Electronic Supplementary Material (ESI) for Chemical Communications.

Electronic Supplementary Information

Promoting Neurite Outgrowth and Neural Stem Cell Migration from Neurospheres using Electrospun Nanofibers Decorated with Nanoscale Protrusions and Galectin-1 Coating

Materials and methods

Materials and reagents

PCL (Mw ≈ 80 000 g mol^{-1}) was obtained from Sigma-Aldrich (America). Hexafluoro-2-propanol (HFIP) was bought from Shanghai Macklin Biochemical Company. Tetraethyl orthosilicate (TEOS) and Ammonium hydroxide solution (NH_4OH) were obtained from Aladdin (Shanghai, China). Poly-D-lysine hydrobromide (Mw ≈ 70000-150000 g mol^{-1}) was purchased from Sigma-Aldrich (America). 4',6-diamidino-2-phenylindole (DAPI) and Dulbecco's phosphate-buffered saline were purchased from Solarbio (Beijing, China). Cell counting kit-8 (CCK-8) was purchased from NCM Biotech (Suzhou, China). Phalloidin-iFluor 488 were purchased from Abcam (Shanghai, China). Dulbecco's modified eagle medium (DMEM) and antibiotic-antimycotic were purchased from Solarbio (Beijing, China). Fetal bovine serum (FBS) was purchased from Pan (Germany). SH-SY5Y cell lines were reanimated from the cryopreserved cells at the Institute of Neuroregeneration and Neurorehabilitation, Qingdao University. DMEM/F-12 medium, StemPro™ Acctuasee™ cell dissociation reagent and B27 were obtained from Gibco made in Life Technologies Corporation (America). Recombinant Gal-1, human recombinant FGF-basic (bFGF) and EFG-basic (bEGF) were acquired from Pepro Tech (Cranbury, NJ, USA). Neutite outgrowth kit was obtained from Thermo Fisher (Shanghai China).

Fabrication and characterization of uniaxially aligned PCL/SiO_2 nanofibers

The electrospun nanofibers were produced by ET-2535H electrospinning equipment from Beijing Ucalery Technology Development Co. Ltd., China. Firstly, we synthesized SiO_2 NPs according to the typical stober method as reported previously [1]. Briefly, ammonium hydroxide (4.5 mL), ethanol (8.125 mL), and deionized water (12.375 mL) were added to a round-bottomed flask. After rapidly magnetic stirring for 10 min, TEOS (2.25 mL) and ethanol (22.75 mL) were mixed in the solution and stirred for 1 min. The reaction process of the mixed solution was continued for 180 min to obtain a milky white suspension under room temperature. The obtained solution was

centrifuged at a speed of 4000 rpm for 30 min. Then, the SiO_2 NPs were obtained after drying for 1 h. The PCL/SiO_2 nanofibers were fabricated using electrospinning. Firstly, the SiO_2 NPs were dispersed into HFIP at different concentrations of 0, 1, 3, and 6% (w/v), respectively, for ultrasonication. Then, PCL was added into the solution at a concentration of 10% (w/v). After stirred overnight, the mixture was pumped into a 5-mL injection syringe with a 22-gauge blunt needle for electrospinning. A flow rate of 1.0 mL h^{-1} and a high voltage of 15 kV were applied. The collection distance between the injection syringe and the colleting drum (2800 rpm) was 15 cm. The obtained nanofibers were fixed on the specimen holder using electric conductive adhesive followed by spraying for 30 s with an ion sputter coater (BV10044, KYKY Technology Co., Ltd.). The mat of PCL/SiO_2 nanofibers was cut to 2×2 mm^2, and the surfaces of the mat were characterized by a scanning electron microscope at the magnification of 15,000× (PW-100-515, ThermoFisher). The average diameters of fibers were analyzed and recorded by ImageJ software. The Fourier transform infrared (FTIR) spectrophotometer (Nicolet 710, Thermo Nicolet Corporation) was used to confirm the successful combination of SiO_2 NPs and PCL in the fibers. The average water contact angle of different nanofibers was respectively measured using data physics OCA (Data Physics, Stuttgart, Germany).

Cell viability and neurite outgrowth

SH-SY5Y cells were chosen as model cells, and the viability and biocompatibility of PCL nanofibers containing different concentrations of SiO_2 NPs were detected by CCK-8 method. The SH-SY5Y cells were cultured in DMEM medium containing 10% FBS. After culturing for 3 days, the cells were seeded at a density of 1×10^4 cells/well in a 24-well plate. The PCL nanofibers containing different concentrations of SiO_2 NPs (0%, 1%, 3%, and 6%, respectively) were used for investigation. After culturing SH-SY5Y cells on the different samples for 1, 3, and 5 days, the cell culture medium in each well was replaced with fresh medium consisting of 10% CCK-8 reagent. After incubating for 3 h, the absorbance values of different samples at 450 nm were measured using a spectrophotometric microplate reader. Neurite extension of SH-SY5Y cells

after cultured on the different nanofibers was also observed. When the cells were cultured for 5 days, each sample was permeabilized by 0.1% Triton X-100 for 5 min and then rinsed 3 times with phosphate buffered saline (PBS). Afterward, the cells were blocked with 1% BSA for 30 min, and the cytoskeletons were stained with Phalloidin-iFluor 488 in PBS solution containing 1% BSA. Finally, cell nuclei were stained by DAPI for observation under an upright fluorescence microscope. The lengths of the extended neurites were measured by ImageJ software.

Coating the PCL/SiO_2 nanofibers with Gal-1 for investigating the cell viability and neurite outgrowth

We first verified the effect of Gal-1 on the survival of SH-SY5Y cells by CCK-8. The SH-SY5Y cells were seeded in a 24-well plate at the density of 1×10^4 cells/well. After cell adhesion, each well was supplemented with a medium containing different concentrations of Gal-1 (0, 10, 20, 50, and 100 ng/mL, respectively). The cell viability was measured and recorded at predetermined time points (1, 3, and 5 days) using the CCK-8 method as described above. After determining the optimal concentration, the Gal-1 was coated on the PCL/SiO_2 nanofibers. The poly-D-lysine solution was prepared by dissolving poly-D-lysine in PBS at the concentration of 0.01 mg/mL. The PCL/SiO_2 nanofibers placed in the wells of 24-well plate were immersed in the poly-D-lysine solution for 24 h so that polycation from poly-D-lysine can make the nanofibers positively charged. The Gal-1 powder was dissolved in double distilled water at the concentration of 50 ng/mL to obtain the Gal-1 solution. Then, the solution was added in the 24-well plate containing nanofibers after discarding the poly-D-lysine solution and washing with PBS for three times. Gal-1 can be absorbed on the surfaces of nanofibers due to the interaction of positively-charged poly-D-lysine coating and negatively-charged Gal-1. Then, the viability of SH-SY5Y cells on the nanofibers was tested by CCK-8. After stained by Phalloidin-iFluor 488 and DAPI, the different samples were observed under the fluorescence microscope. The lengths of the extended neurites were measured by ImageJ software.

Fabrication of radially aligned PCL/SiO_2 nanofibers

The radially aligned nanofibers were prepared to extend the potential of aligned PCL/SiO_2 nanofibers in neural tissue engineering. The fabrication of radially aligned PCL/SiO_2 nanofibers was carried out according to the previous studies [2, 3]. The electrospinning parameters were the same as the case of fabricating uniaxially aligned nanofibers, except that the collection device was changed to a steel ring with a needle placed vertically in the center. The radially aligned PCL/SiO_2 nanofibers with Gal-1 coating was realized as described above. The morphology of the radially aligned nanofibers were observed by the scanning electron microscope.

NSCs culturing and investigation of NSCs migration on radially aligned PCL/SiO_2/Gal-1 nanofibers

The NSCs were extracted from the brain of fetal rats by referring to the previous study[4], which was approved by the Institutional Animal Care and Use Committee of the affiliated hospital of Qingdao University, China. All animal housing and experiments were conducted in accordance with the institutional guidelines for care and use of laboratory animals. Briefly, fetal rats' cerebral cortex was harvested and incubated with StemPro™ Acctuasee™ cell dissociation reagent for 20 min before being blown into single cells. After cell counting, the cells were cultured in medium with 2% B27, 20 ng/mL EGF, 20 ng/mL bFGF and 1% antibiotic-antimycotic at a density of 1×10^6 cells. Half of the culture medium was changed every two days, and the cells were continuously cultured for 4-6 days to obtain neurospheres. We used the 2nd-3rd generation of the neurospheres for cell migration experiments. In this case, individual spheroid of NSCs was seeded in the center of the radially aligned scaffolds and cultured in the medium containing 1% FBS, 2% B27, and 1% antibiotic-antimycotic. At 7 days post culture, the morphology and migration of NSCs were investigated after stained with the Neurite outgrowth kit. Briefly, the stain solution was prepared by diluting sterile Dulbecco's phosphate-buffered saline (DPBS) with the cell viability indicator and membrane stain at a ratio of 1:1000. Staining solution was added to each sample, aspirated after two hours of incubation at room temperature, followed by three-time washing with DPBS. Then, a fresh working background suppression

solution was prepared by diluting background suppression dye (1:100) in sterile DPBS. The working background suppression solution was applied to each sample for 30 min before observation by the fluorescence microscope (Olympus, Tokyo, Japan).

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. The statistical data were presented as mean±standard deviation, and significant differences were determined at $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{\#}P < 0.05$, and $^{\#\#}P < 0.01$.

References

1. K. S. Rao, K. El-Hami, T. Kodaki, K. Matsushige and K. Makino, *J. Colloid Interface Sci.*, 2005, **289**, 125-131.
2. T. Wu, J. Xue, H. Li, C. Zhu, X. Mo and Y. Xia, *ACS Appl. Mate.r Interfaces*, 2018, **10**, 8536-8545.
3. J. Xie, M. R. Macewan, W. Z. Ray, W. Liu, D. Y. Siewe and Y. Xia, *ACS Nano*, 2010, **4**, 5027-5036.
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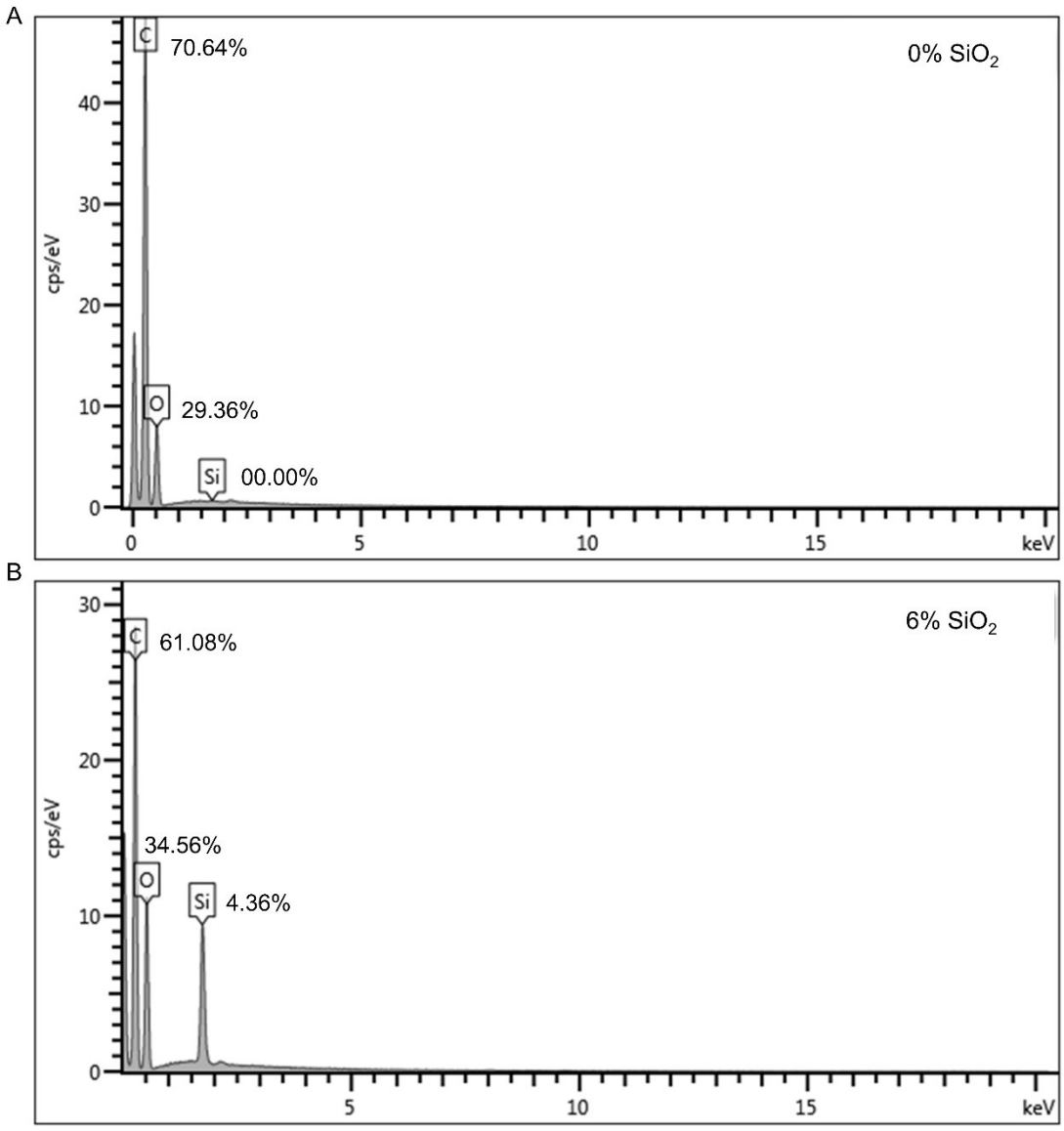

Fig. S1 The EDS analysis of the (A) PCL nanofibers and (B) PCL/SiO_2 nanofibers containing 6% SiO_2 NPs.

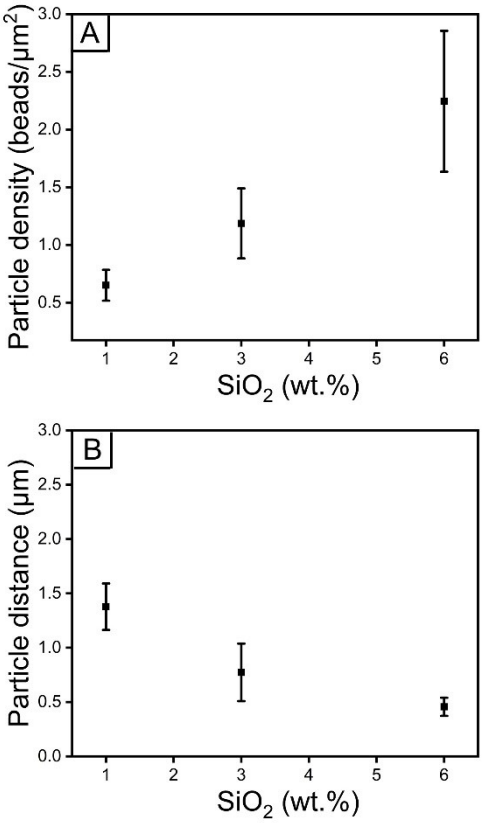

Fig. S2 (A) SiO_2 NPs density per unit area (μm^2) in the PCL/SiO_2 nanofibers. (B) The distance between adjacent SiO_2 NPs in the PCL/SiO_2 nanofibers.

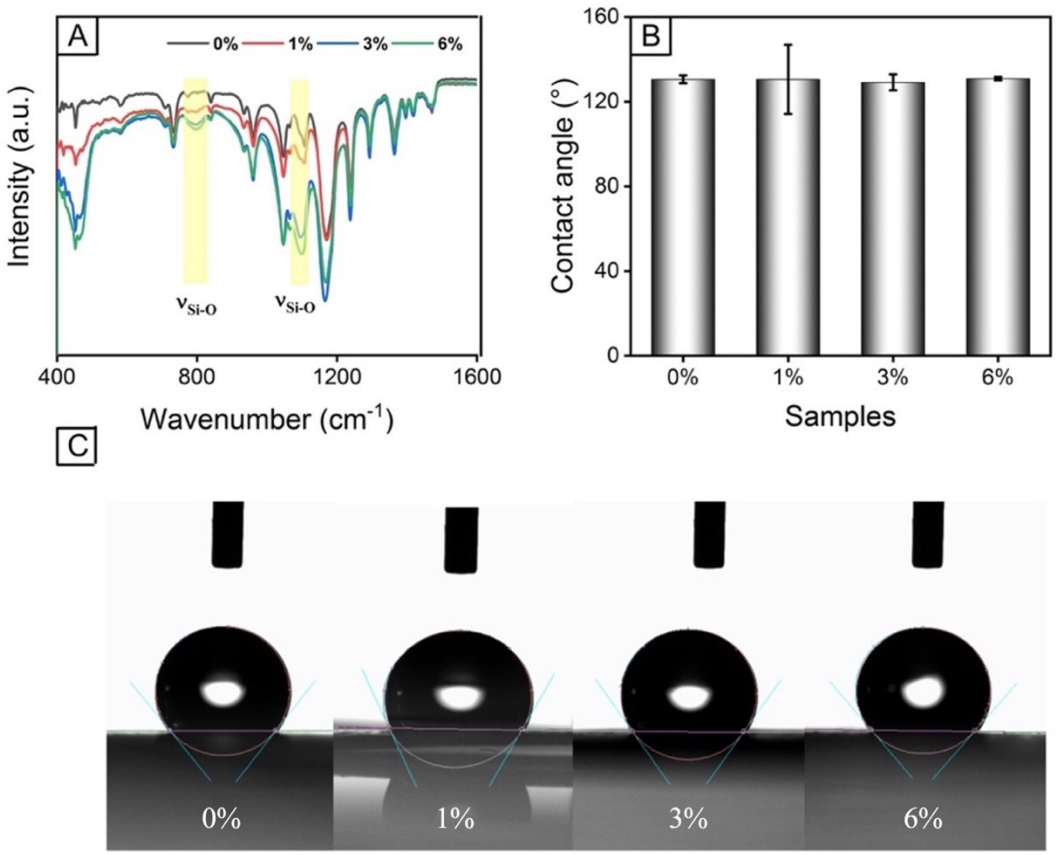

Fig. S3. (A) FTIR spectra of the PCL/SiO_2 nanofibers containing different concentrations of SiO_2 NPs (0%, 1%, 3%, and 6%). (B) Water contact angles and (C) the corresponding photographs of the PCL/SiO_2 nanofibers containing different concentrations of SiO_2 NPs (0%, 1%, 3%, and 6%).

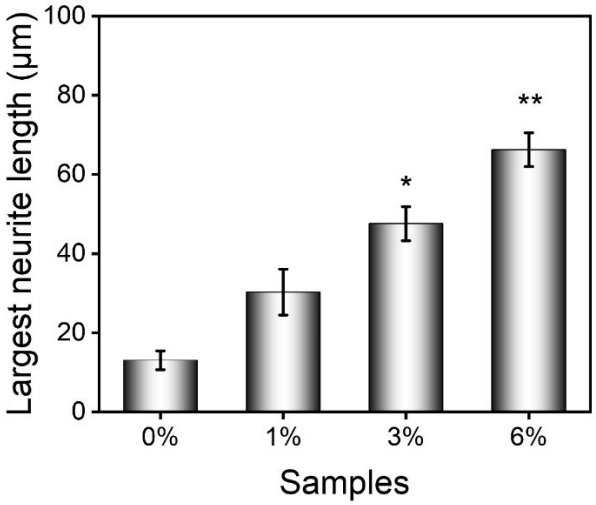

Fig. S4 The largest length of the extended neurites from SH-SY5Y cells after culturing on the different groups which were correlated to groups E to H in **Fig. 1**. $^{**}P < 0.01$ when compared with the other groups. $^{*}P < 0.05$ when compared with the 0% and 1% groups.

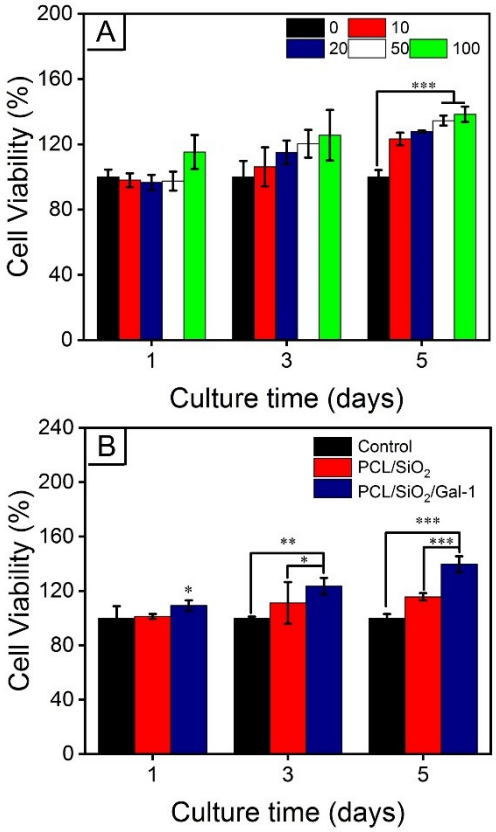

Fig. S5 (A) The effect of Gal-1 (0, 10, 20, 50, and 100 ng/mL) on the viability of SH-SY5Y cells after culturing for 1, 3, and 5 days. ***$P < 0.001$ when comparing the group containing 100 and 50 g/mL Gal-1 with the control group. (B) Viability of SH-SY5Y cells after cultured on the control group, uniaxially aligned PCL/SiO_2 and PCL/SiO_2/Gal-1 nanofibers for 1, 3, and 5 days. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ indicate the significant differences between the compared groups.

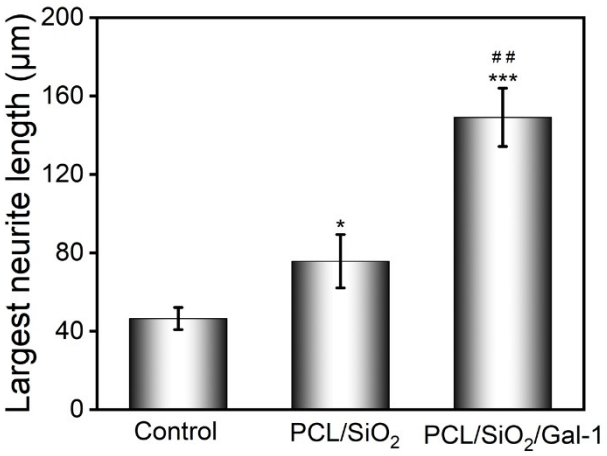

Fig. S6 The largest length of the extended neurites from SH-SY5Y cells after culturing on the different groups which were correlated to groups A to C in **Fig. 2**. ***$P < 0.001$ when comparing PCL/SiO_2/Gal-1 nanofibers with the control group. *$P < 0.05$ when comparing PCL/SiO_2 nanofibers with the control group. $^{\#\#}P < 0.01$ when comparing PCL/SiO_2/Gal-1 nanofibers with PCL/SiO_2 nanofibers.

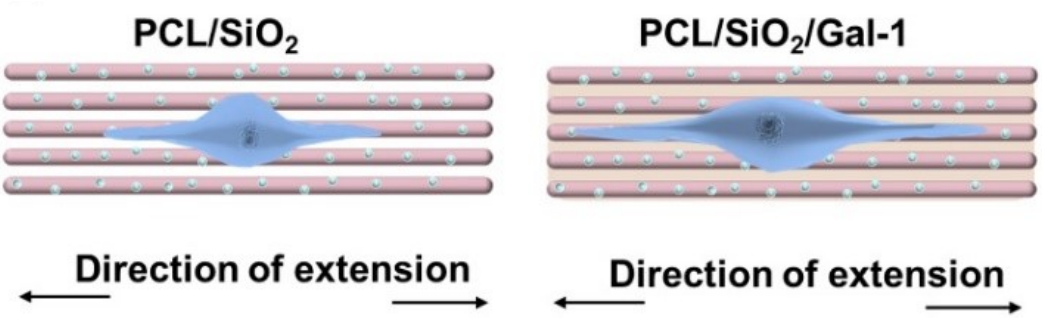

Fig. S7 Schematic illustration showing the neurite outgrowth and after the cells were cultured on the PCL/SiO_2 and PCL/SiO_2/Gal-1 nanofibers.

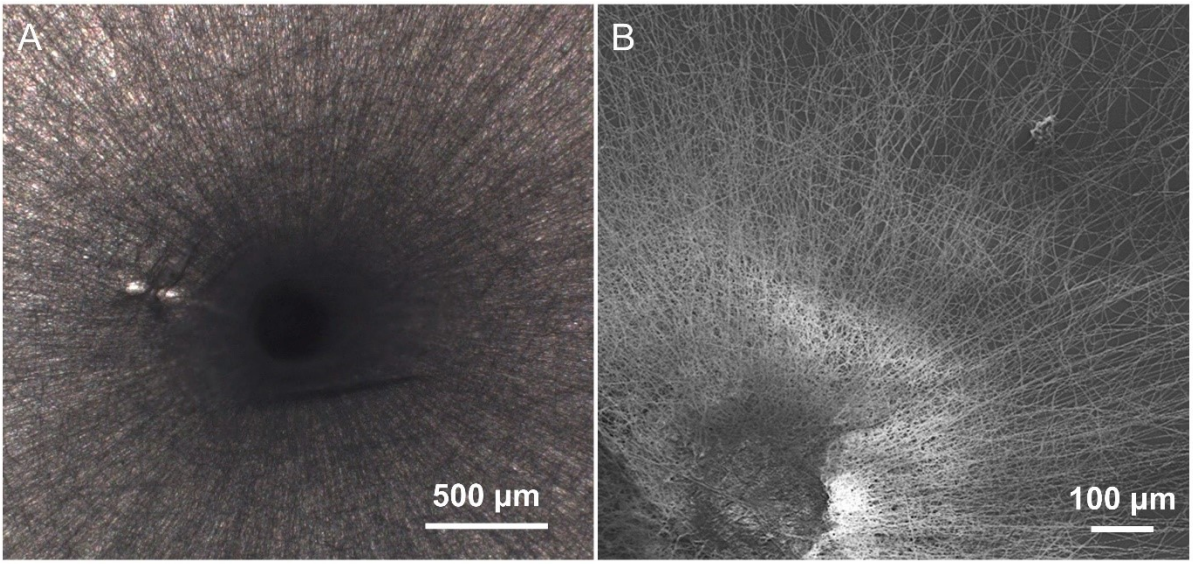

Fig. S8 (A) The optic microscopy and (B) SEM images showing the radially aligned PCL/SiO_2/Gal-1 nanofibers.