

Supporting Information

Polycaprolactone Nanofibers Functionalized with Placental Derived Extracellular Matrix for Stimulating Wound Healing Activity

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2. Materials and Methods

2.1. Extraction and solubilization of the placenta-derived extracellular matrix (sPEM)

The collected human placentas were rinsed with phosphate-buffered saline (PBS) until it is free of blood. The blood-free placenta was then subjected to tissue homogenization. The minced placenta was decellularized using 0.5% sodium dodecyl sulfate (SDS; Sigma-Aldrich, USA) with 0.05% trypsin/ethylenediaminetetraacetic acid (EDTA; Gibco, USA), 200 mg/mL RNase (Sigma-Aldrich, USA), 0.2% DNase (2000 U; Sigma-Aldrich, USA), 1% Penicillin-Streptomycin (5,000 U/mL, Gibco, USA), and 1 mM phenyl methyl sulfonyl fluoride (Sigma-Aldrich, USA) in a sealed rotating vessel. The entire procedure was conducted in a sterile environment, and the solution was changed every 24 h to prevent tissue degradation and contamination. The decellularized placentas were washed with PBS and stored at $-80\text{ }^{\circ}\text{C}$ for further processing.

For Solubilization of ECM, the decellularized human placenta was pulverized in the presence of liquid nitrogen. The resulting powder was immersed and stirred in a 4 M urea buffer (240 g urea, 9 g NaCl, and 6 g Tris base in 1 L distilled water) containing a protease inhibitor cocktail for 24 h. The samples were further subjected to homogenization. The supernatant was collected by centrifuging the samples and subsequently dialyzed using an 8000 MWCO dialysis tubing against Tris-buffered saline (TBS; 6 g tris base and 9 g NaCl in 1 L distilled water and 2.5 ml of chloroform for sterilization) to remove urea. The contents of the dialysis tubes were centrifuged again to remove protein aggregates, and then the supernatant was frozen at $-80\text{ }^{\circ}\text{C}$, freeze-dried for 42 h, to obtain sECM.

2.2. Hemolytic Testing

Direct contact method was performed to determine the hemocompatibility of the fabricated PCL and PCL-sPEM nanofibers mats as described elsewhere¹. Briefly, human blood was collected from volunteer and diluted with an anticoagulant. The diluted anticoagulated blood was centrifuged at 1800 rpm for 15 min, and the RBC pellet was collected which was subsequently dissolved in 19 folds with saline. Nanofiber discs (5 mm diameter) were directly immersed in the diluted blood, incubated for 1 h at 37 °C and subsequently centrifuged at 1000 rpm for 10 min. The resulting supernatant absorbance was measured at 540 nm using a UV spectrophotometer. In this assay, 0.1% Triton-X was used as positive control and saline was used as negative control. The following equation below calculated the hemolysis degree (HD):

$$\text{HD (\%)} = [(D_n - D_0)/(D_1 - D_0)] \times 100\%$$

Where, D_n , D_0 , and D_1 are the absorbance of the sample, the negative control, and the positive control, respectively.

2.3. In Vivo Toxicity Analysis

The in vivo cellular response of PCL-sPEM nanofibers mats was studied by subcutaneous implantation in albino Wistar rats (200 ± 10 g; male; $n = 3$). Hydrated PCL-sPEM nanofibers mats (2 cm X 2 cm X 0.2 mm) was implanted on the dorsal side of rats after dissection and sutured using chromic catgut under aseptic condition. After five days of implantation, the rats were euthanized, and the site of implantation along with the adjacent tissue was retrieved for further examination. The retrieved samples were fixed, dehydrated, and subsequently embedded in paraffin blocks. The paraffin blocks containing the sample were processed and subsequently

stained with H&E, MT, TB (toluidine blue; Sigma- Aldrich, USA), and anti-MPO (anti-myeloperoxidase) antibody (Abcam, USA) according to the manufacturer's protocol.

3. Results

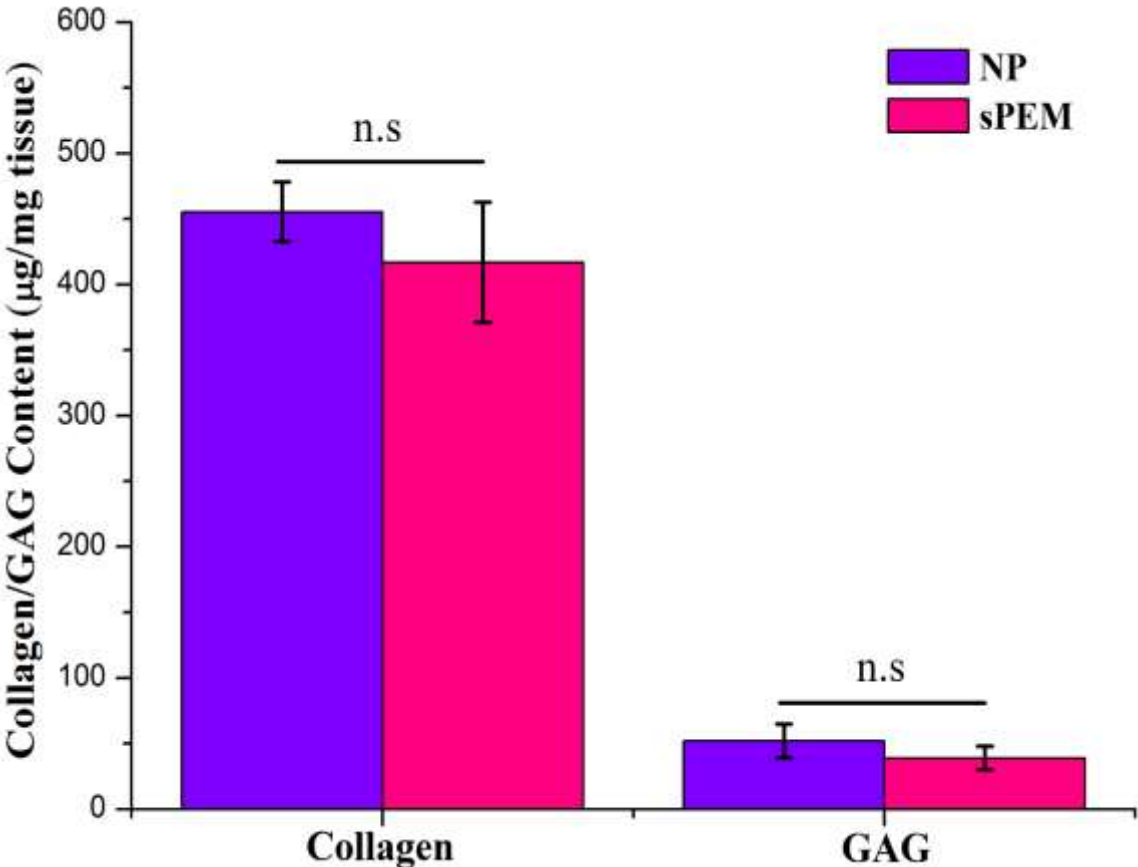


Fig. S1: Collagen/GAG Quantification of NP and sPEM

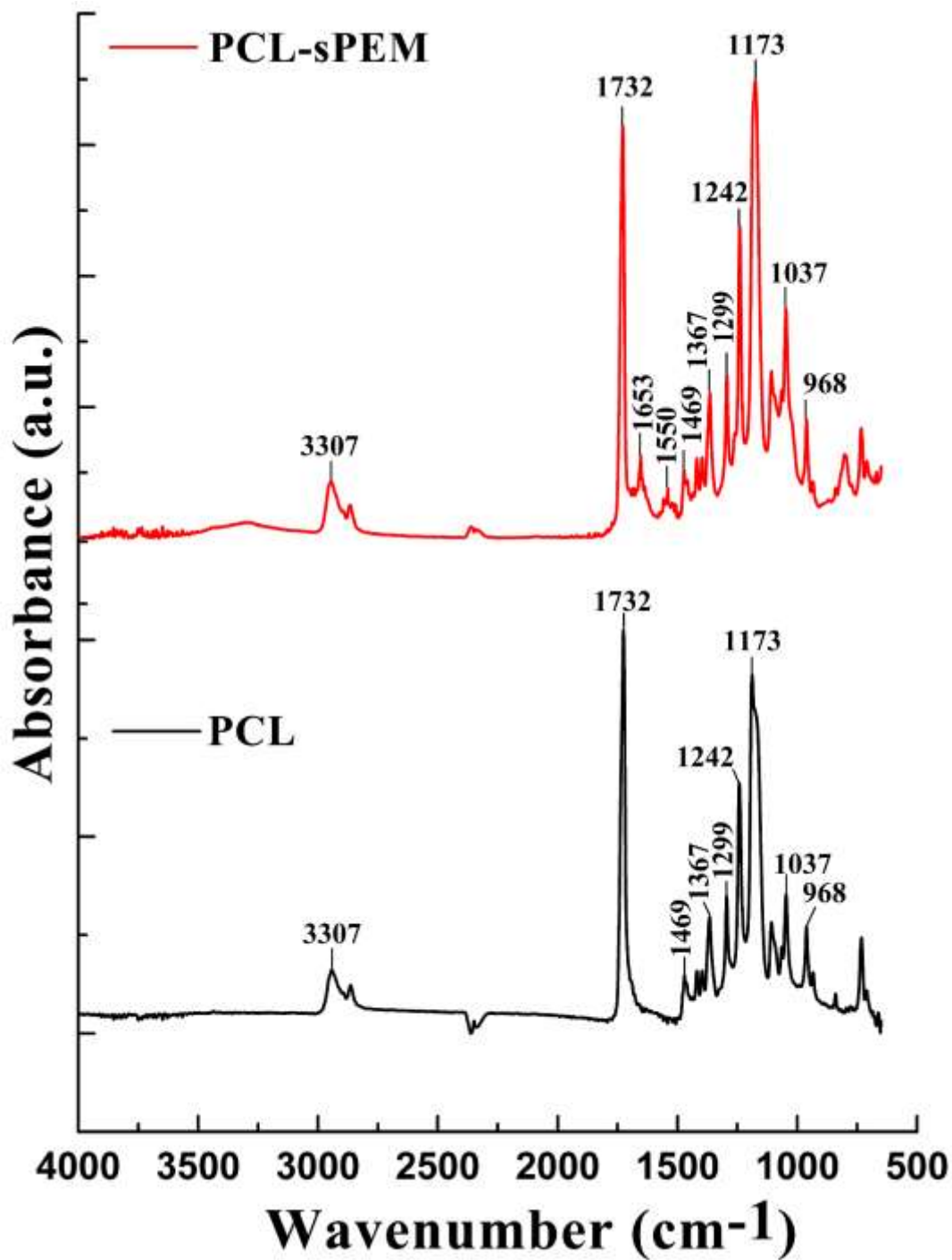


Fig. S2: FTIR Analysis of PCL and PCL-sPEM Nanofiber Mats

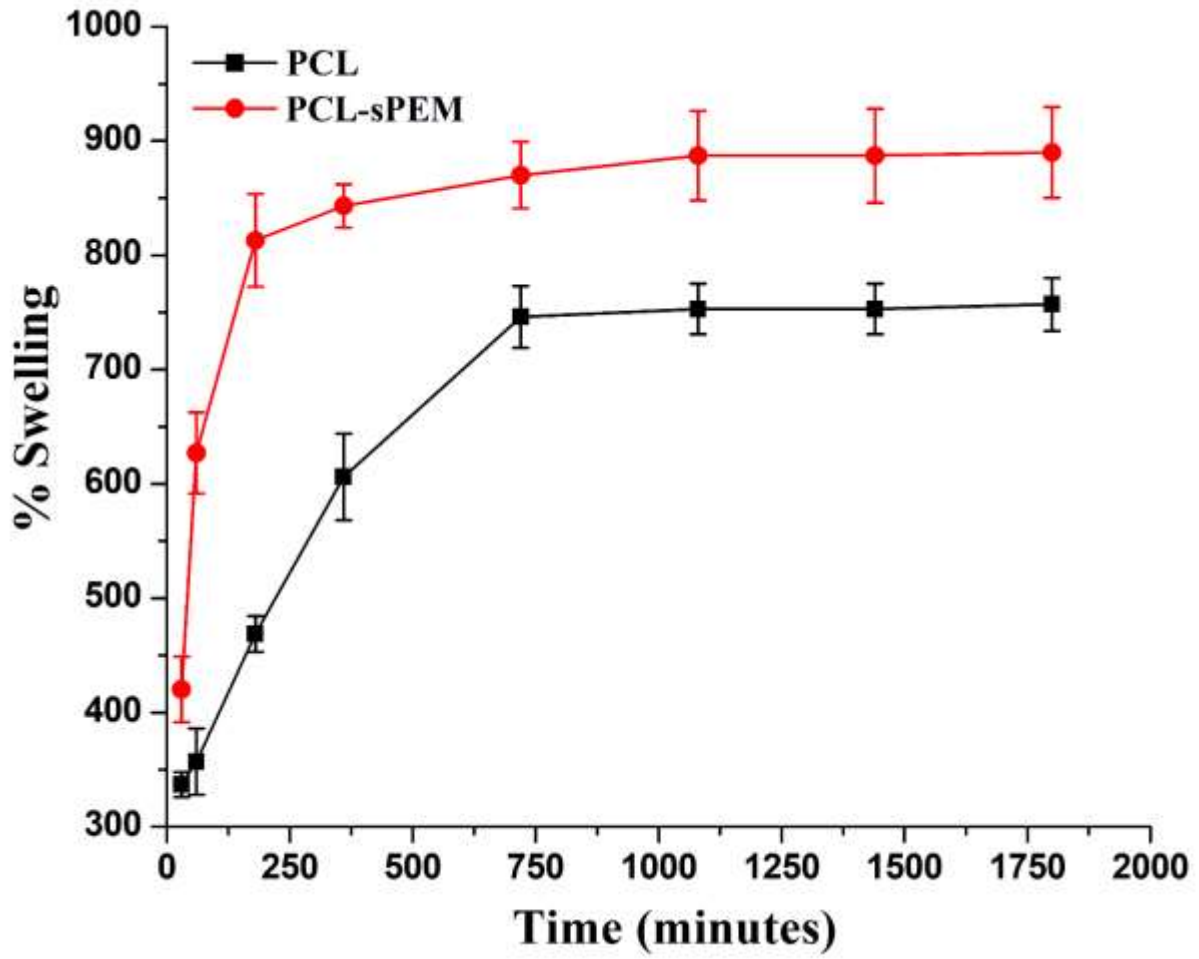


Fig. S3: Swelling Kinetics of PCL and PCL-sPEM at 37 °C in PBS

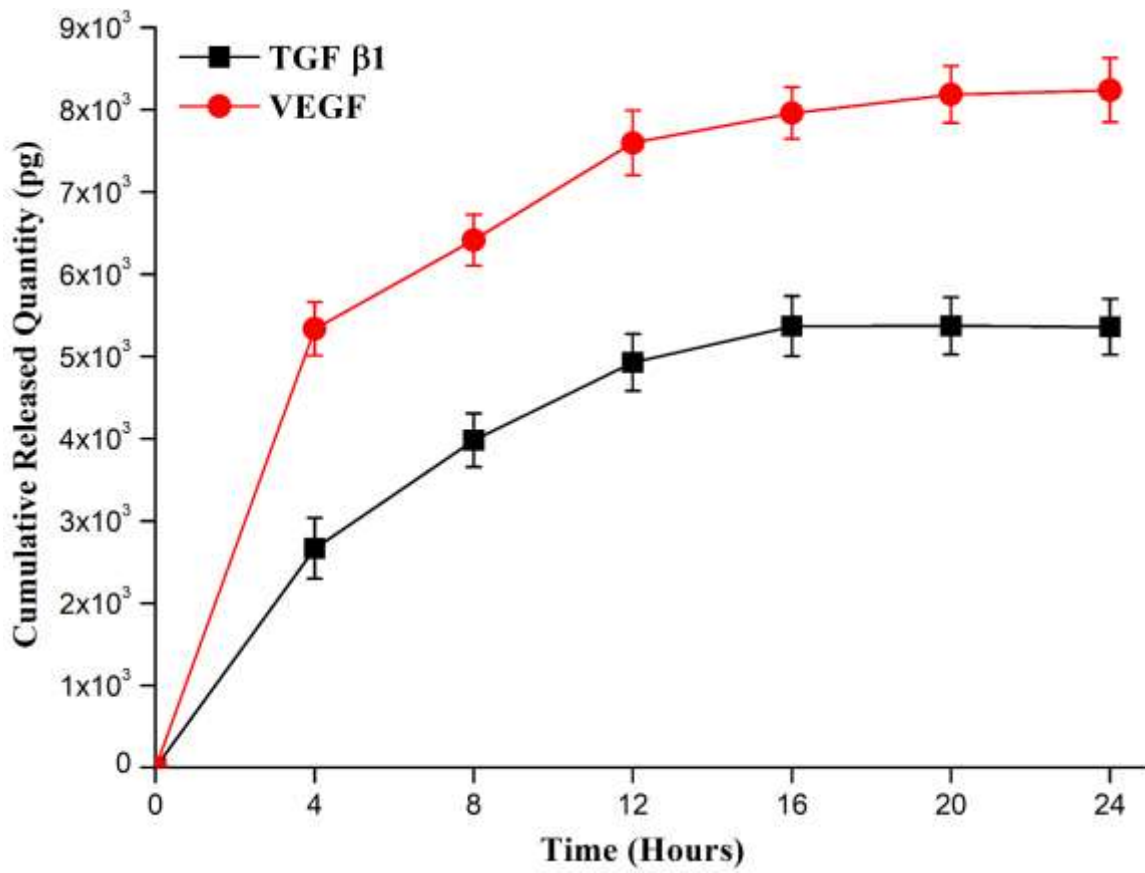


Fig. S4: Cumulative Release Kinetics of Growth Factors from PCL-sPEM Nanofiber Mats

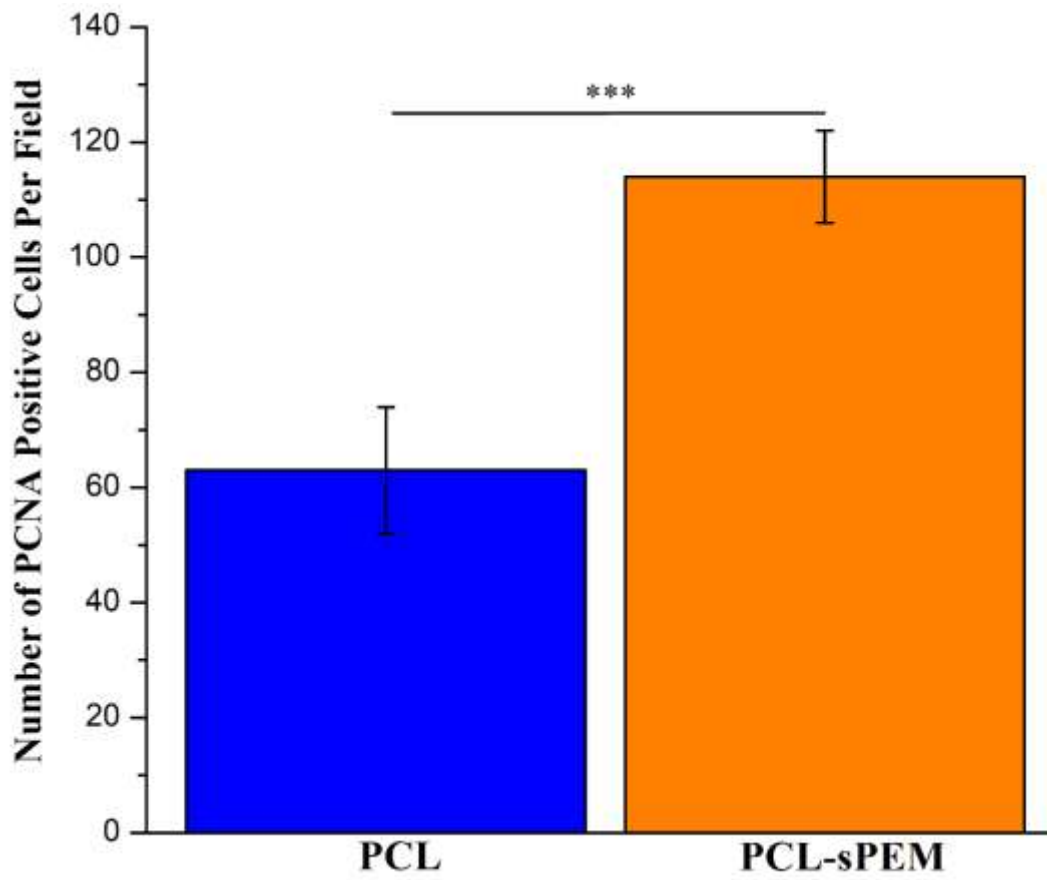


Fig. S5: Quantification of PCNA Positive Cells in PCL and PCL-sPEM

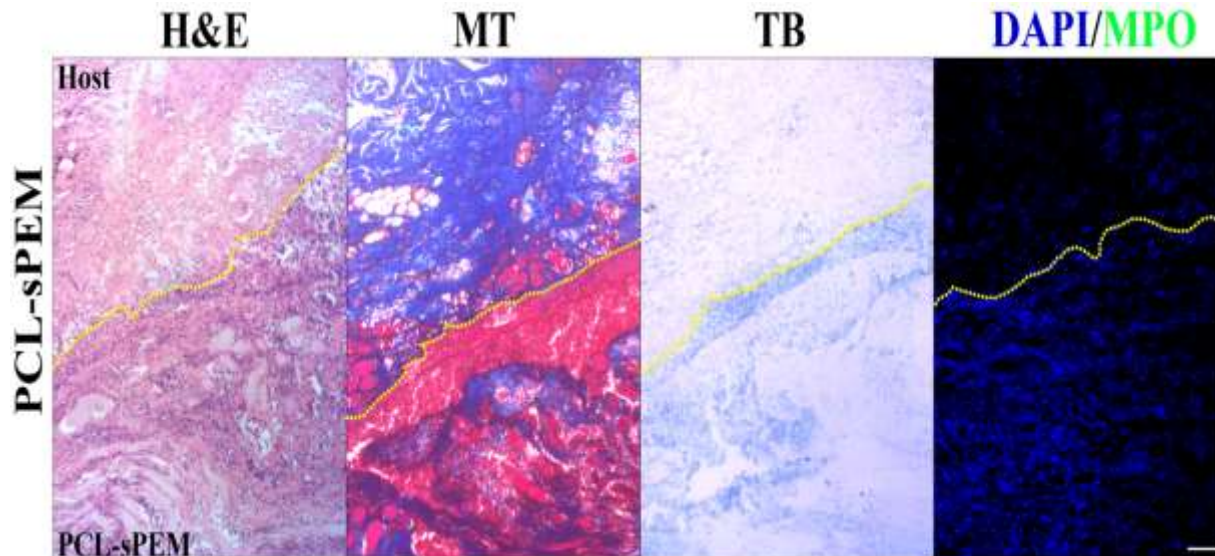


Fig. S6: H&E, MT, TB, and anti-MPO of the explanted PCL-sPEM nanofiber mats on day five after subcutaneous implantation (scale bar represents 50 μm ; blue represents nucleus staining DAPI, and green depicts antibody expression).

The animals with subcutaneous implantation of PCL-sPEM nanofiber mats remained healthy, and no mortality was observed. After five days of implantation, PCL-sPEM nanofiber mat was retrieved with surrounding tissue and histologically stained as shown in Fig. S6. H&E staining revealed tissue integration/in-growth in the implanted PCL-sPEM nanofiber mats. Interestingly, anti-myeloperoxidase (MPO) staining revealed the absence of neutrophils; indicating the absence of immune reaction. From TB staining, few mast cells were visualized in the host region, but no mast cells were found infiltrated into PCL-sPEM nanofiber mats implanted region. From MT staining, no fibrosis or capsular layer formation was detected. These results suggest that PCL-sPEM nanofiber mats did not trigger adverse host immune inflammatory responses in vivo.

Table S1 Gene-Specific Primers for Reverse Transcriptase-PCR (RT-PCR)

Name of Gene	Sequence	Fragment Size (bp)	T_m (°C)
GAPDH	F-CCATGGAGAAGGCTGGGG R-CAAAGTTGTCATGGATGACC	195	54
COL I	F-GCGCCAGAAGAAGTGGTACATCAGCAA R-AAGCGTTTGCCTAGTAATTGCA	100	60
COL III	F-CTGAAATICTGCCATCCTGAAC R-GGATIGCCGTAGCTAAACTGAA	236	58.4
KRT 10	F-CATGAGTGTCCCCCGGTATC R-CAGTATCAGCCGCTTTCAGA	79	59

Reference:

1. P. Punnakitikashem, D. Truong, J. U. Menon, K. T. Nguyen, Y. Hong, *Acta Biomater.* 2014, **10**, 4618.