Supporting Information

Three-dimensional rope-like and cloud-like nanofibrous scaffolds facilitating in-depth cell infiltration developed using a highly conductive electrospinning system

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Materials

The polylactic acid (PLA, 4032D) was provided by Nature Works. Polyacrylonitrile (PAN, Mw=150000) chips were bought from Shanghai Macklin Biochemical Co., Ltd. The suspension of silver nanoparticles (Ag NPs suspension, <15 nm in diameter, 10000 ppm) was purchased from Shanghai Huzheng. Dimethyl formamide (DMF), trichloromethane, glycerin and sodium dodecyl sulfate (SDS) were bought from Shanghai Lingfeng Chemical Reagents

Co., Ltd and used as received. Phosphate buffered saline (PBS), Dulbecco's modified Eagle's medium (DMEM), 4% paraformaldehyde solution, Triton X-100, DAPI (4',6-Diamidino-2-Phenylindole) were obtained from Thermo Fisher, Co.. MTS reagent (CellTiter 96® Aqueous One Solution Cell Proliferation Assay) was purchased from Promega, Co.. The rat bone marrow mesenchymal stem cells (BMSCs) were bought from Shanghai Bioleaf Biotech Co.,Ltd (ATCC, R7500).

Fabrication of scaffolds

The PLA solvent was prepared by mixing DMF and trichloromethane (V_{DMF} : $V_{trichloromethane}$ = 1 : 9). The PLA granules were dissolved in the precursor solvent with addition of glycerin. The PLA, precursor solution and glycerin weight ratio was 20 : 230 : 1. Ag NPs were dispersed in the PLA solution at 0 to 0.9 wt.% based on the weight of PLA. The spinning solutions were loaded into syringe and electrospun in a conditioning chamber at 16~24 kV with an extrusion rate of 1 mL h^{-1}. The distance between the needle tip and the fiber collector was kept at 14 cm. The relative humidity and temperature in the conditioning chamber were maintained at 30 % RH and 20 °C. To prove the hypothesis about the reverse transfer of charges, a plastic airbag was mounted in front of the fiber collector and the spinning process was conducted as described above.

To investigate the mechanism of the SDS solution system, SDS powders (30 wt.% based on the weight of PAN) were dissolved in the PAN solution (14 wt.% in DMF). The polymer PAN was selected to replace PLA because the SDS powders could not be dissolved in the PLA solution in a relatively high concentration, and the PAN was also a synthetic polymer widely used for electrospinning process. During spinning, the high voltage of 15 kV was supplied and other parameters were similar with the spinning process of PLA fibers.

Morphological haracterizations

The conductivities of the spinning solutions were measured by a conductivity meter (S3-StangardKit, Mettler Toledo). The fiber formation processes were recorded by a high-speed

high-resolution camera (i-Speed 716, Mono, IX Camera). The macro-morphologies of scaffolds were recorded by a digital camera (Canon, Power Shot SX40HS, Japan). The micro-morphologies of scaffolds were observed under a scanning electron microscope (SEM, JSM-5600LV, Jeol, Japan). Fibrous scaffolds were also scanned by a confocal laser scanning microscope (CLSM, LSM700, Carl Zeiss, Germany) after staining by the Rhodamine-B solution (AR, Sinopharm, China) and the three-dimensional (3D) images were reconstructed by an imaging software (ZEN2008, Carl Zeiss, Germany). The specific pore volume was calculated by the following equation:

$$V_{sp} = \frac{V_p}{m} = \frac{V_t}{m} - \frac{1}{\rho}$$

where V_{sp} is the specific pore volume, V_p is the pore volume in the scaffold, m is the mass of the scaffold, V_t is the total volume, ρ is the density of the scaffold material. The total volume V_t was measured from the images taken from the top and side views of the scaffold by the Photoshop CC2019. The mass of each scaffold (roughly 70 mg each) was weighted by a 4-digital balance. The volume of the corresponding scaffold was measured by the drainage method. The scaffold was immersed in a measuring cylinder and repeatedly compressed by the custom-made plug to eliminate the air trapped in the scaffold. The change of the water volume was recorded and used to calculate the density of the material ρ with the mass of the scaffold.

Recharging of the as-spun fibers

The PAN/SDS fibers and the PLA/Ag NPs fibers (0.9 wt. % Ag NPs) were located on an aluminum foil which was supplied with high voltages (0 to 9 kV). The states of fibers were recorded by the digital camera and the sizes of fiber bulks were measured.

Electrostatic field simulation

The electric potential and field lines of electrostatic fields generated with and without blocking of the air-bag were simulated by the COMSOL Multiphysics Simulation Software (Version 5.3a, COMSOL Inc., USA). The materials and sizes of each component in the

spinning set-ups were predefined. The decline of electric potential was simulated for 16 kV (applied voltage) at distances of 14 mm. The electric potentials from 500 to 2 kV and the distribution were presented in the image with different colors.

Cell viability and infiltration evaluation

The cell attachment and proliferation on scaffolds were investigated using MTS assays. Five specimens were prepared for each sample with similar weights around 4 mg. BMSCs were seeded onto samples at 2.8×10^4 cells/well and cultured at 37 °C in a humidified 5 % CO_2 atmosphere for designed durations. After incubation, samples were washed with PBS for 10 min, immersed in 20 vol % MTS solutions in DMEM (0.5 mL/well), and incubated at 37 °C for 3 h. Then, 150 μL medium from each well was removed into a 96-well plate. The optical densities were measured at 490 nm on a UV/VIS multiplate spectrophotometer (AMR-100, Hangzhou Aosheng). To eliminate variations, the normalized optical densities were calculated by dividing the optical density values with the sample weights. To investigate cell infiltration to scaffolds, BMSCs were seeded on scaffolds (6.4×10^4 cells/well) and cultured for up to 5 days. At 4 h and 5 days, cells on scaffolds were fixed by 4 vol % paraformaldehyde for 20 min, permeabilized by 0.1 vol % Triton X-100 for 5 min and stained in the DAPI solution (1.25 μg/mL in PBS). Samples were scanned under an inverted confocal laser scanning microscope (LSM700, Carl Zeiss) with a step of 10 μm. The fluorescent emission of cell nuclei was collected at a wavelength of 461 nm.

Statistical analysis

Statistical significance among data was analyzed by a one-way variance with Turkey's pair wise multiple comparisons and a confidence interval of 95 %. As the *p*-value was less than 0.05, the compared data were considered statistically significantly different and labeled with different symbols.

Supporting Figures

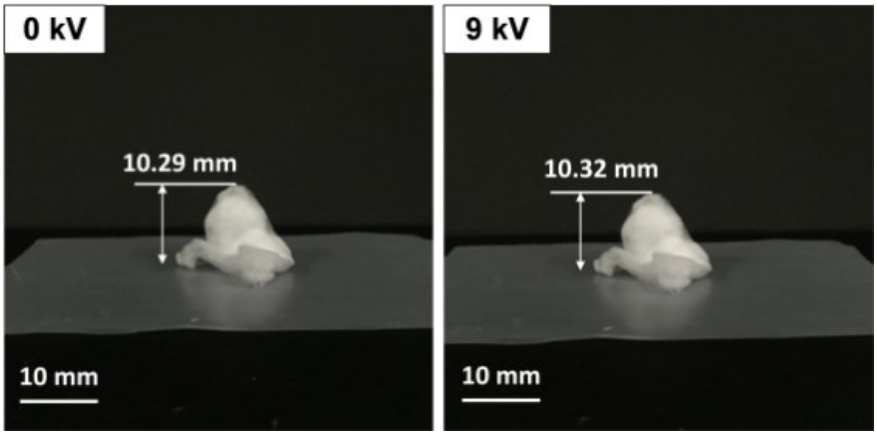

Figure S1. PAN fibers bulk with 30 wt.% SDS supplied with high voltages from 0 to 9 kV.

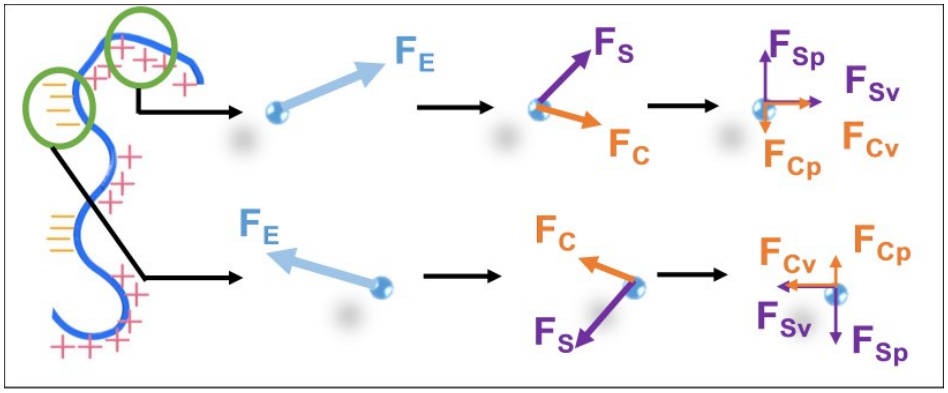

Figure S2. The analysis of the electric field forces on the positively and negatively charged fiber segments.

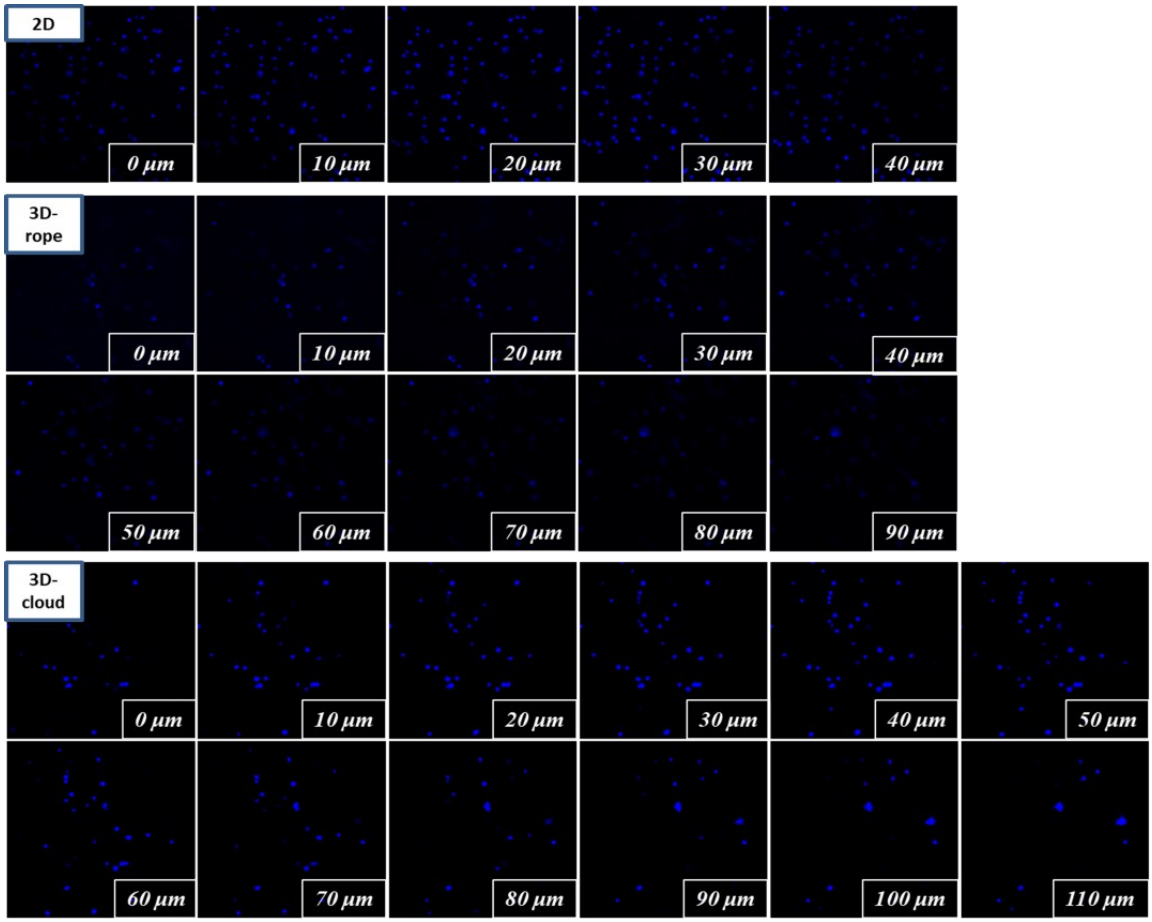

Figure S3. Cell infiltration in the 2D mat, 3D rope and 3D cloud scaffolds at the attachment stage (4h) observed by a CLSM (cell nuclei were fluorescently labeled (blue)).

Supplemental Movie Captions

Movie S1. The 2D electrospinning process of PLA

Movie S2. The electrospinning process of the 3D rope-like scaffold with 0.55 wt.% of Ag NPs

Movie S3. The electrospinning process of the 3D cloud-like scaffold with 0.81 wt.% of Ag NPs

Movie S4. The electrospinning process with blocking of an airbag at the same parameters with the spinning process of the 3D rope-like scaffold