Supporting Information

Hetero-epitaxy of Anisotropic Polycaprolactone Films for the Guidance of Smooth Muscle Cell Growth†

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Experimental details

Materials

Polycaprolactone (PCL, M_n 70 000 ~ 90 000 g mol^{-1}) was obtained from Sigma-Aldrich (Shanghai) Trading Company. Chloroform was purchased from Beijing Chemical Works, which was purified by molecular sieve dehydration before use. Other organic reagents, glass plates and glass tubes were bought from Beijing Chemical Works.

Fabrication of composite PCL/PTFE films

The glass plates or glass tubes were first successively cleaned with pure water, hot acetone, hot concentrated sulfuric acid-hydrogen peroxide solution (concentrated sulfuric acid/hydrogen peroxide = 2:1), pure water, and pure ethanol.

To prepare well aligned thin films of highly aligned PTFE, the friction-deposition technique was utilized. Before rubbing, the friction surface of PTFE rod was fully polished by 1200 mesh sand paper. To rub a glass plate, a PTFE rod with the diameter of 20 mm and the length of 5 cm was used. The deposition was performed at a velocity of 10 mm/s, a temperature of 280 °C, and an applied pressure of 0.5 MPa. To rub a glass tube (diameter 8 mm, length 4 cm), the home-made PTFE rod (a quarter of PTFE rod with the length 8 cm) with the same curvature radius of 8 mm as the glass tube was used. The rubbing was performed at a velocity of 10 mm/s, a temperature of 280 °C, and an applied pressure of around 0.5 MPa with the home-made PTFE rod at a tilt angle of 2º. It should be noted that the temperature and pressure could not be controlled as well as on flat surface when rubbing inside the tube. After self-cooling into room temperature, the glass substrates or glass tubes with aligned PTFE were immersed into the PCL solution (about 0.01 g mL^{-1} in chloroform) for 1 minute and then pulled out. After evaporation of solvent, the thin anisotropic PCL films were formed on the oriented PTFE

surface. The composite PCL/PTFE films were then further annealed at 55 °C for 2 hours to improve the epitaxial crystallinity of the PCL.

Cell Culture

The glass slides with composite PCL/PTFE films were cut into rectangular pieces with size of 1cm × 1 cm, and sterilized by exposing to UV light for 1 hour in a clean hood. The glass tubes were cut into 1 cm in length for easy cell culture. The distance between the UV source and the samples was 30 cm. The samples were placed at the bottom of 12-well cell-culture plate followed by being soaked incomplete medium (high Glucose DMEM (Hyclone) supplemented with 10% calf serum (Gibco) and 1% of penicillin and streptomycin sulphate (Solarbio)) for 24 h. Fibroblast-like L929 cells (mouse fibroblast cell line), rabbit vessel smooth muscle cells (SMCs), bone marrow mesenchymal stem cells (BMMSCs) and Chinese hamster ovary cells (CHO), all obtained from Cell Resource Center (IBMS, CAMS/PUMC), were cultured on different substrates in cell-culture plate. Cells were seeded on the samples after the fresh medium being changed, and incubated at 37 °C in the atmosphere of 5% CO_2 overnight. Fresh medium was replenished according to the color indicator in all cultures.

Cell alignment statistical method

The percentage of cell alignment of SMCs cultured on composite PCL/PTFE films was statistically measured. The angle between the long axis of a cell and the direction of the rubbing was defined as the cell orientation angle. The rubbing direction was set as 0^{o}**.** About 300 of SMCs fixed after 3 days' culturing were measured against the orientation angle. The cells were considered to be aligned with the substrate patterns when the cell orientation angle was less than 15^{o}.

Real-time polymerase chain reaction (PCR)

The SMCs were seeded on various substrates (composite PCL/PTFE films as the experiment group, and pure PCL films as the contrast group) and cultured for 2, 5, 7 days respectively. The total RNA

was extracted with Trizol (Invitrogen, USA). Total RNA was converted to cDNA using the PrimeScript® RT reagent kit (TaKaRa, Japan). In order to evaluate the gene expression of the alpha smooth muscle actin (α-SMA) and osteopontin (OPN), the Real-time PCR reactions were performed using SYBR® Green assay (TaKaRa, Japan) on the Fast 7500® Real-time PCR System (ABI, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), as a housekeeping gene, was used as endogenous normalization controls for protein-encoding genes. The primers are listed in Table 1.

Table 1. The primer sequences used in the experiment were as follows. Note: all primers are the same for rabbit vessel SMCs.

Genes	Forward Primer Sequence (5'-3')	Reverse Primer Sequence (5'-3')
α-SMA	TGTACCCTGGCATTGCTGACCG	TGTGGGCTAGAAACAGAGCAGGG
OPN	CCACACGCCGACCAAGGAACAA	AGCTGCCCGAATCAGCGTGT
GAPDH	CTTCAACAGTGCCACCCACTCCTCT	TGAGGTCCACCACCCTGTTGCTGT

Characterizations

Morphology detections

Tapping mode atomic force microscopy (AFM) images were obtained at ambient conditions using a Bruker, Multimode 8 AFM. Si tip with a resonance frequency of approximately 300 kHz, a spring constant of about 40 N m^{-1}, and a scan rate of 0.8 Hz was used. The orientation of the samples was investigated by polarized optical microscope (POM, Olympus BH-2). The morphology of samples after sputter-coated with gold palladium for 60 s were characterized using a JEOL S-4300 field emission scanning electron microscope (FE-SEM) operated at an accelerating voltage of 10 kV.

Fluorescence staining and imaging

Samples were washed twice (10 min per time) with 0.1 M sterilized phosphate buffered saline (pH 7.2, PBS) at room temperature, followed by being fixed in 4% paraformaldehyde for 30 min, and washed three times with PBS. Then, the α-actin of cells was stained with FITC-Phalloidin (Sigma-Aldrich) and incubated for 1 h, and the nucleus was stained with DAPI (Sigma-Aldrich) for 10 min. Samples were thoroughly washed three times with PBS, and the glycerol/PBS mounting medium was added to the samples before inspection with fluorescence microscope (Observer D1, Carl Zeiss). All the microscope settings were the same, in which the exposure time was 245 ms.

Figures

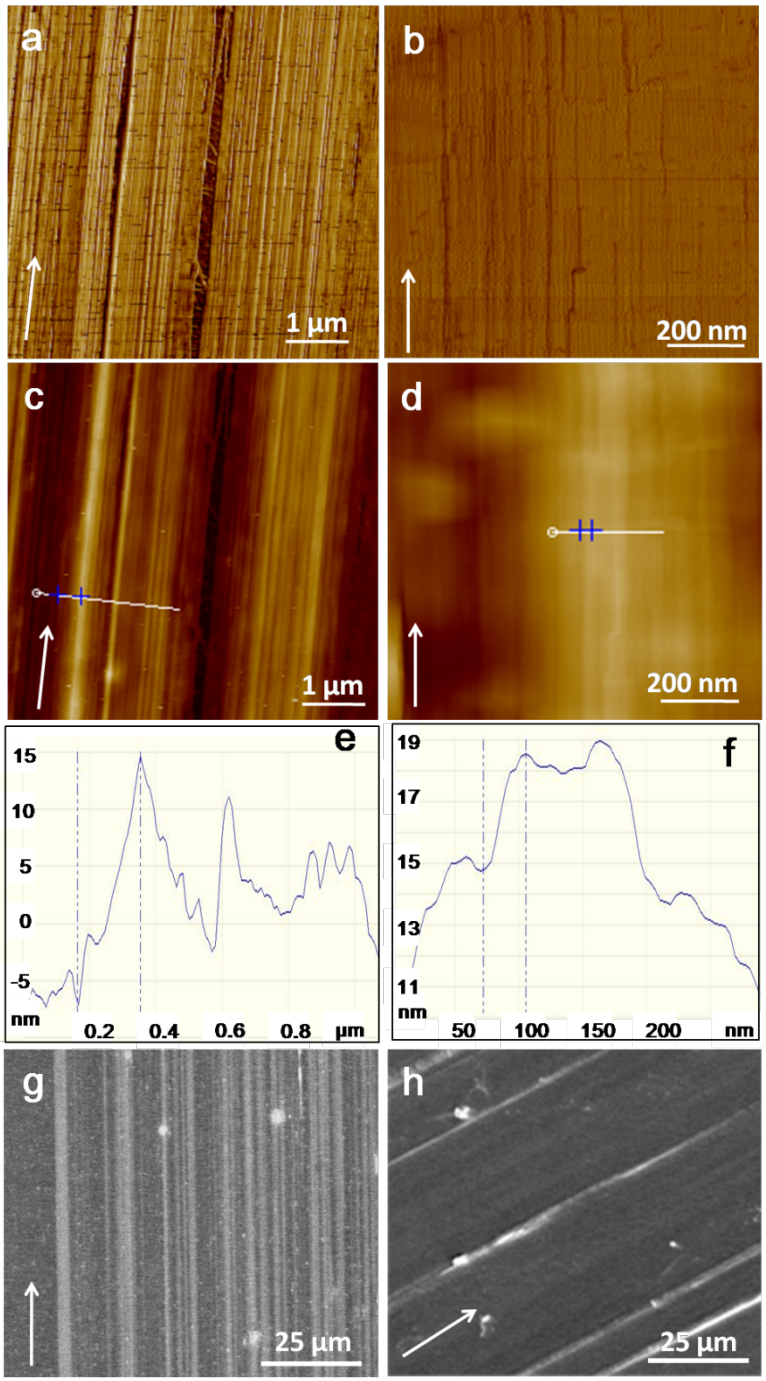


Figure S1. AFM phase (a, b) and height (c, d) images of (left) friction-transferred single PTFE layer and (right) composite PCL/PTFE films on glass substrate, and their corresponding cross-section profiles (e, f), respectively. SEM images of single friction-transferred PTFE layer (g) and composite PCL/PTFE films on glass substrate (h). The arrows indicate the sliding direction of PTFE on glass substrate.

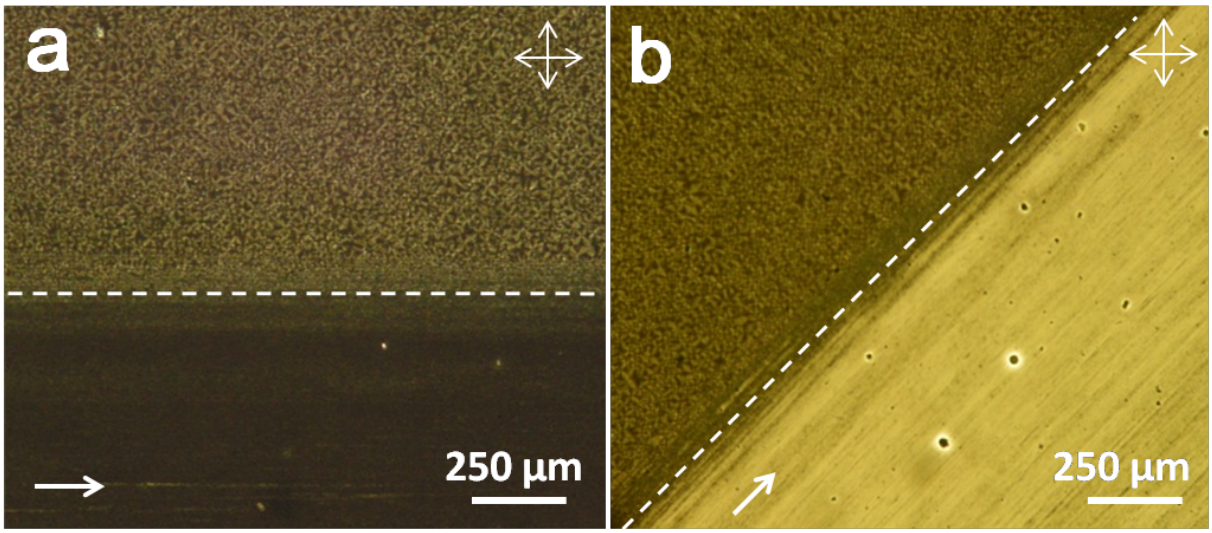


Figure S2. POM images of the sample under crossed polarizers, the anticlockwise ratation angle to the beam axis in which is 0° (a) and 45° (b), respectively. The white dashed line indicates the boundary. Top part is the PCL crystallized on glass substrate (pure PCL films) and bottom part is the PCL crystallized on the PTFE surface (composite PCL/PTFE films). The arrows indicate the rubbing direction.

Large area characterization of the PCL/PTFE film

We selected couple areas to perform large (50 μm× 50 μm) area characterization of the PCL/PTFE film by AFM. As shown a typical AFM height image below (Fig S3), the whole film was very homogenous with surface roughness 8.54 nm and the height difference around 40 nm. These data were added in the revised manuscript. Furthermore, the composite PCL/PTFE film was also checked by the TEM-EDAX, and the selected area electron diffraction pattern (Fig. S4) also indicates that the whole film had the highly homogeneous structure.

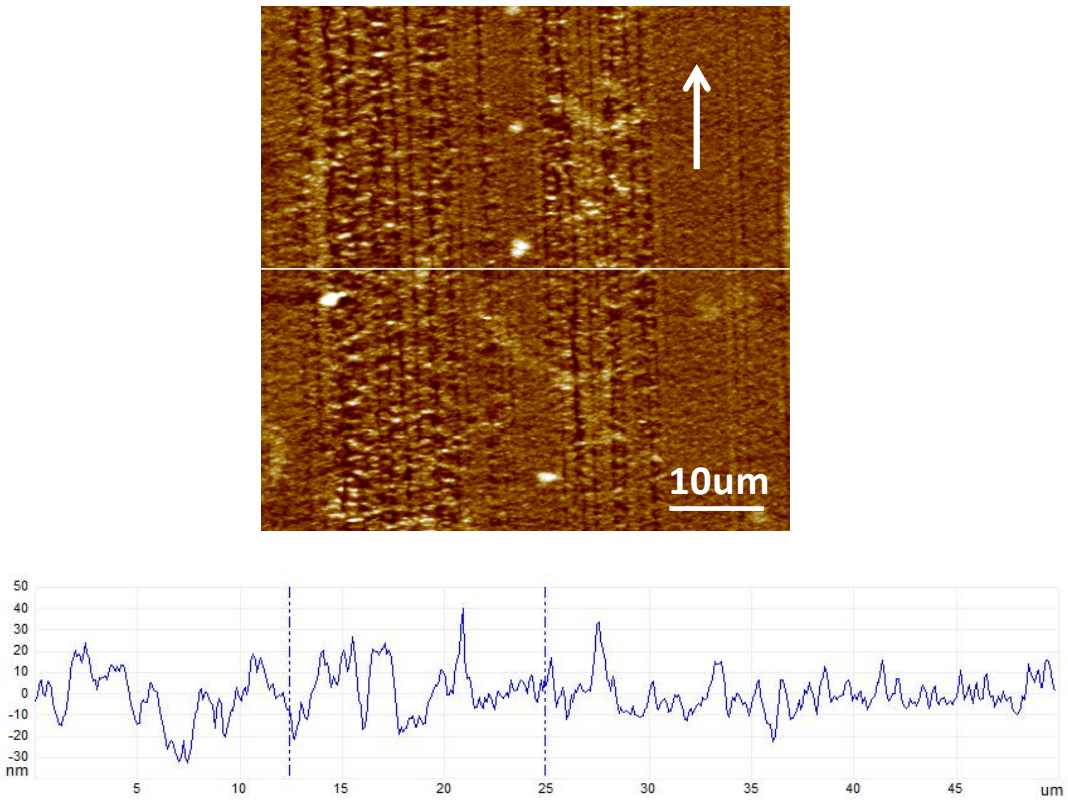


Figure S3. Large scale AFM height image of the composite PCL/PTFE film and the corresponding cross-section profile.

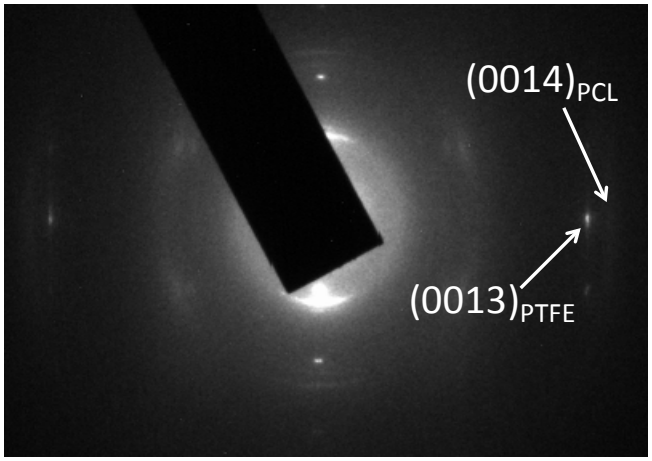


Figure S4. Selected area electron diffraction pattern of the composite PCL/PTFE film.

Isotropic PCL film

The isotropic PCL film on the glass slide with random grown spherulites was employed as the reference. As shown in Fig. S5, sperulites with diameter around 50 μm are randomly grown on the glass slide.

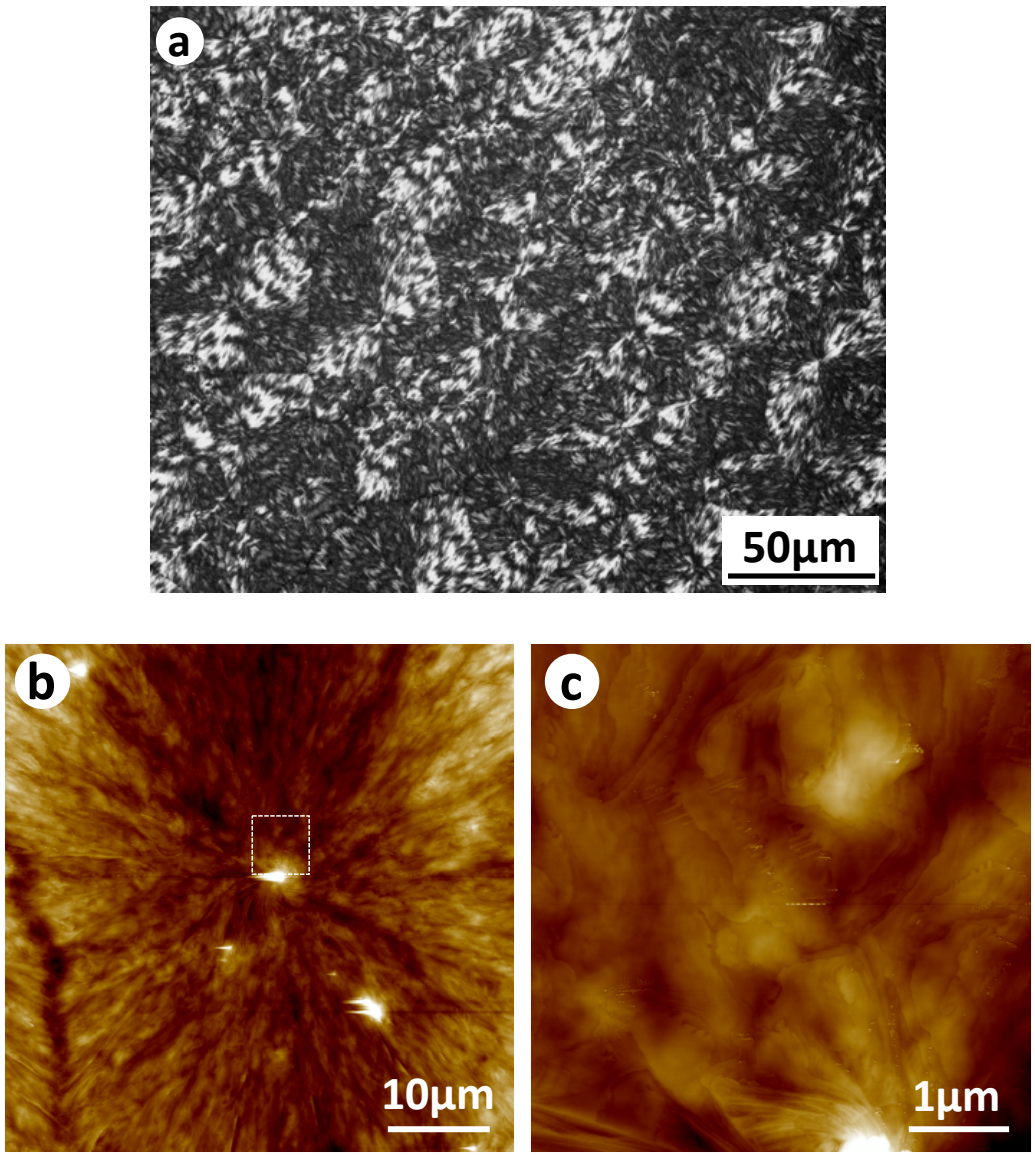


Figure S5. (a) POM micrograph of single PCL film, (b) AFM height image of a spherulite on single PCL film and (b) the zoom-in AFM height image as marked in part (a).

Stability test of the film

The stability of the composite films was tested by treating samples under ultrasonication in PBS for 5 min and incubation in PBS for 2 weeks. As shown in Figure S6b and c, after ultrasonication and incubation, the surface of thin film still maintained the similar morphology as untreated sample (Figure S6a). Furthermore, their corresponding cross-section profiles (Figure S6d, S6e and S6f) clearly show only slight difference of height of treated samples with untreated samples. The surface roughness for these samples is very similar, which is 8.54 nm for untreated sample, 10.6 nm for ultrasonication sample, 10.0 nm for incubation sample, respectively. These results illustrate that the composite PCL/PTFE films were very stable in PBS for at least 2 weeks.

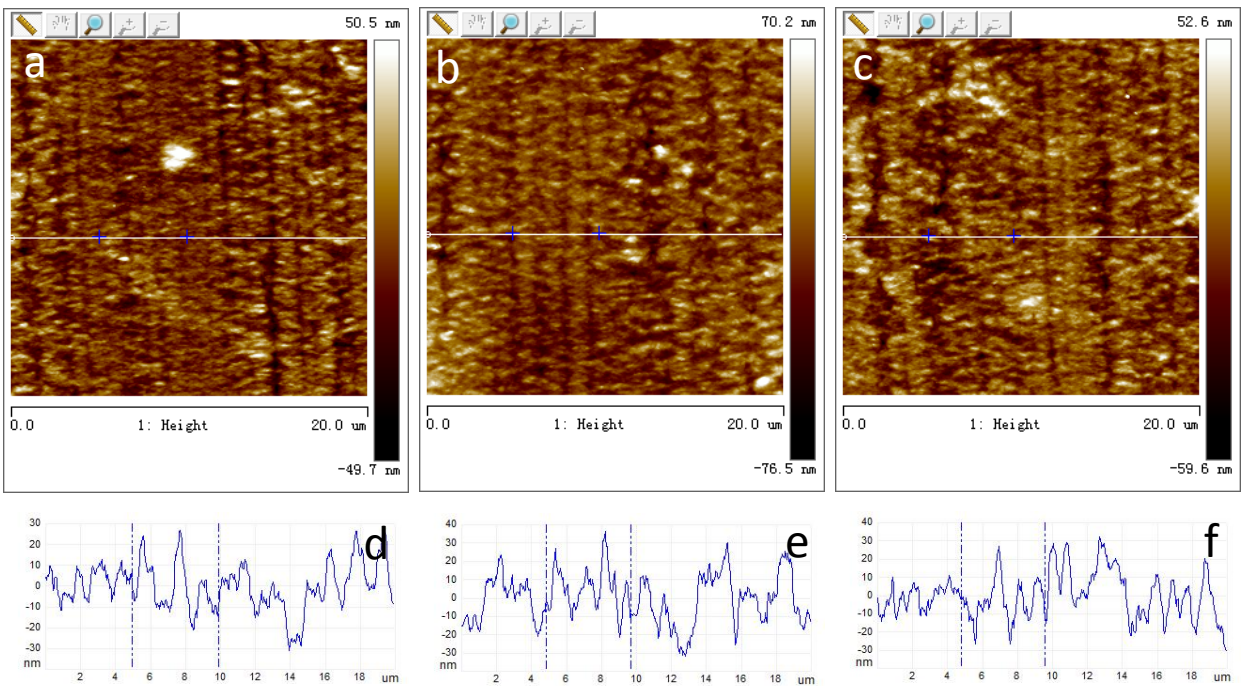
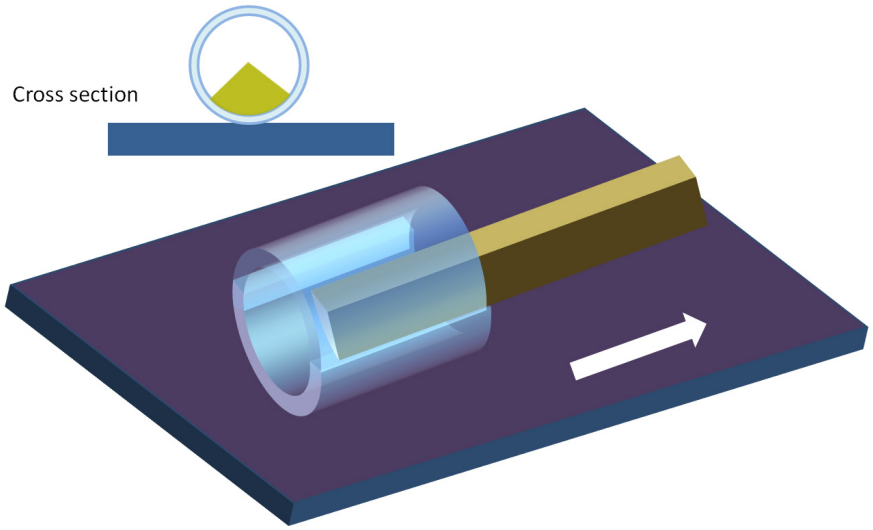


Figure S6. AFM height images of (a) untreated PCL/PTFE thin film, (b) after ultrasonication in PBS for 5 min and (c) after incubation in PBS for 2 weeks. (d), (e) and (f) are corresponding cross section profiles of (a), (b) and (c).

Rubbing the glass tube

As shown in the Scheme S1, to rub a glass tube, the home-made PTFE rod with the same curvature radius of 8 mm as the glass tube was used. The rubbing was performed manually at a velocity of 10 mm/s, a temperature of 280 °C, and an applied pressure of around 0.5 MPa with the home-made PTFE rod at a tilt angle of 2^{o}. It should be noted that the temperature and pressure could not be controlled as well as on flat surface when rubbing inside the tube.



Scheme S1. Schematic illustration of rubbing the glass tube by a PTFE rod.

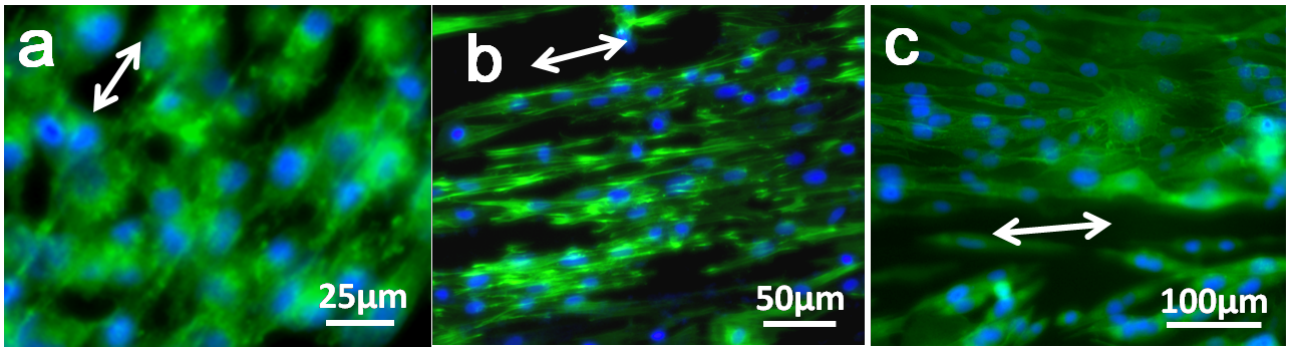


Figure S7. The FM merged images of various cells on composite PCL/PTFE films. (a) L929 cells were cultured for 3 days. (b) BMMSCs were cultured for 7 days. (c) CHO cells were cultured on 5 curved composite PCL/PTFE films for 7 days. Arrows indicate the PTFE rubbing direction.