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Supporting Information

Silk Fibroin Tissue Engineering Scaffolds with Aligned Electrospun Fibers in Multiple Layers

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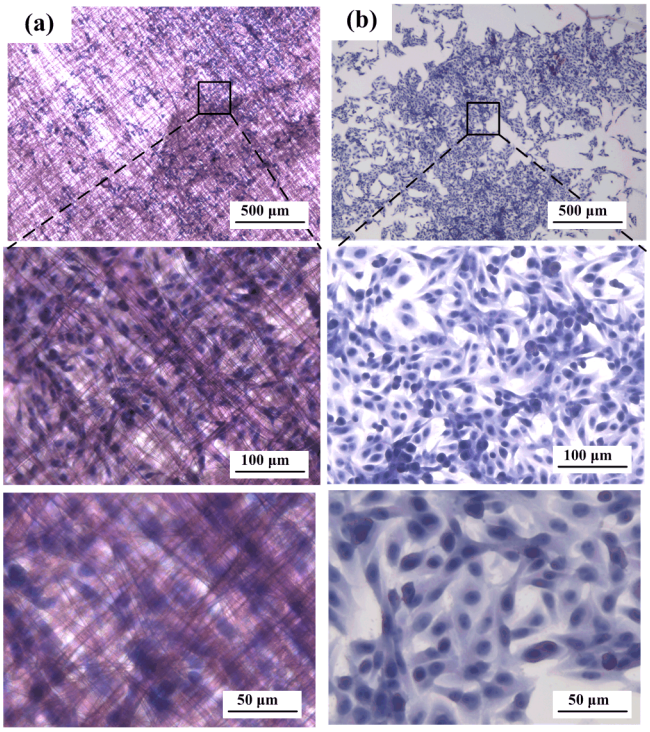
Hematoxylin and Eosin (H&E) Staining and MTT Analysis

PIECs were cultured in cell culture flasks with culture medium. The media were replaced every two days, and the culture were maintained in a humidified incubator at 37 °C with 5% CO_2. Electrospun mats were prepared on circular glass cover slips (14 mm in diameter) and fixed with steel rings in the 24-well cell culture plates. The mats were sterilized by 75% ethanol for 2 d and then washed with phosphate-buffered saline solution (PBS) for three times and immersed with culture media for 30 min, and lastly washed with the media. Cells were trypsinized when their density reached 80-90% of the cell culture flask and counted with a hemocytometer.

The HE staining was used to investigate cell attachment and proliferation. After three days incubation, the PIECs were fixed in 4% paraformaldehyde for 10 min, and then washed twice with distilled water for 2 min. Before washing with running water for 10 min, the PIECs were immersed in hematoxylin for 10 min to stain necleus. The PIECs were then dehydrated in 95% alcohol, and the cytoplasm were stained by eosin for 5 min. Finally, dehydrate the cells twice in 70% alcohol prior to dry. The stained PIECs were observed and photographed using Olympus phase contrast microscope (Model IX71, Japan).

To evaluate cell adhesion and proliferation, PIECs were seeded onto cover slips and electrospun mats (n = 4), respectively, at a density of 10×10^5 mL^{-1} for 1 h, 2h, 4 h, 8 h, 1 d, 3 d, 5 d , and 7 d for methylthiazol tetrazolium (MTT) assay. At the appointed time the culture media were removed and the samples were washed three times with PBS to remove the residual culture media. Each sample was added with 360 μL serum-free DMEM medium and 40 μL MTT solution (5 mg mL^{-1} MTT stock solution in PBS), and incubated at 37 °C for 4 h to gain formazan. Thereafter, the culture media were extracted and 400 μL dimethylsulfoxide (DMSO) was added to dissolve

formazan crystals. Then 100 μL formazan solution of each sample was added into a 96-well plate and tested by an Enzyme-labeled Instrument (MK3, Thermo, U.S.) at a wavelength of 492 nm.



Supplementary Figure 1| **HE staining images of PIECs**: cultured on (a) multilayered grill-like SF mats and (b) cover slip.