

Supplementary information

Experimental section

Materials

Poly(ϵ -caprolactone) (PCL) (Mw 65,000), 2-hydroxyethyl methacrylate (HEMA), 2-bromoisobutylate bromide (BIBB), copper bromide (CuBr, CuBr₂), 2, 2'-bipyridyl (Bpy), bovine serum albumin (BSA), lysozyme, and 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) were purchased from Sigma-Aldrich (St. Louis, MO). 2-sulfoethyl methacrylate (SEMA) was obtained from Polyscience, Inc. (Warrington, PA). Pyridine was purchased from Daejung Chemical Co. (Cheongwon, South Korea). A Bicinchoninic acid (BCA) Protein assay was purchased from Thermo Fisher Scientific Inc. (Rockford, IL). Epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) were purchased from PeproTech (Rocky Hill, NJ). Cy5.5-NHS ester was purchased from Lumiprobe (Hallandale Beach, FL). The formaldehyde solution was purchased from Wako Chemicals (Osaka, Japan). A mouse embryonic fibroblast cell line, NIH3T3, was purchased from Korea Cell Line Bank (Seoul, South Korea). Dulbecco's modified Eagle's medium (DMEM), streptomycin/penicillin, trypsin/EDTA, fetal bovine serum (FBS), and Alexa Fluor[®] 568 Phalloidin were purchased from Invitrogen (Carlsbad, CA). All other chemicals were of analytical grades.

Electrospinning and fabrication of PCL nanofibrils

Polymeric nanofibrils were fabricated via the partial-digestion of electrospun PCL nanofibers at alkaline conditions. The PCL solution was electrospun into PCL nanofibers as described in our previous study [21]. Briefly, a 15% (w/v) polymer solution in

chloroform/methanol (3/1, v/v) was electrospun at 15kV and a steady flow rate of 1 ml/h through a 27G needle. The electrospun nanofiber was collected on an aluminum foil ground at a ground-to-needle distance of 15 cm. The PCL nanofibers were subsequently hydrolyzed into fibril structures in 1.0 M sodium hydroxide at 37°C for 12 h. The hydrolyzed PCL nanofibrils (hPCL NF) were collected by centrifugation at 12,000×g and washed with distilled water (DW).

Surface modification of nanofibrils by surface-initiated atom-transfer radical polymerization (SI-ATRP)

hPCL NF (2 g) was pre-hydrated with methanol (3 ml) and dispersed in dried hexane (50 ml). The hydroxyl groups of the hPCL NF were brominated with BIBB (4 ml) and pyridine (2 ml) for the surface-initiation of ATRP. The reaction was performed with gentle stirring at 0°C for 2 h, and the reaction mixture was then further incubated at room temperature for 12 h. The initiator-immobilized hPCL NF was then washed repeatedly with a methanol/DW mixture (5/1, v/v). For SI-ATRP on hPCL NF, HEMA or SEMA, catalyst, and ligand were added to the initiator-immobilized PCL NF in a methanol/DW mixture (5/1 (v/v), 20 ml) (molar ratio of a monomer: CuBr: CuBr₂: bpy= 100: 1: 0.2: 2), and the reaction was carried out at room temperature for 12 h under nitrogen. A HEMA, SEMA, or HEMA/SEMA mixture (1:1 molar ratio) was surface-polymerized on the hPCL NF to prepare poly (HEMA-SEMA), poly (HEMA), and poly (SEMA) on the hPCL NF (HS@NF, H@NF, and S@NF). The reaction mixtures were washed thoroughly with methanol to completely remove the unreacted chemicals and then freeze-dried.

Characterization of surface-modified PCL NFs

The polymerization of HEMA or SEMA on the surfaces of hPCL was confirmed both by NMR spectroscopy and X-ray photoelectron scattering (XPS) spectroscopy. The HEMA or SEMA-polymerized hPCL NF was dissolved in CDCl₃ and subjected to 400MHz ¹H-NMR spectroscopy (Bruker, DPX, Germany) at the central laboratory of Kangwon National University. The surfaces of the hPCL NF were also characterized by X-ray photoelectron scattering (XPS) spectroscopy with non-monochromated Al K α radiation (VG Scientific, ESCA Sigma Probe, USA). Survey scan spectra of C_{1s}, O_{1s}, and S_{2p} were obtained, and each spectrum was de-convoluted into the respective composition. The morphology of NFs was observed by field-emission scanning electronic microscopy (FE-SEM) (Hitachi, S-4300), Japan) and energy filtering-transmission electron microscopy (EF-TEM) (Carl Zeiss, LEO 912 AB, Germany) at the Korea Basic Science Institute. The water adsorption capacity of the NFs was measured via the swelling ratio. After swelling in DW at 37°C for 1 h, the NFs were weighed, and the swelling ratio of NFs was calculated according to the following formula: swelling ratio (%) = [(W_s-W_i)/W_i] x 100%, where W_s is the weight of swollen NFs and W_i is the initial weight of NFs.

Protein binding study

The protein adsorption capacity of the surface-modified NFs was tested using a BCA assay and fluorescence imaging. The NFs (5 mg) were incubated with various protein solutions (1 ml) in 1.5ml-eppendorf tubes, including BSA (100 μ g/ml), lysozyme (100 μ g/ml),

and FBS (1%, v/v) in phosphate buffered saline (PBS, pH 7.4) for 3 h with gentle shaking at 37°C. To calculate the protein adsorption efficiency, the amount of unbound protein in the supernatant was quantified using a BCA assay. Briefly, BCA reagent (50µl) was mixed with the supernatant (50µl) and incubated at 37°C for 30 min before the absorbance was measured at 590 nm (Thermo Fisher Scientific, Mutiskan GO, USA). Cy5.5-labeled proteins were prepared to visualize protein adsorption to the NFs. Briefly, Cy 5.5-NHS ester (200µg) in DMSO (100µl) was slowly added to the protein solution (1 mg) in 0.1 M sodium bicarbonate (pH 9.0) (5 ml). The reaction was performed at 4°C for 12 h, and the unreacted Cy5.5-NHS esters were subsequently removed by dialysis (MWCO=1,000). Cy5.5-labeled BSA, lysozyme, and growth factors (EGF and bFGF) were incubated with the NFs and imaged using an in vivo imaging system (IVIS) (Caliper Life Sciences Inc., Xenogen IVIS 200, USA) (ex=673 nm, em=700 nm).

Cell culture with the nanofibrils

A suspension of NIH3T3 cells (1 ml, 5×10^5 cells/ml) was blended with the pre-hydrated NFs (10 mg) by gentle pipetting and seeded on a 48-well cell culture plate in DMEM supplemented with 10% FBS (v/v) and streptomycin/penicillin. After 7 days, MTT solution (10 µl, 5 mg/ml) was added to each well and incubated for 6 h. The formazan crystals were dissolved with DMSO (500 µl) after removing the cell culture medium, and the absorbance was measured at 570 nm for each well. The cell viabilities were determined with respect to the cells cultured with hPCL NF. The rheological properties of the NFs were monitored using a rotating rheometer (Malvern Instruments, Bohlin Advanced Rheometer,

UK). A mixture of the NF (10 mg) and cells ranging from 10^3 to 10^6 cells at 7 days was harvested and placed on a parallel plate rheometer (diameter=20 mm). The contribution of the elastic modulus (G') was recorded with a frequency range from 0.1 to 100 Hz at a constant stress of 100 Pa. The G' max was calculated by averaging the elastic modulus values from 20 to 100 Hz. The morphology of the NF/cell composite was observed via FE-SEM and fluorescently visualized via confocal laser scanning microscopy (CLSM) (Olympus, FV1000 SPD, USA). To prepare the SEM samples, a NF/cell composite was fixed in 3.7% formaldehyde solution for 3 h and freeze-dried. For the CLSM images, the cells were stained with Alexa Fluor[®] 568 Phalloidin for the cytoskeleton and DAPI for the nucleus. The stained cells were visualized via CLSM with a HeNe G laser (543 nm) and emission filters of BA560-620 nm to observe the cytoskeleton. A LD 405 laser (405 nm) and the emission filters of BA430-470 nm were used to observe the nucleus.

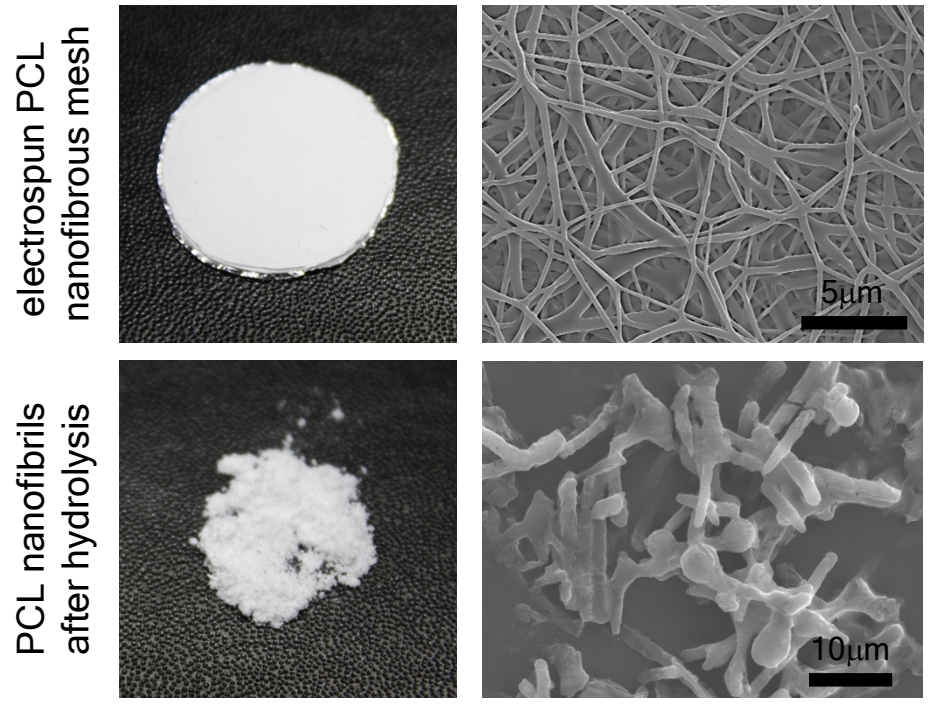


Figure S1. Macro images (left) and SEM images (right) of nanofibrous scaffolds before (upper) and after treatment of NaOH (below).

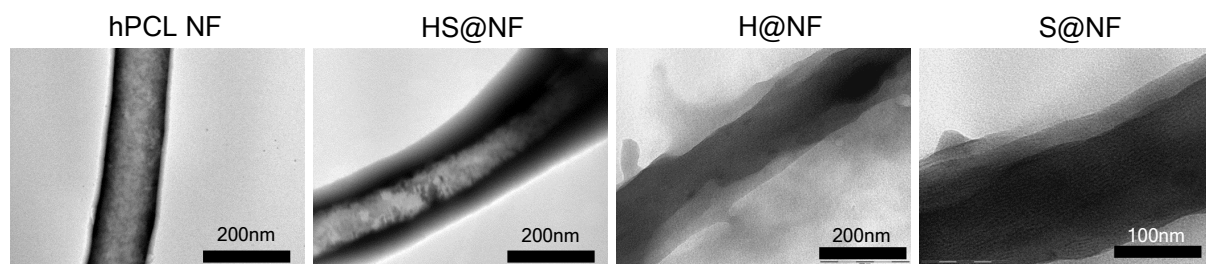


Figure S2. TEM images of hPCL NF and surface-modified NF. We could visualize the thin layers of poly(methacrylate) derivatives surrounding the core fibrils in HS@NF, H@NF, and S@NF.

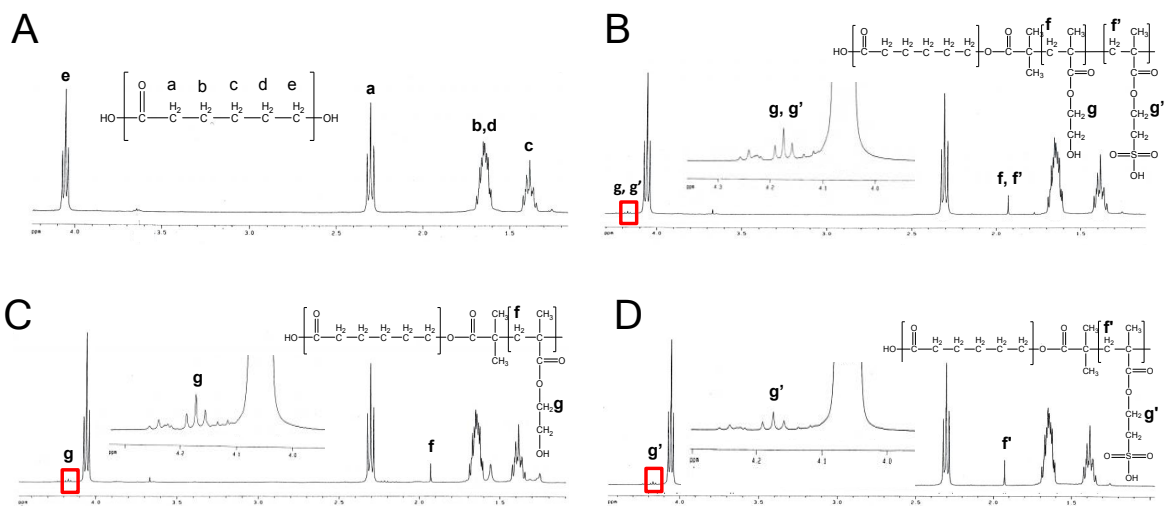


Figure S3. $^1\text{H-NMR}$ of (A) hPCL NF, (B) HS@NF, (C) H@NF and (D) S@NF. Distinguishable peaks of the methacrylate groups were detected at 1.9ppm and 4.2ppm in surface-modified NFs.

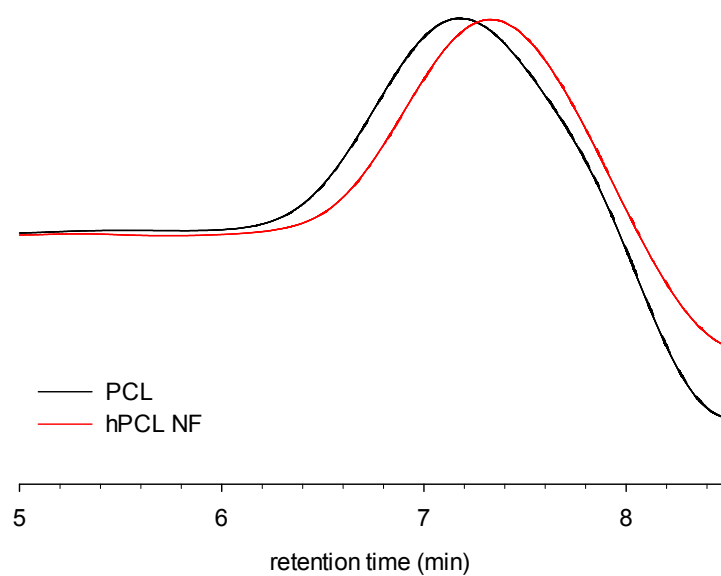


Figure S4. GPC curves for PCL (black) and hPCL NF (red). The molecular weight of PCL and hPCL NF determined by GPC is 60KDa (7.2min) and 56KDa (7.3min), respectively.

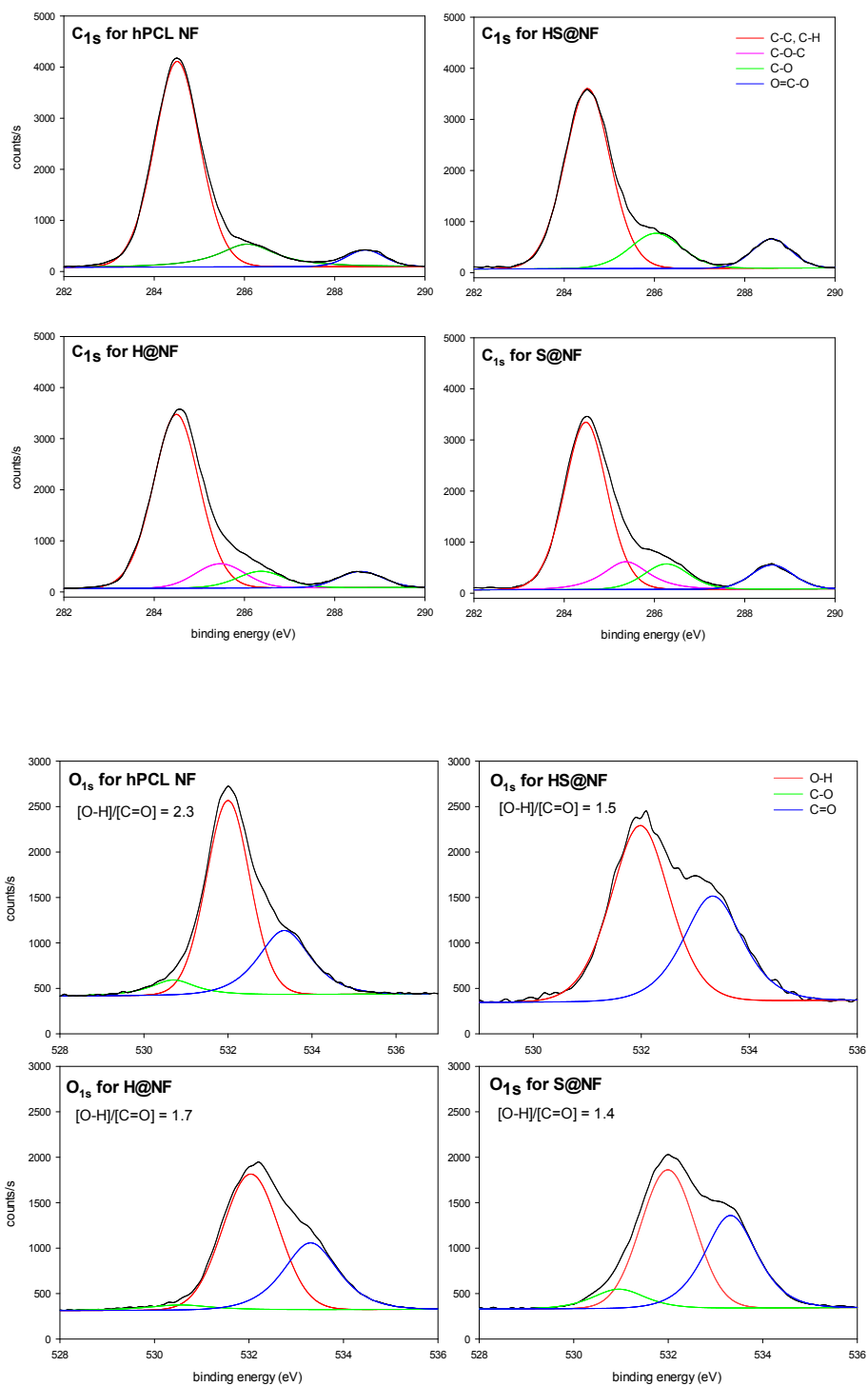


Figure S5. Core-level XPS spectra of C_{1s} and O_{1s} for hPCL NF and the surface-modified NF.

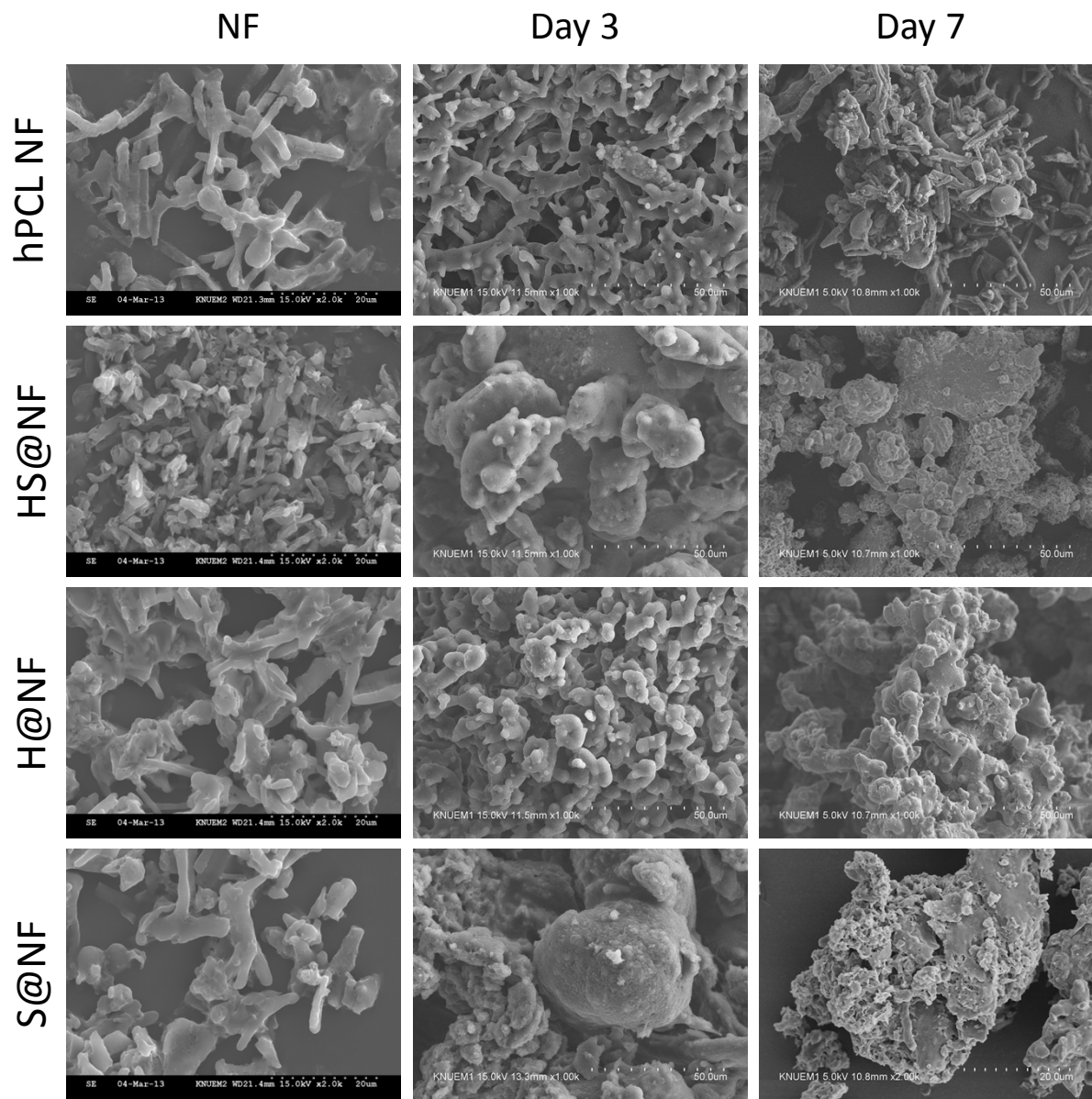


Figure S6. SEM images of the NF/cell composite cultivated for 3 and 7 days.

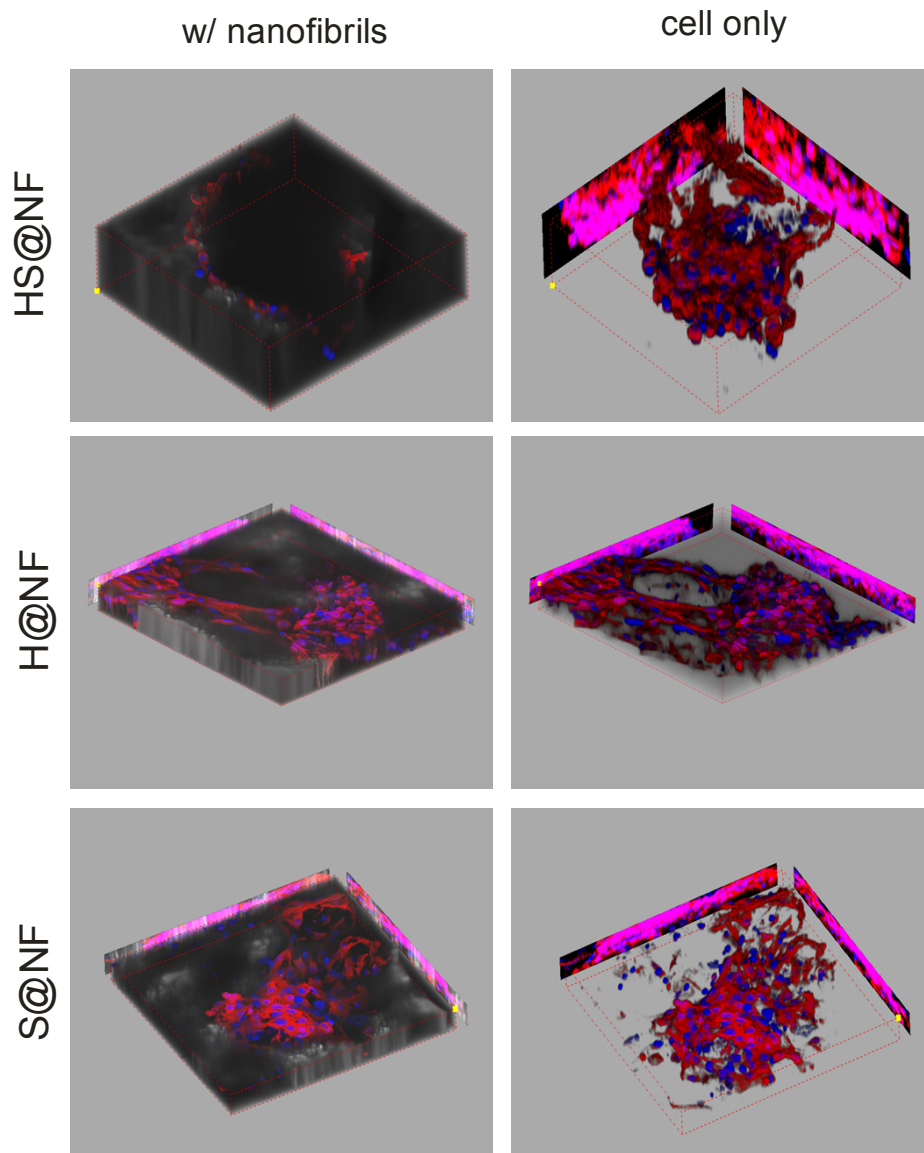


Figure S7. 3-dimensional CLSM images of the NF/cell composite at day 7. Ten slices of z-stacked CLSM images were merged in each group.

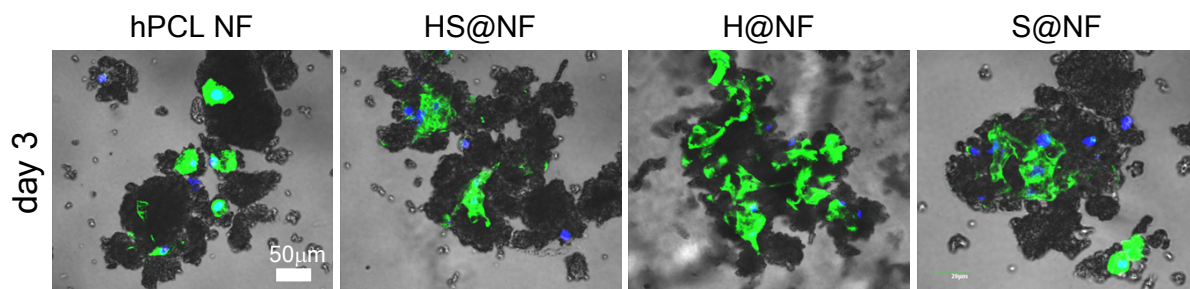


Figure S8. CLSM images of the NF/cell composite at day 3. The cells were stained with Oregon green[®] 488 Phalloidin for the cytoskeleton and DAPI for the nucleus.

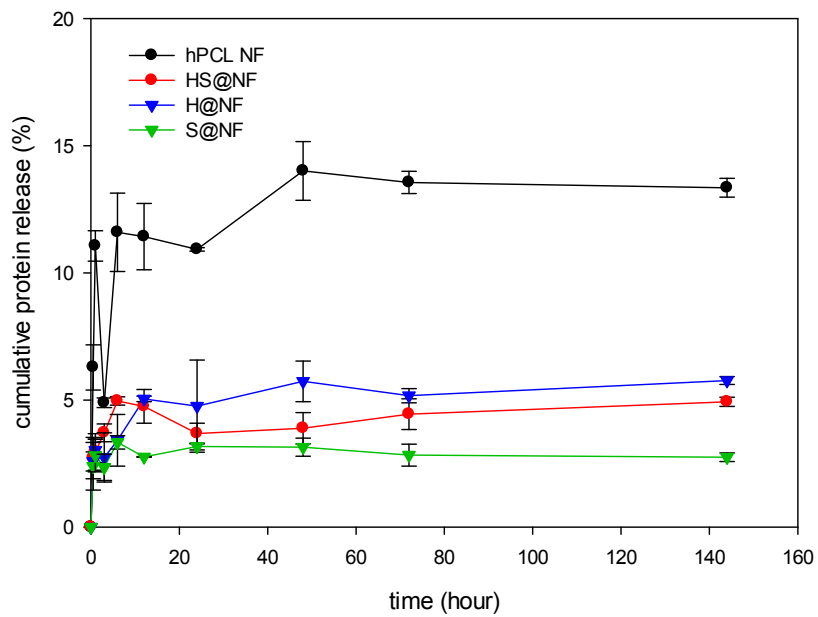


Figure S9. Protein release profile of the protein-sequestered NFs. After NFs (5mg) were incubated in 1% FBS for 3 h, protein releases was performed in PBS (pH 7.4) for 6 days.

Table S1. Swelling ratio of the NFs after 1h in distilled water.

NF	swelling ratio (w/w, %)
hPCL NF	607.5 ± 43.3
HS@NF	735.8 ± 78.6
H@NF	843.1 ± 76.7
S@NF	690.4 ± 67.5