Electronic Supplementary Information

Hyaluronic Acid-Functionalized Electrospun PLGA Nanofibers Embedded in a Microfluidic Chip for Cancer Cell Capture and Culture

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Materials

Poly(lactic-co-glycolic) acid (PLGA) (LA:GA = 50:50; Mw = 81,000 g/mol，η = 0.58) was obtained from Jinan Daigang Biomaterial Co., Ltd. (Jinan, China). Hyaluronic acid, fluorescein isothiocyanate (FITC), 1-ethyl-3-(3-dimethylaminoproyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS) were supplied by Sigma-Aldrich (St. Louis, MO). Polydimethylsiloxane (PDMS) (Sylgard 184) was purchased from Dow Corning (Midland, MI). Negative photoresist SU-8 was obtained from MicroChem (Newton, MA). DMSO, N,N-dimethylformamide, and all other chemicals and solvents were acquired from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Human epithelial carcinoma HeLa and mouse fibroblast L929 cells were purchased from Institute of Biochemistry and Cell Biology (the Chinese Academy of Sciences, Shanghai, China). Fetal bovine serum (FBS), Dulbecco's Modified Eagle's medium (DMEM), and antibiotics (streptomycin and penicillin) were from Hangzhou Jinuo Biomedical Technology (Hangzhou, China). CellTrace™ Calcein red-orange AM stains were obtained from Thermofisher. Red blood cell lysis buffer was purchased from Biosharp (Shanghai, China). Water used in all experiments was purified using a Milli-Q Plus 185 water purification system (Millipore, Bedford, MA) with a resistivity higher than 18.2 MΩ cm. Blood sample was donated by volunteers ranging from 20 - 30 years old. The blood collection was performed at Huashan Hospital, Fudan University.

Materials characterization

Scanning electron microscopy (SEM, JEOL JSM-5600LV, Tokyo, Japan) at 10 kV operating voltage was used to visualize the morphology of the PLGA nanofibrous membranes. Ten nanometer thick gold film was sputter coated onto the nanofibrous samples prior to testing. To measure nanofiber diameter, three hundred nanofibers were randomly selected from each sample. The distribution of fiber diameter was analyzed using ImageJ software (https://imagej.nih.gov/ij/download.html). UV-vis

absorption spectrum of the PEI-FITC was measured with a UV-vis spectrophotometer (Perkin Elmer Lambda 25 spectrometer, Waltham, MA). Fourier transform infrared (FTIR) absorption spectra were collected with Nicolet Nexus 670 FTIR spectroscopy (Thermo Nicolet Corporation, Denver, CO) in the range of 600-4000 cm^{-1} in a transmission mode. The modification of PEI-FITC on PLGA nanofibers was characterized by an inverted fluorescence microscope (Carl Zeiss Axio Vert. A1, Jena, Germany). The water contact angles of raw PLGA, PEI-modified PLGA, and HA-modified PLGA nanofibrous membranes were measured with an optical contact angle goniometer (Dataphysics OCA40Micro, Damstadt, Germany).

Protocol S1. Detailed procedures for cytotoxicity assay

Cytotoxicity assay was employed to study the cytocompatibility of PLGA and PLGA-PEI-HA nanofibers. Cells were cultured in 25 cm^2 tissue culture flasks with 5 mL of DMEM containing 10% FBS, 100 U mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin in a humidified incubator at 37 °C and 5% CO_2. Before cell seeding, PLGA and PLGA-PEI-HA nanofibrous mats (collected on 14 mm diameter round coverslips) were placed in a 24 well tissue culture plate and fixed with stainless steel rings to prevent nanofibrous mat from floating in the medium. The nanofibrous mats were then UV treated for 2 h and washed with PBS. Next, the nanofibrous membranes were submerged in DMEM overnight prior to cell seeding. Tissue culture plates (TCPs) and cover slips were used as controls. The seeding density on all substrates was 7000 cells per well. Four hundred microliters of cell culture medium was placed in each well and replaced with fresh medium every 2 d. At the end of 1, 3, or 7 d, the cells were washed with PBS for three times and added with Cell Counting Kit-8 (CCK-8) solution (250 μL per well), followed by incubation for another 4 h. Then, one hundred microliters of the resulted solutions from each sample was moved into corresponding wells of a 96-well plate and examined using a Microplate Reader (MK3, Thermofisher, Waltham, MA) at 450 nm.

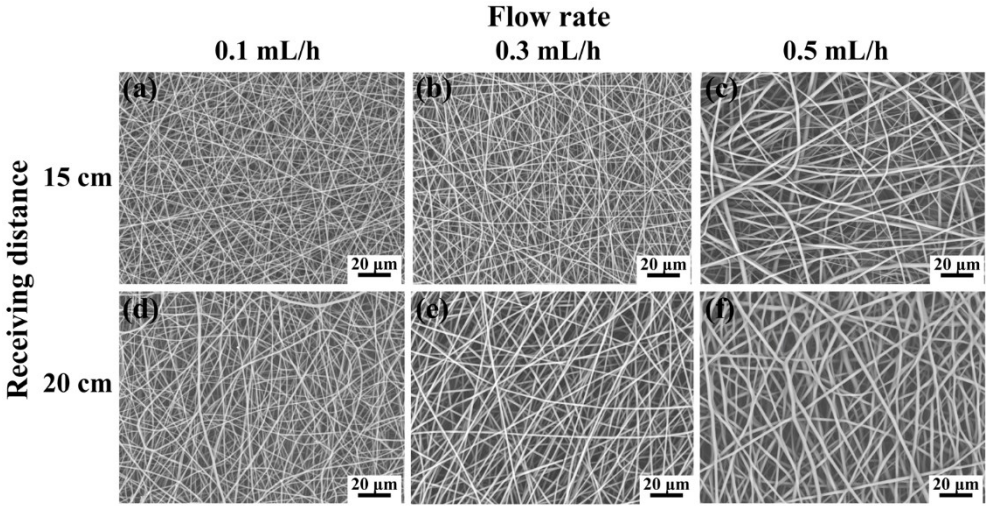

Fig. S1. SEM micrographs of electrospun PLGA nanofibers prepared at different flow rates and receiving distances.

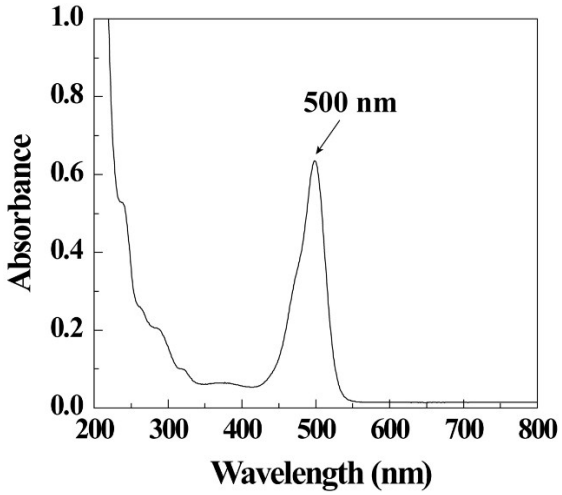

Fig. S2. UV-vis spectrum of a PEI-FITC solution.

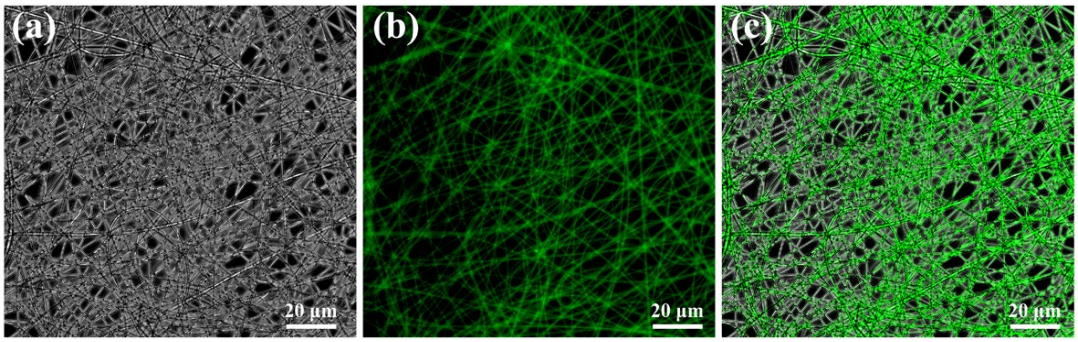

Fig. S3. Phase contrast (a), fluorescence (b), and overlay (c) microscopic images of PEI-FITC-modified PLGA nanofibers.

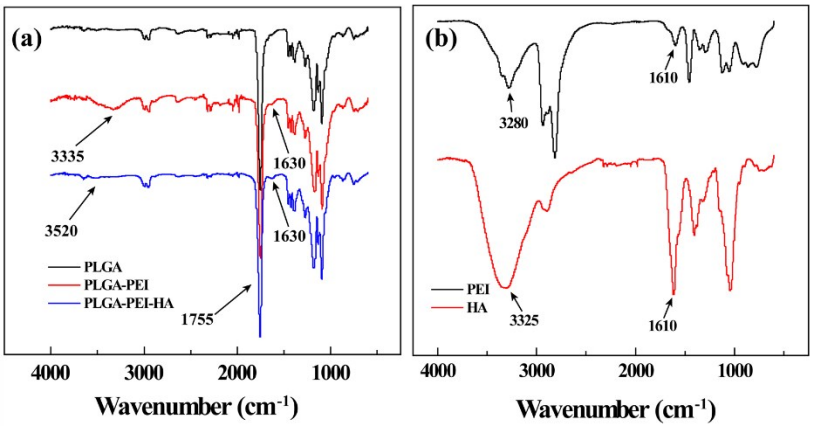

Fig. S4. FTIR spectra of PLGA (black line), PLGA-PEI (red line), PLGA-PEI-HA (blue line) nanofibrous membranes (a), pure PEI (black line), and HA (red line) (b).

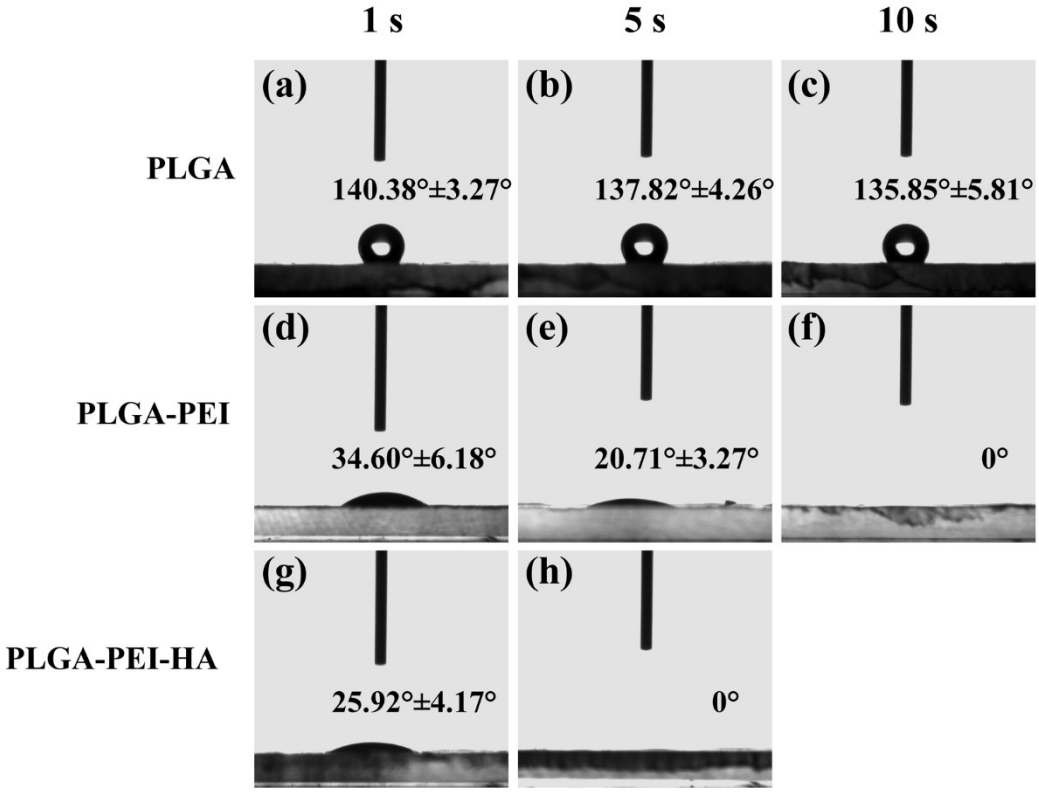

Fig. S5. Water contact angles of PLGA, PLGA-PEI, and PLGA-PEI-HA nanofibrous membranes.

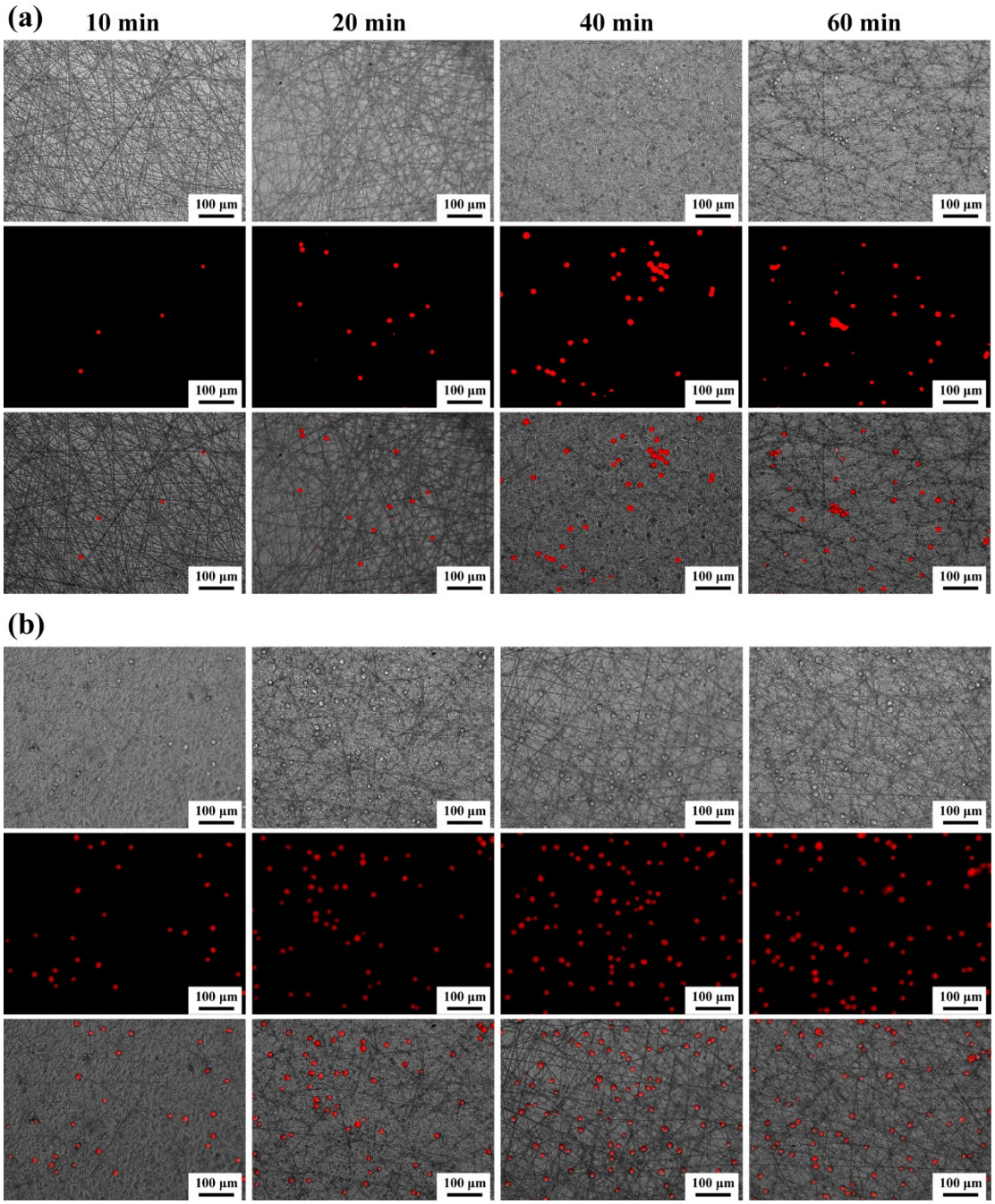

Fig. S6. Phase contrast and fluorescent microscopic images of L929 cells (a) and HeLa cells (b) captured by PLGA-PEI-HA nanofibers under static condition at different time points.

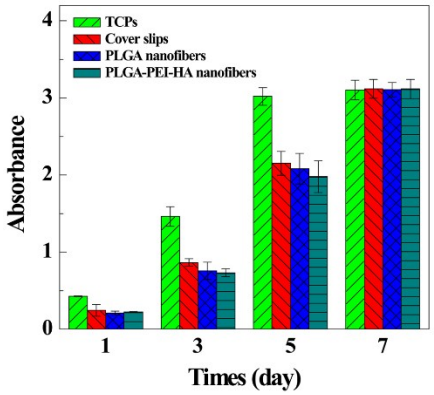

Fig. S7. Cytotoxicity assay of the nanofibrous materials. Cells were cultured on TCPs, cover slips, PLGA nanofibrous membranes, and PLGA-PEI-HA nanofibrous membranes for up to 7 days. All error bars show standard deviations (n≥3).

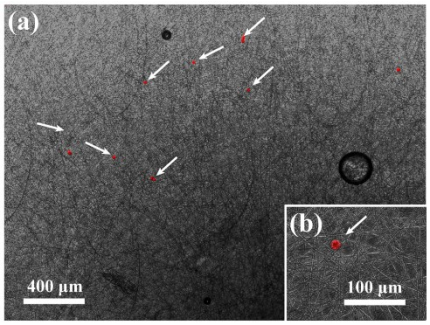

Fig. S8. An optical microscopic image of HeLa cells captured by microchip embedded with PEI-HA-modified PLGA nanofibers at a flow rate of 1.0 mL h^{-1} (a). Zoomed image showing the captured cells (b).

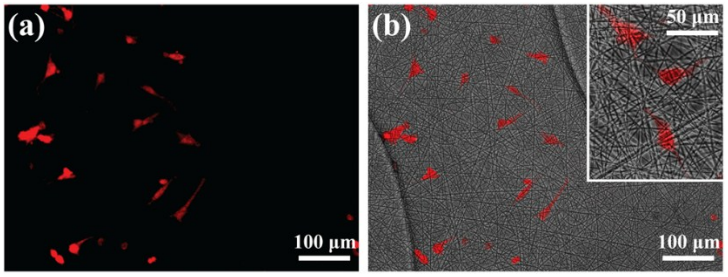

Fig. S9. The fluorescent and overlay microscopic images of HeLa cells cultured for 4 days on HA-modified PLGA nanofibers in microfluidic chip. Images were taken after 4 days of perfusion culture. The inset was a zoomed image, showing cells spread on nanofibers.