Supporting Information

"Cell climbing stone" - varying the surfaces of electrospun nanofibers with protrusions as secondary structures to manipulate neural cell behaviors

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Experimental Details

Preparation of SiO_2 nanoparticles

SiO_2 nanoparticles (SiO_2 NPs) were synthesized through the Stöber process by initially dissolving specific concentrations of ammonia water, anhydrous ethanol, and deionized water in a round-bottom flask, followed by vigorous stirring with a magnetic stirrer for 10 minutes to form Solution A. Solution B was prepared by uniformly mixing ethyl orthosilicate (TEOS) with anhydrous ethanol in a separate beaker. Solution B was then promptly added to Solution A. The reaction was sealed and allowed to proceed for 2-5 hours. The resulting SiO_2 nanoparticles were subsequently separated by centrifugation, washed meticulously, and dried. Varying the concentration of ammonia water enabled the production of SiO_2 particles with controlled diameters of 200 nm, 400 nm, and 600 nm.

Preparation of PCL/SiO_2 hybrid aligned nanofibers with nanoscale protrusions on surfaces

A dispersion of 200 nm, 400 nm, and 600 nm SiO_2 nanoparticles with concentrations of 1%, 3%, 6%, and 9% (w/v) was prepared by ultrasonication in Hexafluoro-2-propanol (HFIP) for 3 hours, respectively. The suspension was ensured to be uniformly dispersed without the presence of any sediment. Subsequently, poly(caprolactone) (PCL) was introduced at a concentration of 10% (w/v), and the mixture was stirred magnetically until complete dissolution, yielding a PCL/SiO_2 hybrid slurry. This slurry was further sonicated for additional 2 hours, and the PCL/SiO_2 hybrid uniaxially oriented fibers were fabricated by electrospinning. The electrospinning process was conducted at a voltage of 10 to 15 kV and with a collection distance of 18 cm. Pure PCL nanofibers were produced using identical electrospinning parameters.

Characterization of PCL/SiO_2 hybrid aligned nanofibers with nanoscale protrusions on surfaces

To achieve precise characterization of the morphological characteristics of PCL/SiO_2 nanofiber arrays with nanoscale protrusions across varying experimental conditions,

gold coatings were deposited onto the samples *via* ion sputtering. Subsequently, a comprehensive analysis of the nanofiber morphology was conducted using a scanning electron microscope to provide detailed imaging and structural insight. Meanwhile, to enable a quantitative analysis of the fiber diameter, we utilized ImageJ software to measure and record the average diameter values of the fibers.

Cell viability on the different PCL/SiO_2 hybrid aligned nanofibers

To verify the cell compatibility of PCL/SiO_2 aligned nanofibers with nanoscale protrusions at different parameters, Schwann cells (SCs) were seeded onto different nanofibers, including pure PCL, at a density of 5×10^3 cells per well. Blank glass served as the control group. After culturing in the culture medium for 24 hours, the cells were washed with PBS twice. Under light-shielded conditions, 400 μL of fresh complete culture medium containing 10% CCK-8 reagent was added to each well. The cells were then incubated in a cell culture incubator at 37℃ with 5% CO_2 for 2 hours. Afterward, the supernatant was transferred into 96-well plates with 100 μL per well, and the absorbance at 450 nm was measured by an enzyme-linked immunosorbent assay reader.

Migration of SCs on the different PCL/SiO_2 hybrid aligned nanofibers

To investigate the influence of PCL/SiO_2 aligned nanofibers with nanoscale protrusions on the migration of SCs across a range of parameters, the experimental groups containing PCL were adhered to square coverslips (20 mm × 20 mm) utilizing a biological adhesive. The coverslips were then subjected to sterilization through vaporization with 75% alcohol for 6 hours, followed by 1 hour of ultraviolet radiation treatment. Subsequently, the sterilized coverslips were placed into 6-well plates and incubated overnight in a 37°C cell culture incubator within poly-D-lysine (PDL) solution. Square blank coverslips were employed as the control group. Polydimethylsiloxane (PDMS) rectangular molds with the same sizes were separately positioned at the left edge of each sample, with the leftward region designated for cell seeding. The seeding density for SCs was established at 5×10^5 cells per well. Upon confirming cell adhesion, the PDMS substrates were carefully removed to allow for the

migration of SCs.

Observation of cell motility *via* fluorescence microscopy staining was conducted using Phalloidin-iFluor 488 and 4',6-Diamidino-2-Phenylindole (DAPI). The culture medium was discarded from the well plate, and the cells were rinsed twice with PBS. The cells were fixed with 4% paraformaldehyde at ambient temperature for 30 minutes. Then, a 5-minute treatment with 0.1% Triton X-100 was administered, followed by washing with PBS for a total of three times. Finally, the cells were incubated in 1% BSA for 1 hour to prevent non-specific binding, followed by aspiration of the BSA and three additional washes with PBS. The staining working solution was prepared by combining Phalloidin-iFluor 488 with 1% BSA/PBS solution at a 1:1000 ratio. 200 μL staining solution was added into each experimental well and incubated with the cells in dark at ambient temperature for 30 minutes. Subsequently, cell nuclei were stained using a DAPI-based mounting medium and proceed with slide mounting. The samples were imaged using a Nikon upright fluorescence microscope, and the ImageJ software was explored to assess the migrating cells based on the DAPI-staining fluorescence micrographs.

Migration of neural stem cells (NSCs) on the different PCL/SiO_2 hybrid aligned nanofibers

The regulatory effect of PCL/SiO_2 oriented nanofibers with nanoscale protrusions on the migration of NSCs was investigated using consistent nanofiber treatment protocols across groups as previously described. Regarding cell culture procedures, NSCs were cultured in a complete neural stem cell culture medium and allowed to form cell spheres after a period of 4 to 6 days of continuous cultivation. These cell spheres were subsequently seeded onto oriented PCL/SiO_2 nanofibers with varying parameters and subjected to culture in a differentiation medium supplemented with 1% FBS. Following a 5-day incubation period, the cells were stained with GFAP, MAP-2, and DAPI. The stained samples were then examined and photographed using a laser scanning confocal microscope. The maximum migration distance was measured from fluorescence microscope images using ImageJ software to analyze the migration of NSCs.

The outgrowth of neurites from primary neurons on the different PCL/SiO_2 hybrid aligned nanofibers

The different PCL/SiO_2 nanofibers were treated according to the aforementioned method. Embryonic rat primary neurons were isolated from cerebral cortex and then seeded at a density of 10^4 cells per well in 24-well plates. On days 7 and 14 post culture, the samples were stained with MAP-2, SYP, and DAPI. The neurons were observed and photographed using a laser confocal microscope, and the length of neurites was measured from the fluorescence microscopic images using ImageJ software. 100 neurites were randomly selected per group to calculate the average neurite length and determine the maximum neurite length.

Statistical analysis

The multiple comparison procedures between groups were performed using one-way ANOVA with Origin 2018, and each group was repeated at least three times. Statistical results were expressed as means ± standard deviation (SD). To observe the significance of differences between the test groups, Student's t-test was used for all pairwise comparisons. $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ represent the significant differences.

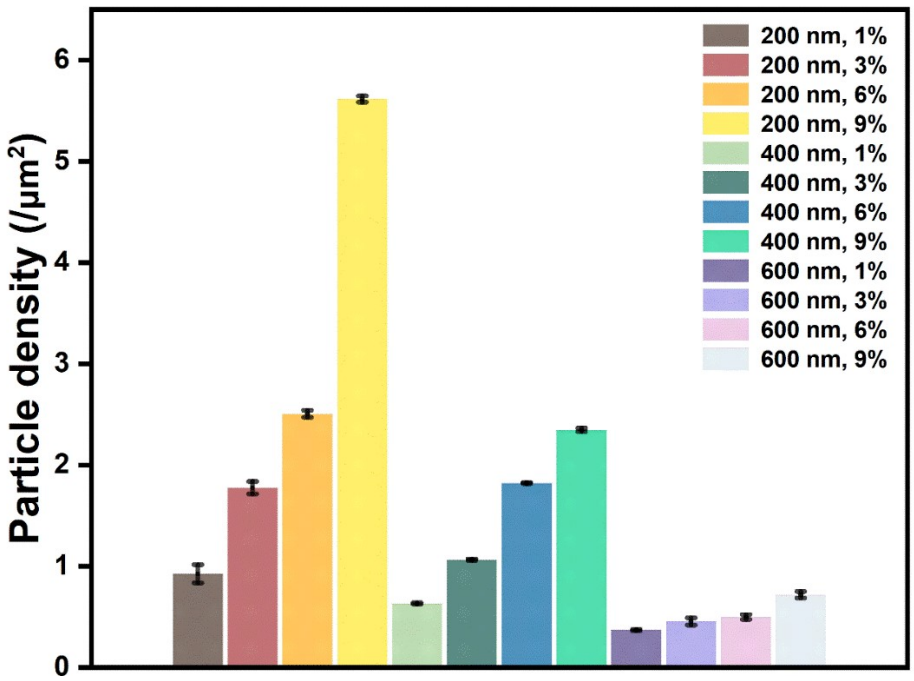

Fig. S1 Particle density of SiO_2 for the different uniaxially aligned PCL/SiO_2 hybrid nanofibers.

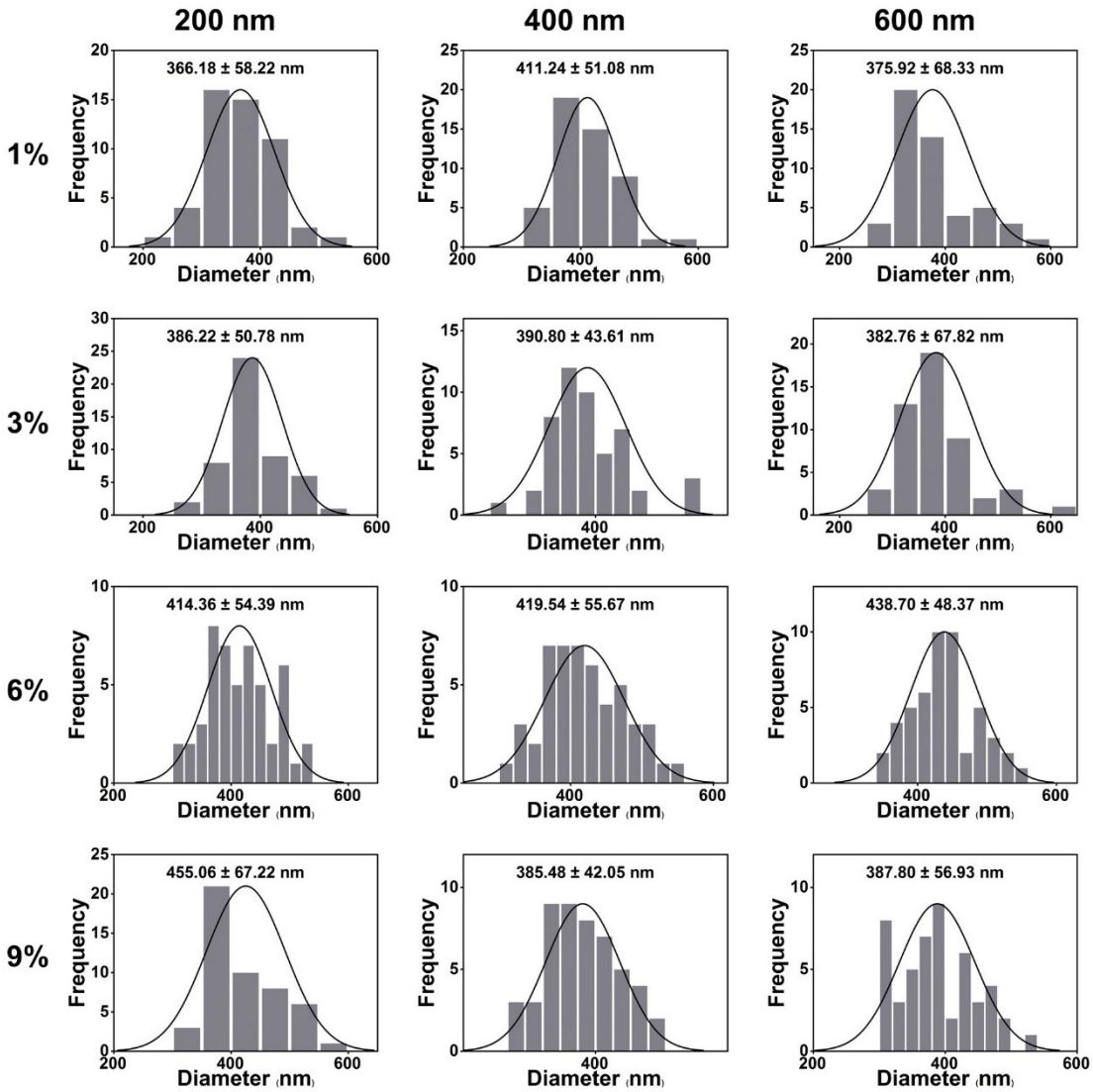

Fig. S2 Diameter distribution of the different uniaxially aligned PCL/SiO_2 hybrid nanofibers.

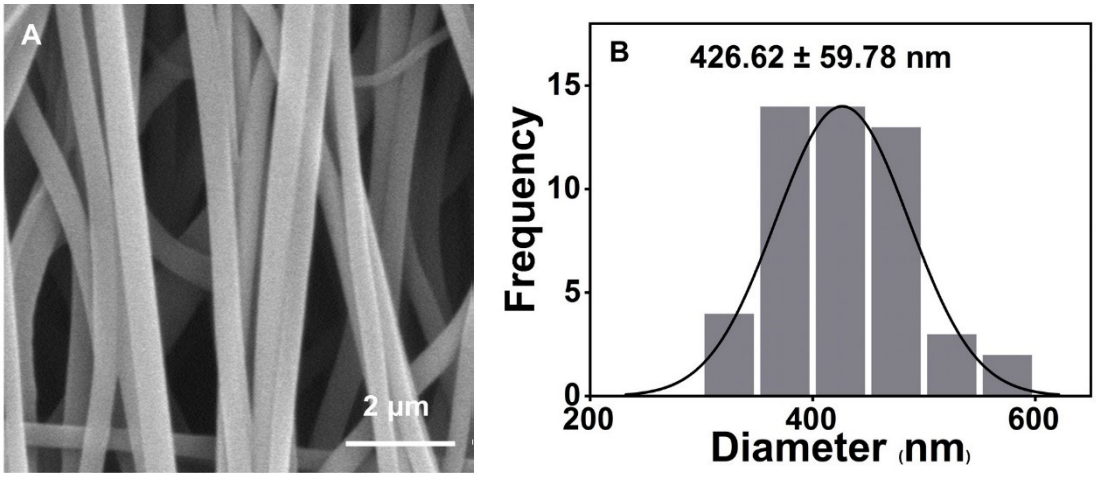

Fig. S3 (A) SEM image and (B) diameter distribution of the aligned PCL nanofibers.

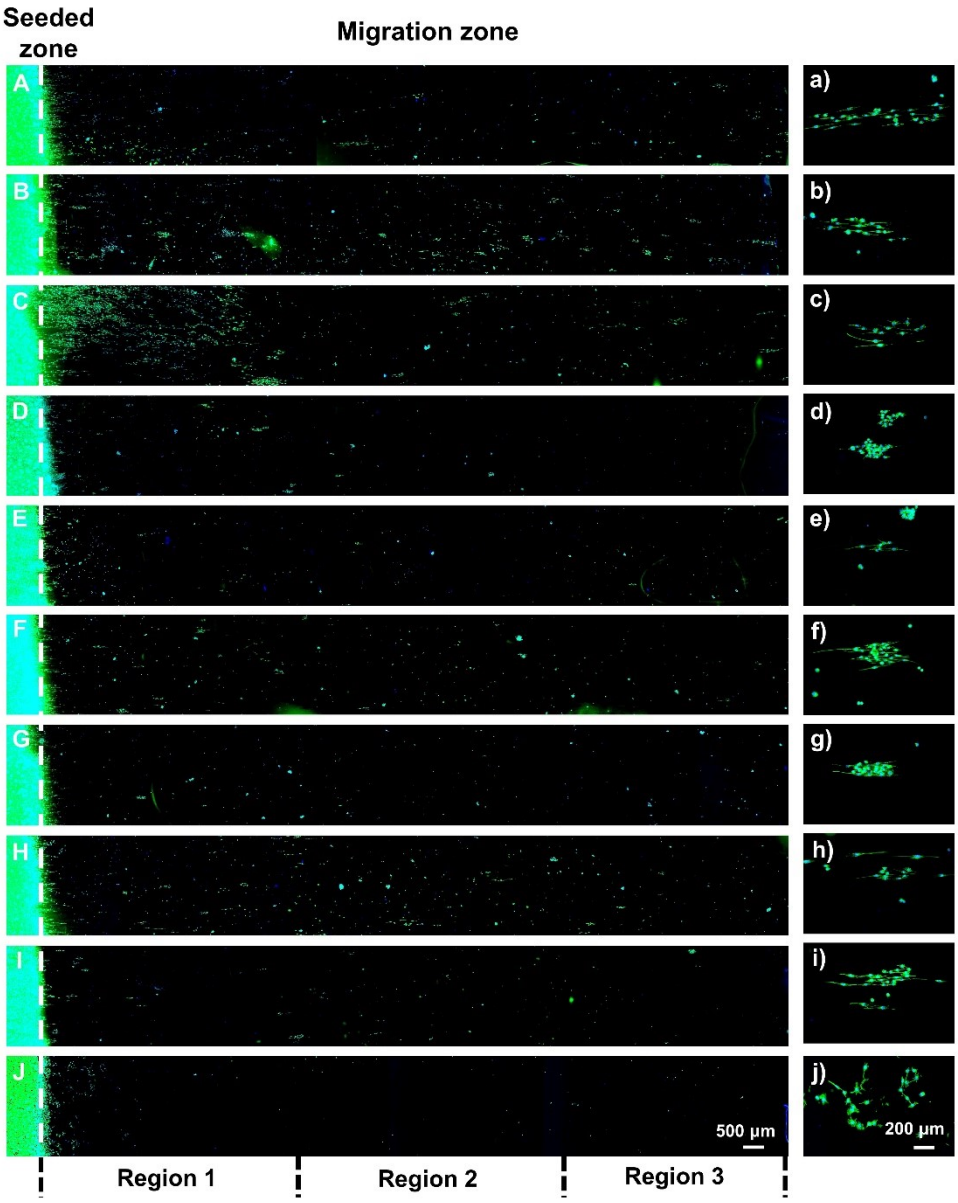

Fig. S4 Fluorescence images of the migration of SCs on the different fibers after culture for 3 days: (A) group 400 nm, 1%; (B) group 400 nm, 3%; (C) group 400 nm, 6%; (D) group 400 nm, 9%; (E) group 600 nm, 1%; (F) group 600 nm, 3%; (G) group 600 nm, 6%; (H) group 600 nm, 9%; (I) group PCL; (J) group Con. The magnified images in (a) to (j) show the morphologies of SCs in the migration zone. Green: Phalloidin-iFluor 488; Blue: DAPI. Note that this investigation was conducted in the same batch for group 200 nm (Figure 2), 400 nm, 600 nm, PCL, and Con.

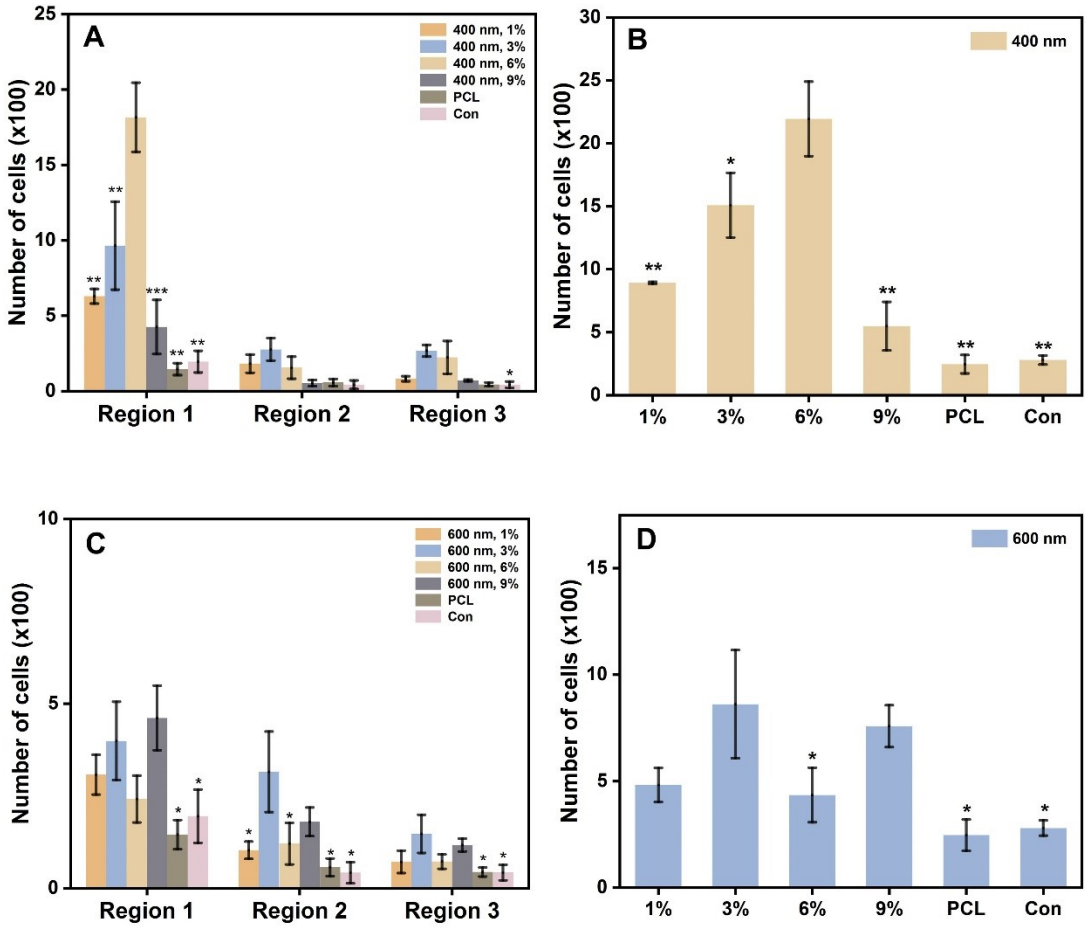

Fig. S5 (A, C) The number of SCs in the different migration zones of (A) group 400 nm and (C) group 600 nm. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ as compared with (A) group 400 nm, 6% and (C) group 600 nm, 3%. (B, D) Statistics of total migrated SCs of (B) group 400 nm and (D) group 600 nm. *$P < 0.05$ and **$P < 0.01$ as compared with (B) group 6% and (D) group 3%.

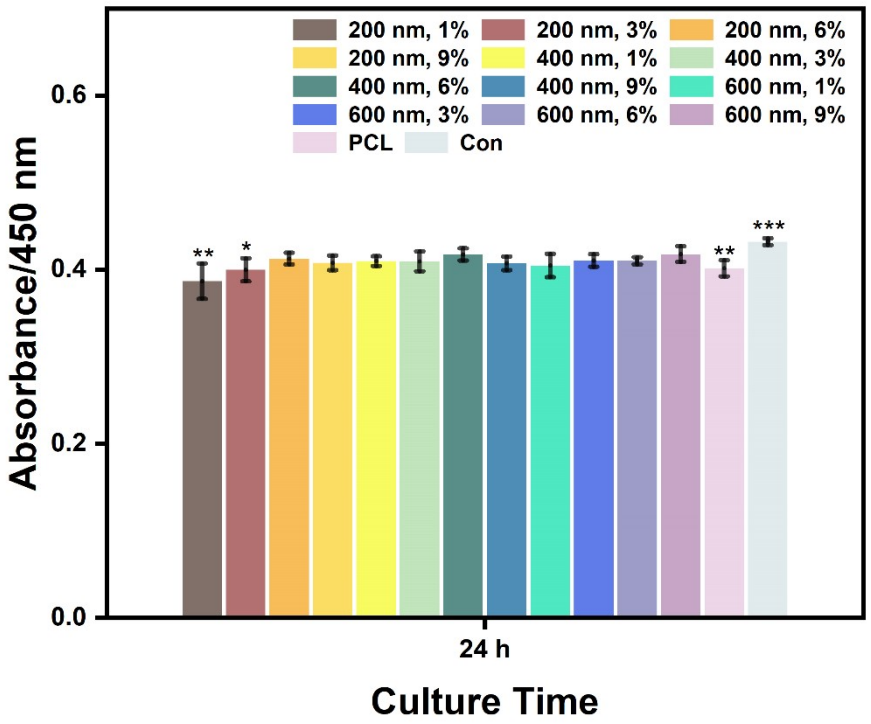

Fig. S6 The viability of SCs after culture on the different groups for 24 h. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 as compared with group 200 nm, 6%.

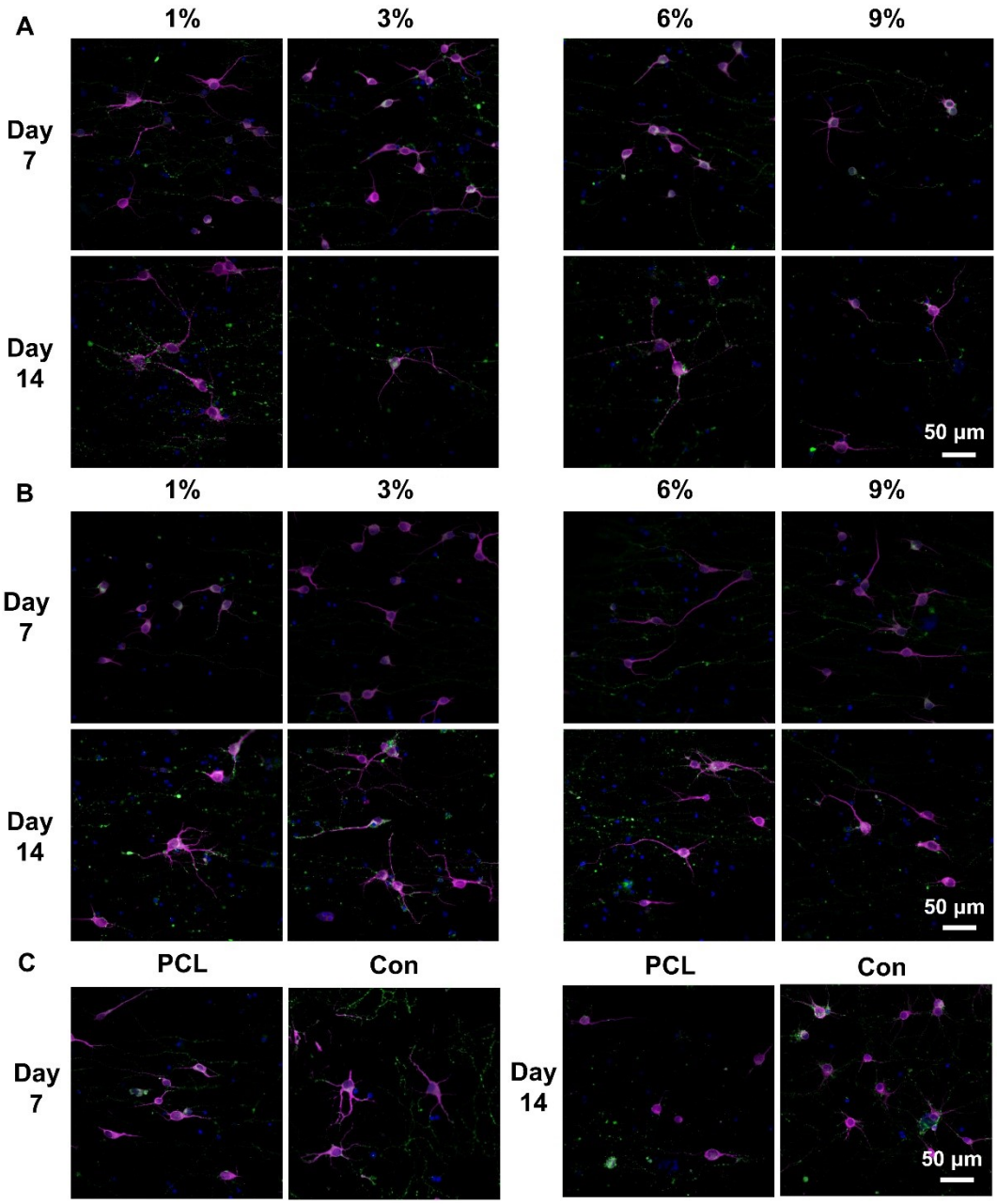

Fig. S7 Fluorescence images of the neurite outgrowth from neurons on the different fibers after culture for 7 and 14 days: (A) group 400 nm, 1%, 3%, 6%, and 9%; (B) group 600 nm, 1%, 3%, 6%, and 9%; (C) group PCL and Con. Purple: MAP-2; Green: SYP; Blue: DAPI. Note that this investigation was conducted in the same batch for group 200 nm (Figure 5), 400 nm, 600 nm, PCL and Con.

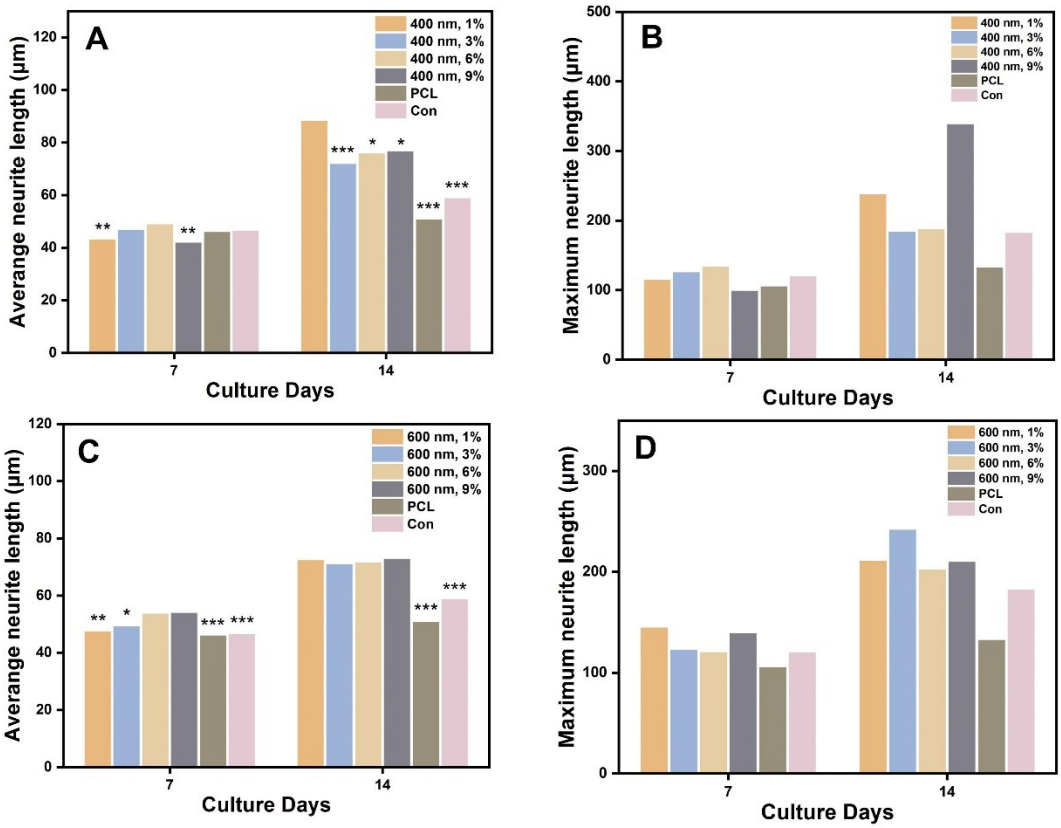

Fig. S8 (A) The average and (B) maximum neurite length of the neurons cultured on group 400 nm. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 as compared with group 400 nm, 6% (day 7) and group 400 nm, 1% (day 14). (C) The average and (D) maximum neurite length of the neurons cultured on group 600 nm. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 as compared with group 600 nm, 9% (Days 7 and 14).