Supplementary Information

Electrospun poly (ε-caprolactone) scaffold modified with matrix metalloproteinase for cellularization and vascularization

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1. Experiment section

Characterization of scaffold surface

For preparation of transmission electron microscope (TEM) samples, electrospun PCL fibers were directly deposited onto copper grids (HCF300-Cu, Hengxinkeyi, China). The self-assembled protein layer and collagenase on the surface of PCL were examined by TEM (HT7700 Exalens, HITACHI, Japan) at 100 kV.

The collagenase function test

Smooth muscle cells (SMCs) were cultured in DMEM/F-12 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL

streptomycin at 37°C in a 5% CO_2 incubator. SMC suspension in medium was seeded into the sample wells (48-well TCPS, Corning) at a concentration of 1×10^4 cells/well. After incubation for 24 h, 100 μL of collagenase solution (1 mg/mL in serum free medium, sterilized by a 0.2 μm sterile filter) was added into each well and images were taken under a microscope (CKX41, Olympus, Japan) at 3 time points (0 h, 1 h, 2 h, $n = 4$).

Immunofluorescence staining of the macrophage

To perform immunofluorescence staining, the membranes were dehydrated in 30% sucrose, embedded in OCT and cross sectioned on a freezing microtome ($n = 4$). Then the sections were fixed in cold acetone for 10 min, dried in air, rinsed twice in 0.01 M PBS. After incubation with 5% normal goat serum for 30 min at room temperature, the sections were incubated with the primary antibody (CD68, 1 : 150, Abcam) overnight at 4°C. After rinsed five times in 0.01 M PBS, the sections were stained with the secondary antibody (Alexa Fluor 594 goat anti-mouse IgG, 1 : 200, Invitrogen) for 2 h at room temperature. The nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI)-containing mounting solution (DAPI Fluoromount G, Southern Biotech, UK). Images were recorded by a fluorescence microscope (Zeiss Imager M2, Germany).

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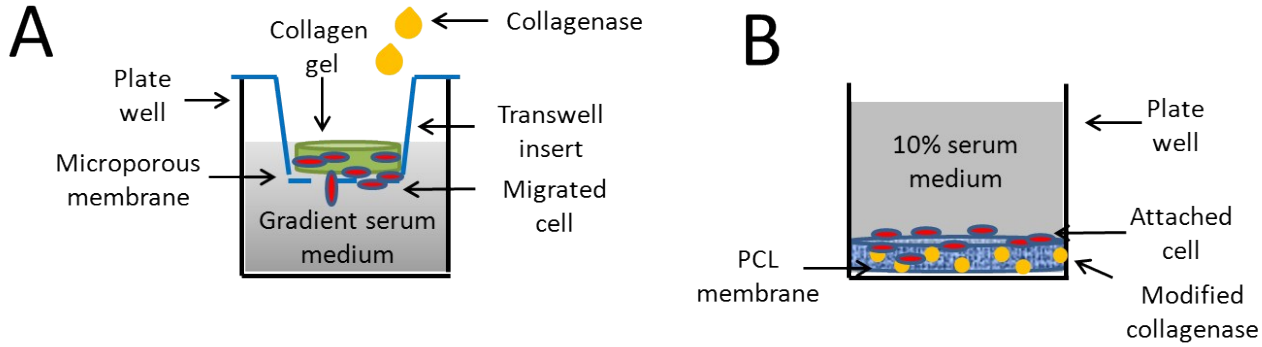

Fig. S1 The scheme of experimental setups for (A) Cell invasion and (B) Cell infiltration.

(A) We first prepared the collagen solution into gel in the Transwell upper compartment, then placed the 10% serum medium in the lower compartment and seeded cells in the upper compartment. Then collagenase solution was added into the upper compartment to degrade the gel. After 48 h of incubation, the migrated cells were stained and counted under a microscope.

(B) The membranes were put into 48 well plates. Then we seeded DiI-labeled cells onto the membranes. After incubation for 24 h, the membranes were dehydrated and cross sectioned on a freezing microtome. The cells were counted under a fluorescence microscope.

2. Experimental results

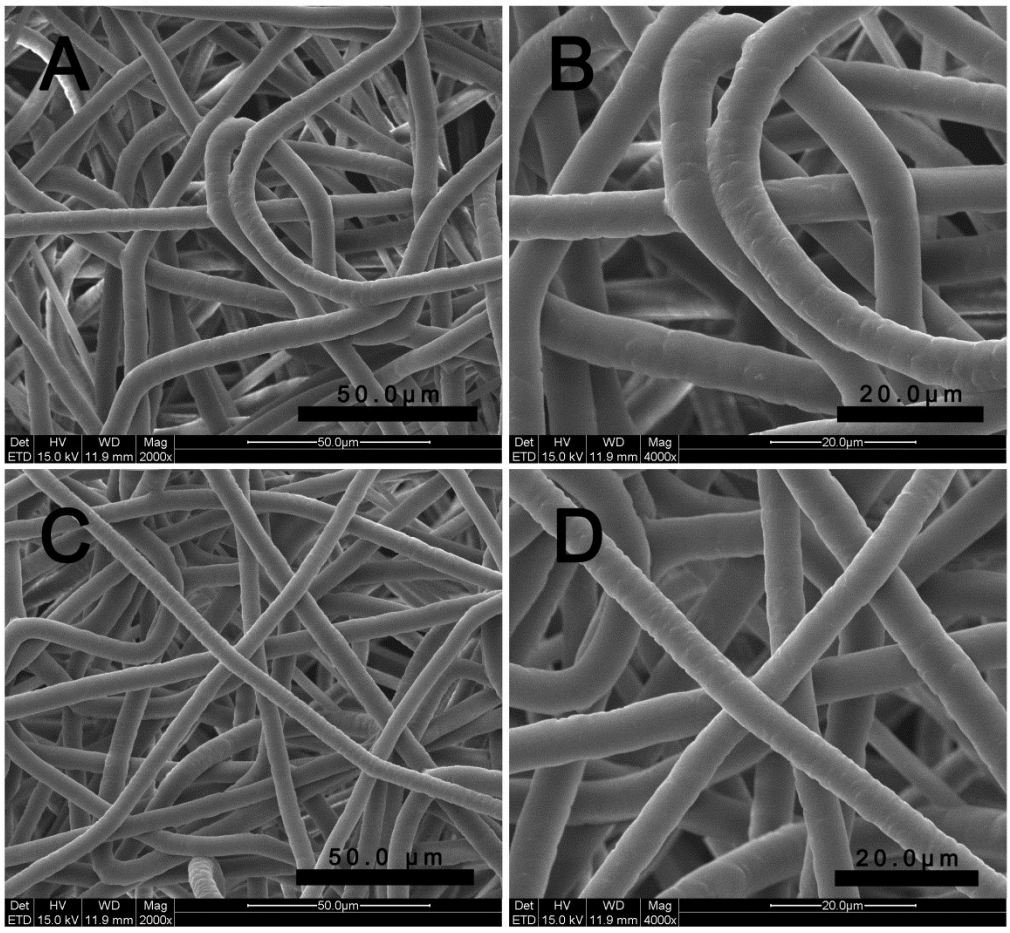

Fig. S2 SEM images of PCL-H (A and B) and PCL-C (C and D) membranes.

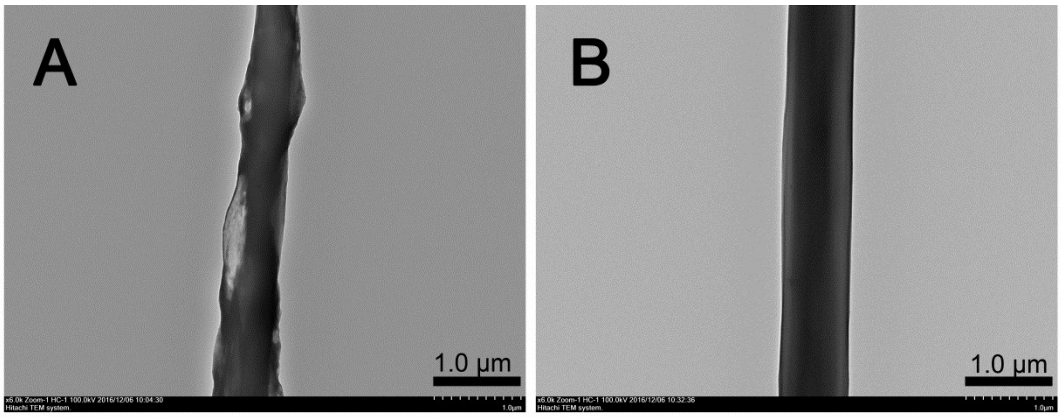

Fig. S3 TEM images of PCL-H (A) and PCL (B) membranes.

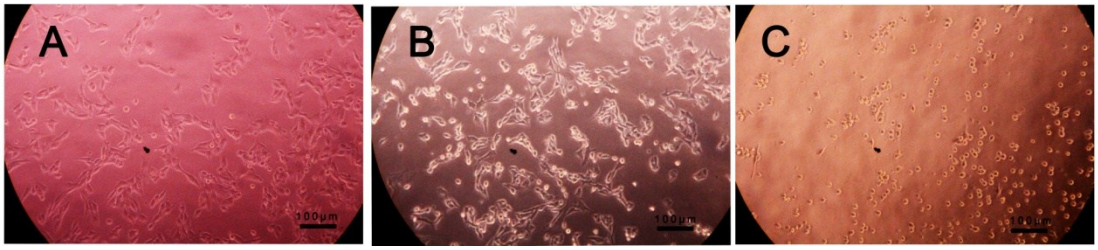

Fig. S4 Enzymolysis on SMC's connection of collagenase. Collagenase treatment after 0 h (A), 1 h (B), and 2 h (C).

The images showed that after 1 h of incubation with collagenase, a part of SMCs were separated (Fig. S4 B), while after 2 h of incubation most cells were separated and showed a round morphology (Fig. S4 C), indicating that the collagenase had the effect of enzymolysis on cell connection with adjacent cells and the well.

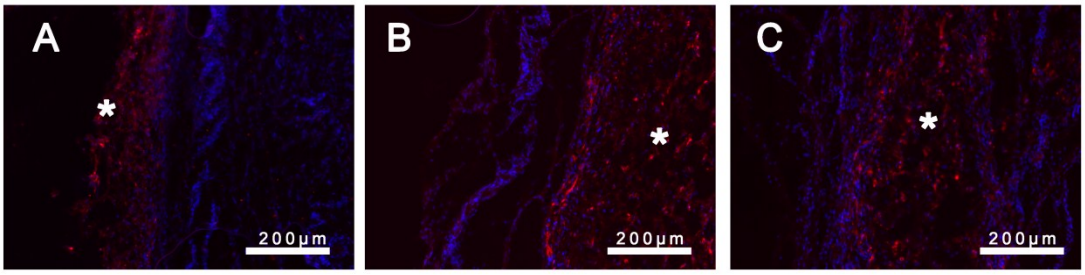

Fig. S5 Immunofluorescence staining of macrophages in acute inflammation test. (A) PCL, (B) PCL-C and (C) PCL-H-C.(Red: CD68; blue: nuclei).* shows the inner part of membranes.

Images of immunofluorescence staining showed that macrophages concentrated at

the material tissue interface in the control (Fig. S5 A), while more macrophages distributed in the inner of modified grafts (Fig. S5 B and C).