

Electrospinning of Nanofibres with Parallel Line Surface Texture for Improvement of Nerve Cell Growth

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Electronic Supplementary Information

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Experimental details

Materials: Cellulose acetate butyrate (CAB, viscosity average molecular weights $M_{\eta} \approx 70,000$ and $M_{\eta} \approx 30,000$), cellulose acetate (CA, $M_{\eta} \approx 50,000$), polyvinylidene fluoride (PVDF, $M_w \approx 534,000$), poly (methyl methacrylate) (PMMA, $M_w \approx 350,000$), polycaprolatone (PCL, $M_w \approx 70,000-90,000$), and poly(vinylidene fluoride-co-hexafluoropropene) (PVDF-HFP, no M_w information provided by the supplier) were obtained from Aldrich and polystyrene (PS, $M_w \approx 100,000$) from BDH Chemicals. Acetone (Chem-Supply), N, N'-dimethylacetamide (DMAc, Aldrich), dichloromethane (DCM, Aldrich), tetrahydrofuran (THF, Aldrich), N, N'-dimethylformamide (DMF, Chem-Supply) and methanol (MERCK) are all of reagent grade. All chemicals were used as received. Rat Schwann cells (SCs, CRL-2768) and culture media were obtained from American Type Culture Collection. All other reagents used for cell culture were purchased from Gibco Life Technologies, USA.

Electrospinning: Electrospinning was performed using a purpose-made electrospinning setup ^[17]. During electrospinning, unless otherwise specified, the applied voltage, flow rate and electrospinning distance were set at 20 kV, 0.8 ml/h and 20 cm, respectively. Aligned CAB fibres were electrospun using a grounded rotating mandrel (surface linear velocity, 26 m/s) as the collector. After electrospinning, CAB fibres were kept in vacuum at 80 °C overnight to remove the solvent residues.

Physical characterizations: Fibre morphologies were observed under a scanning electron microscope (SEM, Zeiss SUPRA 55VP). The fibre diameter was measured based on the SEM images using an image analysis software (ImagePro+6.0). The cell morphologies and the evaporation behaviour of polymer solutions were observed using a laser scanning confocal microscopy (Leica TCS SP5) equipped with Argon lasers. Molecular orientation was evaluated by polarized Fourier transform infrared spectrophotometer (VERTEX 70 FTIR spectrometer equipped with an MIR polarizer, Bruker Biosciences Pty Ltd). The optical transmittance was measured using

an Ocean Optics Inc PX-2 UV-VIS spectrophotometer. The viscosities of the CAB solution were determined by a digital rotational viscometer (D443 Rheology International).

Cell culture and cell morphology observation: Schwann cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum and 1% antibiotic–antimycotic under standard culture conditions (*i.e.* 37 °C, in a humidified atmosphere containing 5% CO₂ and 95% air). After plasma treatment for 3 minutes, the CAB nanofibre mats were punched into circular shapes (14 mm in diameter) and placed individually into a 24-well culture plate, then secured with stainless rings. After sterilisation in an autoclave at 121 °C for 15 minutes, the scaffold samples were washed 3 times with PBS and once with culture medium. Cells were then seeded onto the scaffolds at a density of 1.0×10^4 cells/well and the culture medium was replenished every three days.

For observing cells using the confocal microscopy, the cultured matrices were first washed with sterilised PBS for three times to remove medium and unviable cells, followed by immersing in a PBS solution containing 2% paraformaldehyde for 10 minutes at room temperature. After rinsing with PBS for three times, the matrices were placed in a permeabilization solution (0.2% Triton X-100 in PBS, Sigma-Aldrich) at room temperature for 10 minutes and rinsed again with fresh PBS for three times. The matrices were then stained with 4, 6-diamidino-2-phenylindole (dilution ratio of 1:100, vol/vol) and phalloidin alexa 568 (dilution ratio of 1:100, vol/vol) overnight in dark environment. After rinsing with PBS for three times to remove the residual fluorescent dye, the matrices were ready for imaging.

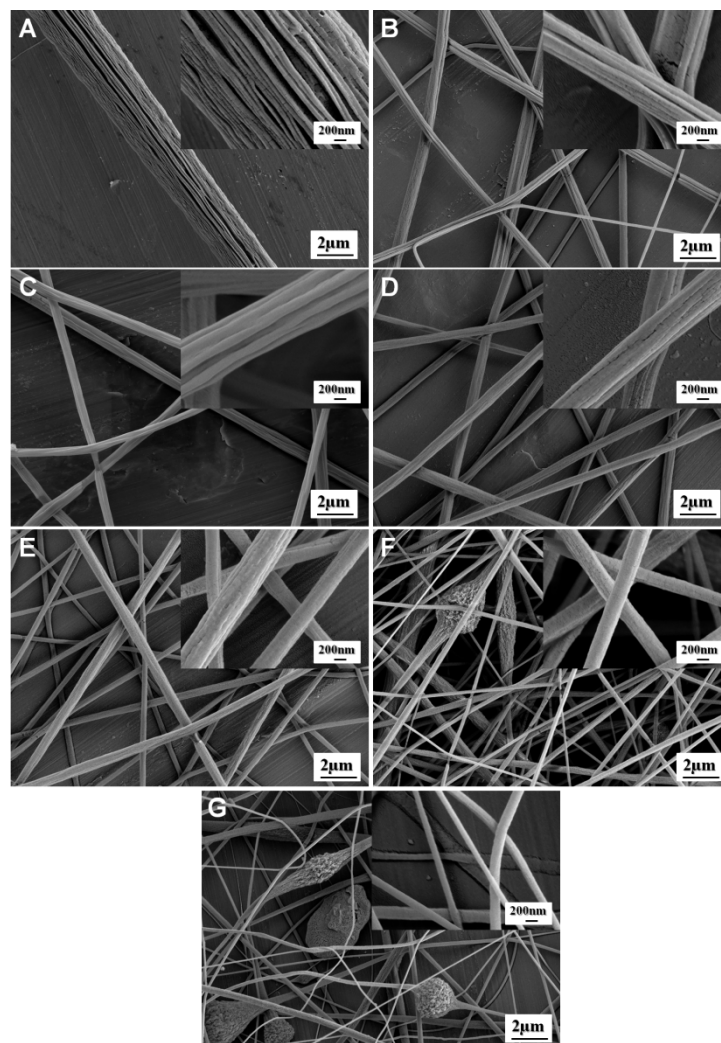


Fig. S1 SEM images of CAB fibres electrospun from 15wt% CAB acetone/DMAc solution with different acetone/DMAc ratios, A) 9/1, B) 4/1, C) 2/1, D) 1/1, E) 1/2, F) 1/4, and G) 1/9 (vol/vol). The respective average fibre diameters are 2350, 492, 483, 430, 401, 273, and 186 nm.

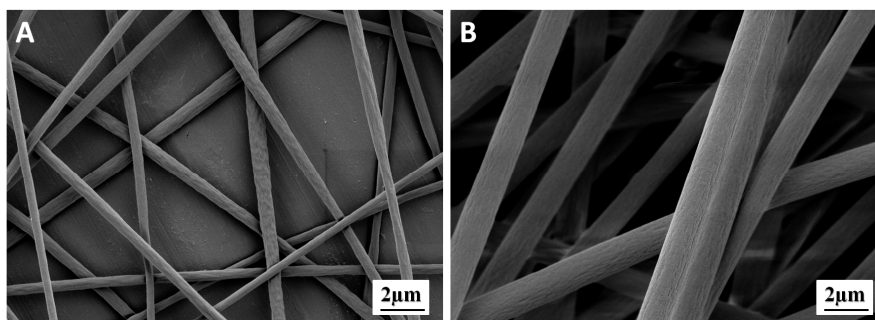


Fig. S2 SEM images of CAB fibres electrospun from CAB having a low molecular weight ($M_{\eta} \approx 30,000$), A) 30 wt% and B) 50 wt%. (Solvent, acetone/DMAc=2/1, vol/vol).

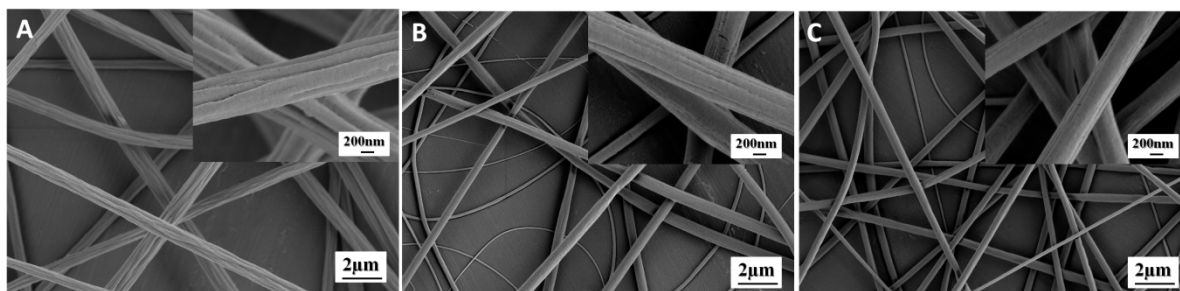


Fig. S3 SEM images of CAB fibres electrospun from different solvent systems, A) Acetone/DMF (2/1, vol/vol), B) Methanol/DMAc (2/1, vol/vol), and C) Methanol/DMF (2/1, vol/vol). The respective average fibre diameters are 566, 417 and 329 nm.

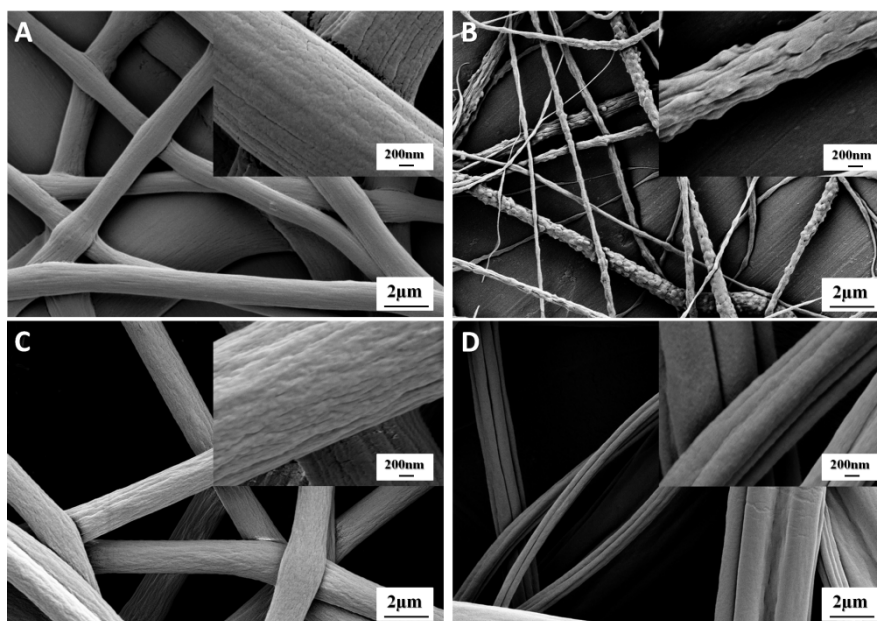


Fig. S4 SEM images of electrospun A) CA (30 wt%), B) PVDF (10 w%), C) PMMA (15 wt%), and D) PS (15 wt%) fibres. The respective polymer concentration for electrospinning is given in the brackets. (Acetone/DMAc=2/1, vol/vol).

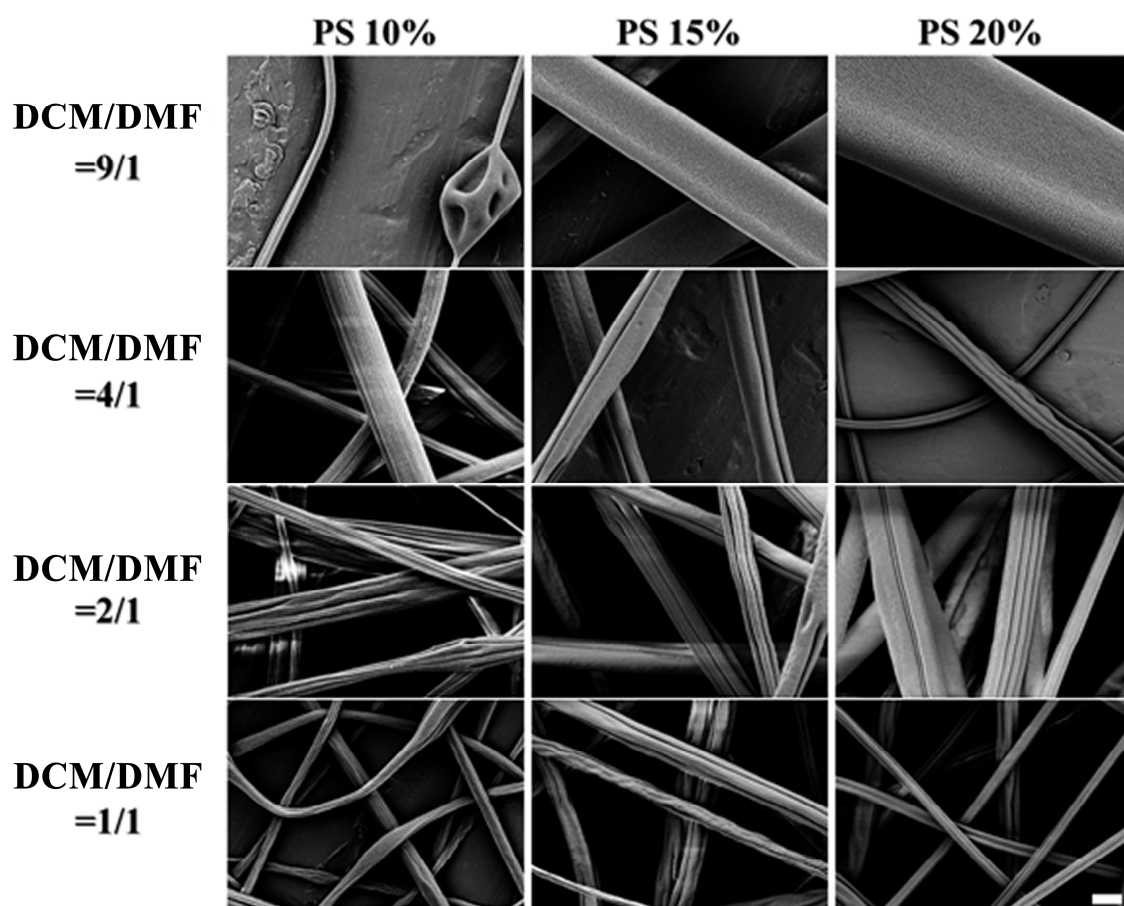


Fig. S5 SEM images of electrospun PS fibres (solvents: DCM/DMF). Scale bar = 2 μ m.

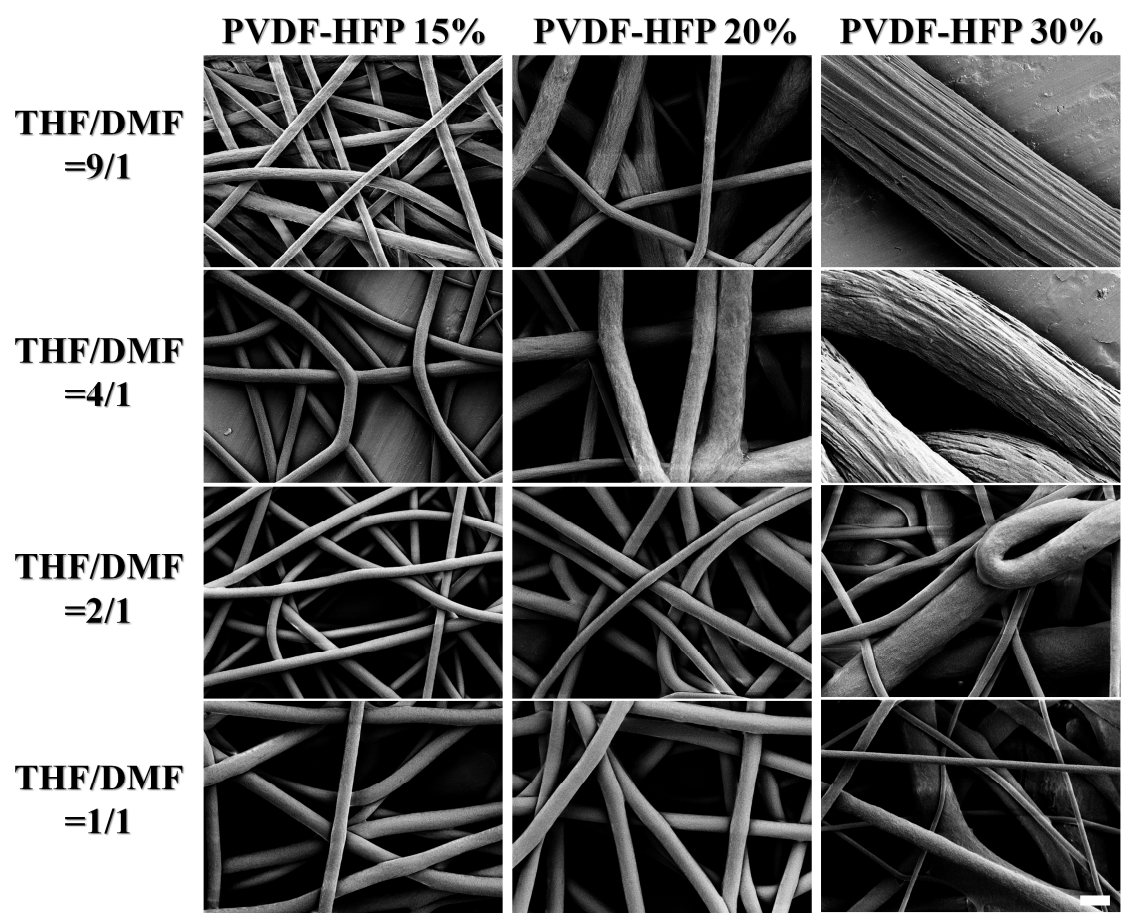


Fig. S6 SEM images of electrospun PVDF-HFP fibres (solvents: acetone/DMF). Scale bar = 2µm.

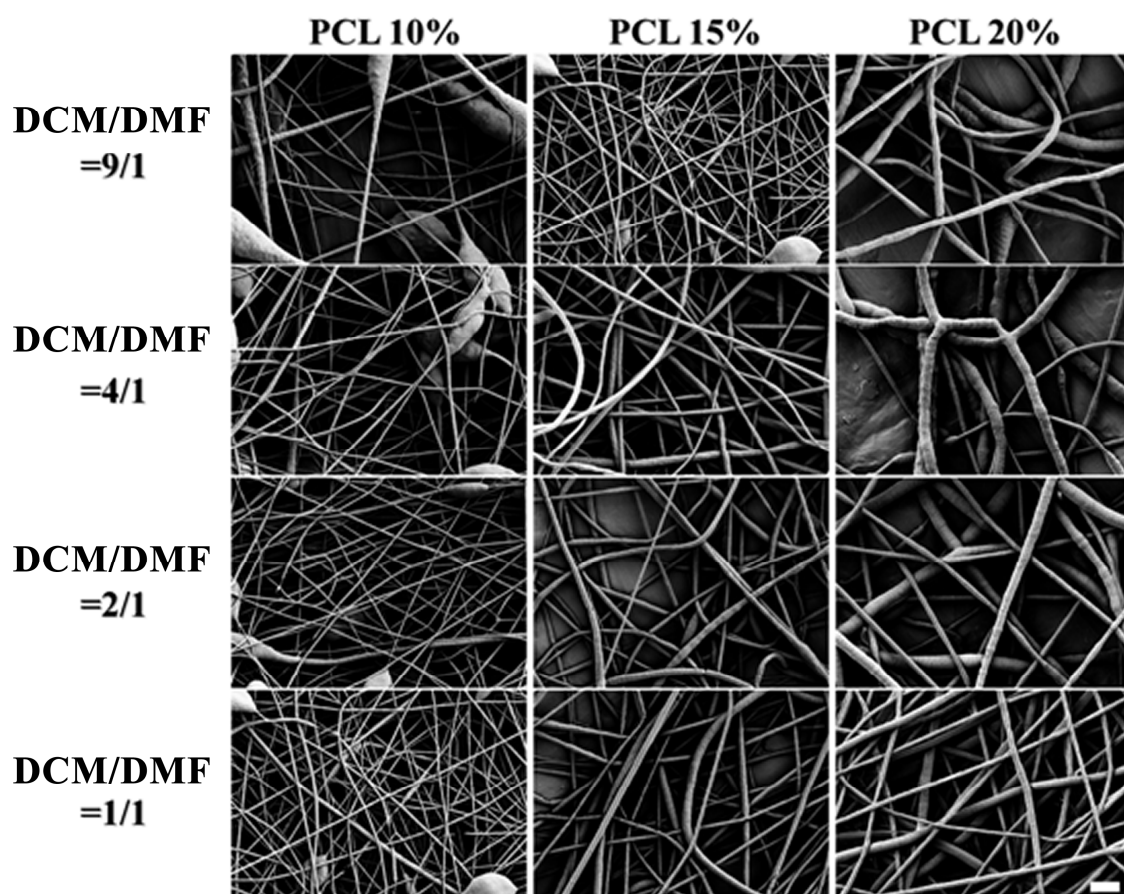


Fig. S7 SEM images of electrospun PCL fibres (solvents: DCM/DMF). Scale bar = 2 μ m.

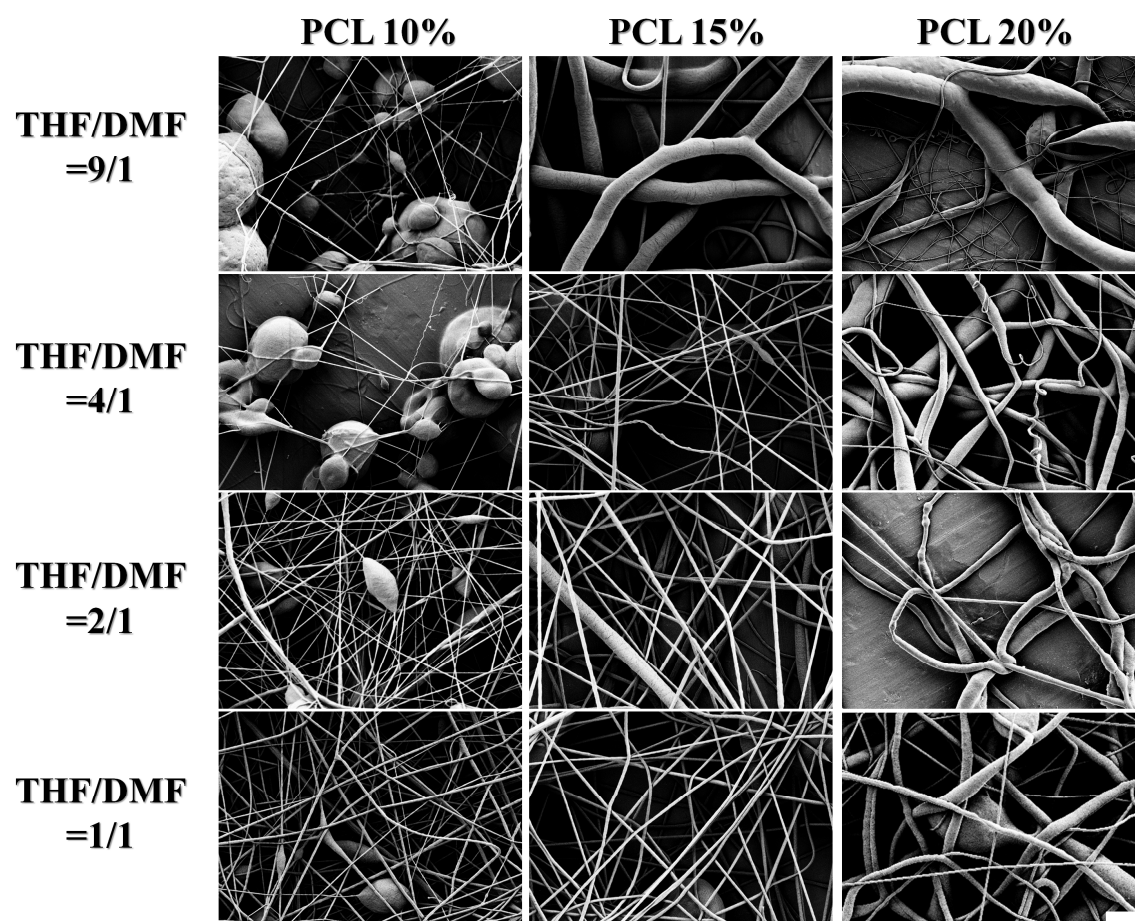


Fig. S8 SEM images of electrospun PCL fibres (Solvents: THF/DMF). Scale bar = 2 μ m.

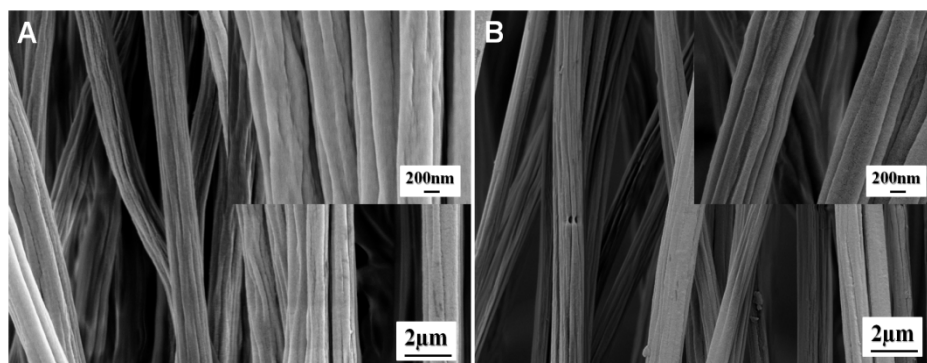


Fig. S9 SEM images of aligned CAB fibres electrospun using a high-speed rotating mandrel as a collector (linear velocity, 26 m/s). A) electrospinning distance 14 cm, and B) electrospinning distance 20 cm. (Electric field intensity of 1 kV/cm).

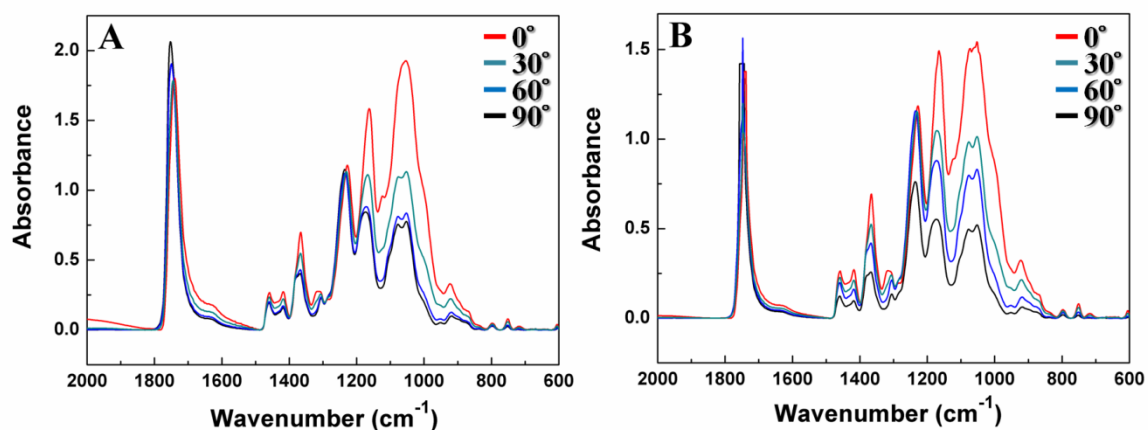


Fig. S10 Polarized FTIR spectra of aligned CAB nanofibres with the applied voltage of, A) 14 kV, and B) 25 kV. (CAB $M_n \approx 70,000$)

Table.S1 Vibrations and dichroic ratios of CAB fibres ($M_n \approx 70,000$)

Wavenumber (cm^{-1})	Vibrations	Dichroic ratio (R)	
		14kV	25kV
1745	$\nu\text{C=O}$ (carboxylate)	0.91	0.97
1367	$\delta\text{sC-H}$	1.73	2.67
1230	$\nu\text{C-O}$ (carboxylate)	1.06	1.60
1163	$\nu\text{asC-O-C}$ (bridge)	1.98	2.81
1070	$\nu\text{C-O-C}$ (pyranose ring)	2.48	3.05
920	νas (pyranose ring) or	3.46	5.25

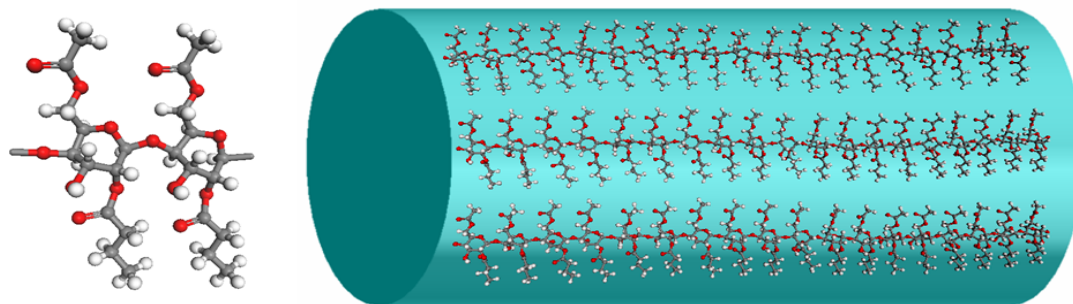


Fig. S11 Schematic diagram showing possible chain conformation in the electrospun CAB nanofibre

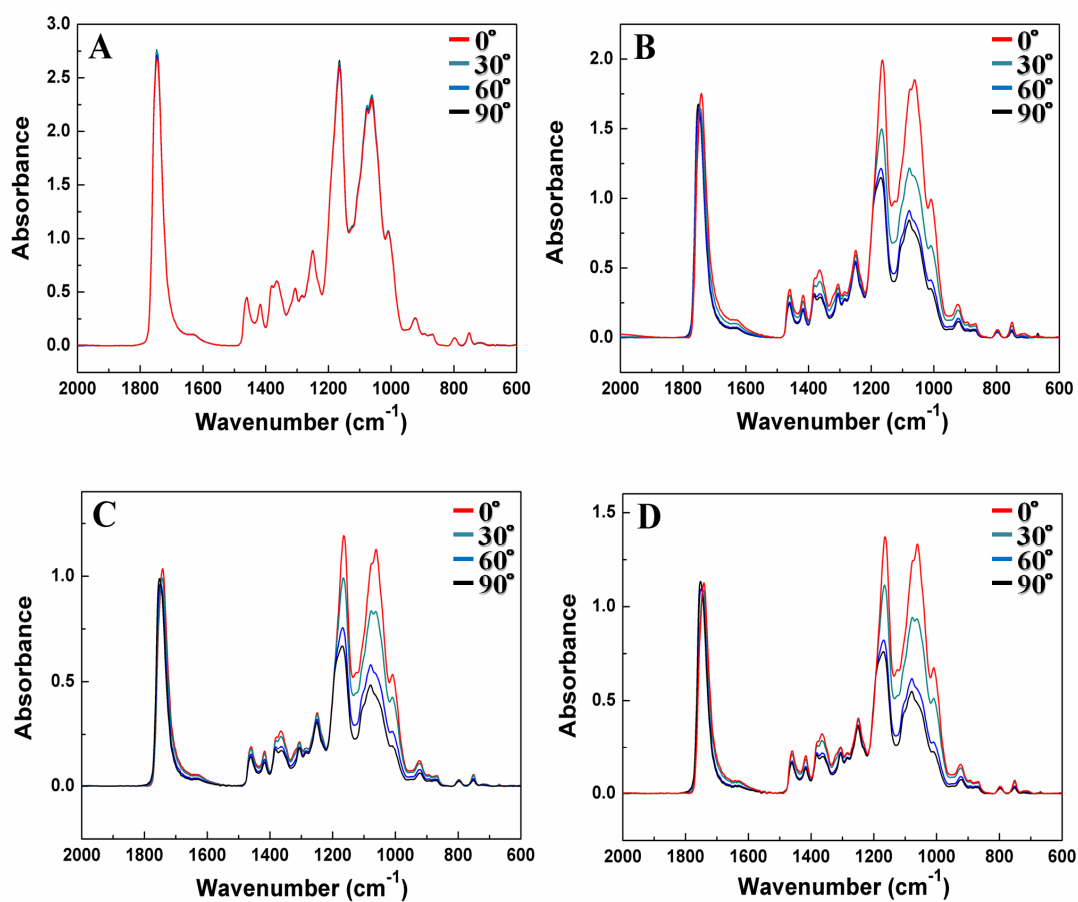


Fig. S12 Polarized FTIR spectra of A) randomly oriented and B~D) aligned CAB nanofibres with the applied voltage of A) 20 kV, B) 14 kV, C) 20 kV, and D) 25kV. (CAB $M_n \approx 30,000$)

Table.S2 Vibrations and dichroic ratios of CAB fibres*

Wavenumber (cm ⁻¹)	Dichroic ratio (R)			
	20 kV	14 kV	20 kV	25 kV
	Random	Oriented	Oriented	Oriented
1745	0.98	1.07	1.07	0.98
1367	0.99	1.70	1.59	1.66
1230	1.00	1.21	1.19	1.15
1163	0.98	1.80	1.85	1.72
1070	0.98	2.27	2.37	2.46
920	0.99	2.02	1.92	2.03

*The fibres were electrospun from a low molecular weight CAB ($M_n \approx 30,000$), which have a smooth surface.

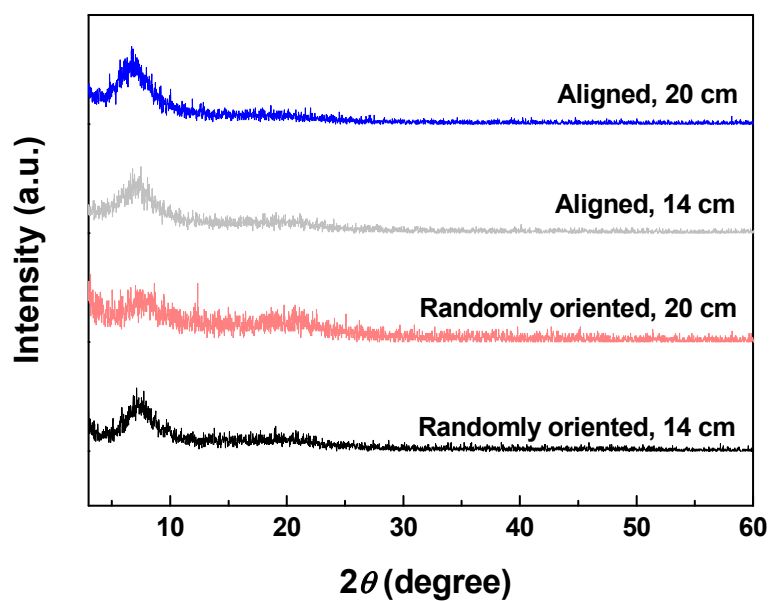


Fig. S13 Wide angle X-ray scattering patterns of CAB nanofibres collected at different electrospinning distances (Electrical field intensity = 1 kV/cm).

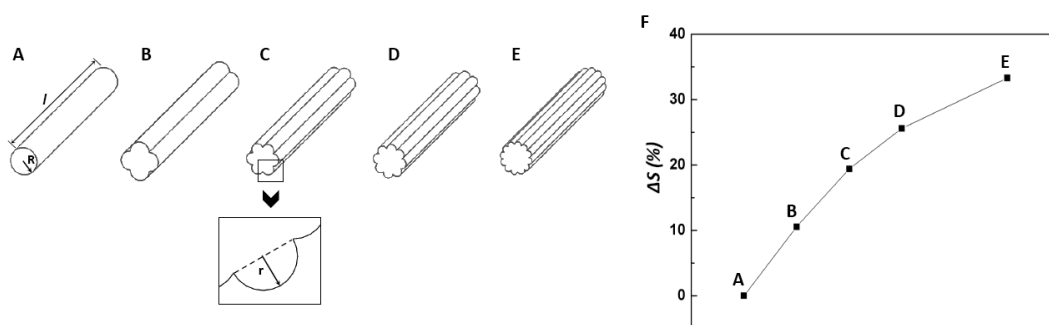


Fig. S14 Illustration of fibres with different number of parallel lines on the fibre surface, A) 0, B) 4, C) 6, D) 8, E) 12. F) The increment of surface area with the number of parallel lines.

To simplify the calculation of surface area, we assume that all fibres have a dense and homogenous structure, and that the parallel lines on the fibre surface have the same semi-circle cross-sectional shape. If the fibres have equal weight and length, the ratio of surface area between the fibre with a parallel line surface and the one with a smooth surface is equal to the ratio of their cross-sectional perimeters (P).

$$\text{Surface area ratio} = \frac{S_{\text{line}}}{S_{\text{smooth}}} = \frac{P_{\text{line}} \times l}{P_{\text{smooth}} \times l} = \frac{P_{\text{line}}}{P_{\text{smooth}}} = \frac{P_{\text{line}}(r)}{2\pi R}$$

Since the weight of the fibres is equal, the fibres should have the same volume.

$$V_{\text{smooth}}(R) = V_{\text{line}}(r)$$

For the equal volume fibres having equal length, their cross-sectional area should be equal.

Therefore,

$$\pi R^2 = \frac{n\pi r^2}{2} + A(r)$$

Where R is the radius of the smooth fibre, and r is the radius of semi-circle cross-sectional shape of parallel lines. $A(r)$ is the area of internal polygon with the equal side length of $2r$. n is side number of the polygon, which is the half number of the parallel lines. $A(r)$ can be easily calculated by standard geometry formula. The increased surface area ΔS can be calculated as:

$$\Delta S = \text{Surface area ratio} - 1$$