a), b), and c) images of A549 cells on fibers; d), e), and f) images of 293T cells on fibers. Calcein-AM stain the live cells and PI stain the dead cells. Scale bar is 100 microns.