Supporting information for:

Nanocomposite Gold-Silk Nanofibers

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Materials and Methods

SNF and AuNPs doped SNF preparation

SNF preparation followed previously published methods.[1] In brief, 5 g of Bombyx mori silk cocoons were cut into 1cm X 1cm pieces, and were boiled for 30 min in 0.02 M Na_2CO_3 in order to degum the silk from unwanted sericin protein. The resulting degummed silk was washed in deionized water for 20 min three times. The silk was dried completely and was dissolved in 11.5 mL of 9.4M LiBr (Sigma-Aldrich) for 4 hours then dialyzed for 72 hours in a 3500Da MW cut-off dialysis cassette (Fisher Scientific) to eliminate the LiBr. Approximately 25-30 mL of 8-12 % w/v silk fibroin solution was collected, centrifuged twice at 9000 RPM for 20 min, and stored for up to one month in a 4°C refrigerator.

To make the solution to be electrospun into SNF, 800 mg of polyethylene oxide (Sigma-Aldrich) was dissolved in 1.6 mL of DI water, followed by addition of 6.4 mL of silk fibroin solution, and was stirred for 10-20 min in a 4°C refrigerator.

To make the solution to be electrospun into SNF_{seed}, 800 mg of PEO was dissolved in 1.6 mL of condensed seed solution (see below), followed by addition of 6.4 mL of silk fibroin solution, and stirred for 10-20 min in a 4°C refrigerator.

Au seed particles synthesis

180 mL DI water and 1.2 mL of 1M NaOH (Sigma-Aldrich) were mixed for 5 min. 48 μL of tetrakis(hydroxymethyl)phosphonium chloride **(**THPC, 80% in water, Sigma-Aldrich) were added to this solution and mixed for an additional 5 min. 7 mL of 1% w/w $HAuCl_4$ aqueous solution was added to the THPC solution to immediately form a AuNPs seed solution (see Figure S1). The resulting solution was condensed by overnight dialysis in 10% polyethylene glycol solution (30kDa, Sigma-Aldrich). 30 mL of seed solution as made was placed in a 12 mL 3500 Da dialysis cassette overnight (Fisher scientific). 1.5-4 mL of condensed solution was collected and used on the same day.

SNFs electrospinning

The SNF solutions were loaded in a 6 mL syringe (outer diameter of 12.1 mm) and mounted to a syringe pump (Harvard Apparatus). The syringe needle was connected to a DC voltage source and the sample collecting plate was kept at ground, and was positioned 25 cm away from the syringe tip. The DC voltage was set to 20 kV and the solution flow rate was 20-40 μL per

min. The samples were spun for 30-40 min. After the electrospinning process SNFs samples were soaked in 90% MeOH (Sigma-Aldrich) for 20-30 min, following by drying in a dry box. Dried SNF and Au seed doped SNF ($AuNP_{seed}$) samples were kept in ambient conditions prior to experiments.

AuNP formation in SNF fibers

$AuNP_{seed}$ were incubated for 3-4 days in a Au^{+} solution consisting of 48 mL DI water, 130 mg K_2CO_3 (Sigma-Aldrich), and 100 mg of $HAuCl_4$ (Sigma-Aldrich). The resulting AuNP-doped SNF (SNF_{Au}) samples were washed twice with DI water, and left in DI water overnight to remove excess Au^{+}.

UV/VIS experiments

Samples of both SNF and SNF_{Au} on glass cover slip were loaded in a UV/VIS spectrophotometer (Cary 50, Agilent-Varian). For all readings, background was subtracted and data were plotted as normalized absorbance (Abs in arbitrary units) versus the wavelength in nm.

TGA experiment

4 samples from each group were prepared and dried prior to the experiment. All the samples were loaded into a thermogravimetric analysis machine (Pyris 1 TGA, Perkin-Elmer), heated to 700°C (20°C/min) and cooled down back to room temperature (200°C/min). The data are presented as a weight loss calculated versus the initial weight.

SEM imaging

The SNF and SNF_{Au} samples were loaded to an ultra-high resolution SEM (Supra 55 VP, Carl Zeiss) without application of any coating, and imaged usually with an accelerating voltage of 1-2 kV to avoid any sample damage.

EDS elemental mapping.

SNF_{Au} were prepared in a Si / 600 nm SiO_2 substrate. The substrate was loaded to an ultra-high resolution SEM (Supra 55 VP, Carl Zeiss) with an EDX detector and imaged at an accelerating voltage of 20 kV and a working distance of 8.5 mm. For EDS the spectra were acquired with a pixel dwell time of 500 μs, and image size of 512 X 400 pixels. The mapping was done for Si, O, C and Au. The presented data is an integration of 8 images that were collected on the same sample.

TEM imaging

In the case of AuNP seed solution, 30 μL of the condensed solution were applied onto a thin carbon TEM grid (Ted Pella) and left to dry in ambient conditions. In the case of SNF_{Au} cross-sectional samples, the $SNFs_{Au}$ were embedded in an epoxy resin (EMBed 812, EMS) and sections of approximately 50 nm were cut using a microtome (Ultramicrotome, Leica). The sections were mounted on a TEM grid (Ted Pella). Both section and seed particles samples were imaged in Cs corrected TEM (Libra 200, Carl Zeiss).

AFM mechanical investigation

The mechanical properties of SNF fibers were investigated using an AFM microscope (ICON, Bruker), under the peak force quantitative nano mechanics mode (PFQNM). In brief, we worked with an AFM cantilever with a nominal spring constant of 200 N/m (TAP 525, Bruker) to match the possible range of mechanical properties of silk. Prior to every experiment, we characterized sensitivity, spring constant, and size of the cantilever tip. In our experiments, they were 113 nm/V, 230 N/m, and 13 nm respectively. These values are stored in the AFM software and are further used to calculate the Young's modulus of the sample. The samples were scanned at 1-1.5 Hz, and all images were acquired at 512 X 512 line resolution. In this mode the mechanical properties of the sample are derived from the MDT model fit.[2] We further analyzed the elastic modulus maps to evaluate the modulus of each sample by using a box average with a fixed width of 190 nm.

Assessment of membrane toxicity in vitro

PC12 cells (CRL-1721, ATCC) derived from rat adrenal medulla pheochromocytoma were cultured at 37˚C and 5% CO_2 in F-12K media (Gibco) supplemented with 2.5% fetal bovine serum (Gibco), 15% horse serum (Gibco), and 1% penicillin-streptomycin (Gibco). SNF and SNF_{Au} membranes were placed in 24-well plates and cells were seeded onto each sample at a density of 5×10^4 cells/cm^2. To induce neuronal differentiation, nerve growth factor (Invitrogen) was added 24 hours after seeding. Cell viability was then assessed using the MTS (Promega) colorimetric assay every 24 hours for a total of 96 hours. At each time point, 150 μL of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium solution was added to the wells of six samples per membrane type and allowed to incubate at 37 ˚C for 2 hours. After incubation, 100 μL of developed MTS-media solution in each well was transferred

to a 96-well plate, which was then read for absorbance at λ = 490 nm. Cells were also visually inspected to confirm the validity of colorimetric readings. Results are presented as percent survivability normalized to control cultures where cells were not exposed to either of the two membrane types that were also assayed at each time point.

SNF surface modification

The surface of SNFs and SNF_{Au} were modified with a peptide containing the adhesion sequence RGD3. A free sulfhydryl group on the cysteine residue in the RGD peptide[3] (GCGYGRGDSPG, Genscript) was utilized to immobilize RGD peptide on AUNPs doped SNFs. In brief, SNF_{Au} were immersed in 50 µM RGD solution in PBS for 4 hours at room temperature followed by washing in DI water three times. SNF without gold were treated the same way to test if non-specific adsorption of RGD peptide affects the immobilization chemistry.

Determination of individual hMSC area(cell area) and adherent cells density (cell density)

Human mesenchymal stem cells (hMSCs) were purchased from Lonza (Basel) and expanded in MSC basal media (Lonza) in T75 flasks at 37˚C and 5% CO_2. The media was replaced every 3-4 days during the culture. hMSCs at passage 3 were used for culture on the silk-based substrates at 5 x $10^3/cm^2$. To examine the adhesion of hMSCs on each substrate through fluorescence microscopy, the cells were fixed after 24 hours with 4% formaldehyde in PBS for 15 minutes and washed three times in PBS. After permeabilization of the cells using 0.1% Triton-100 for 5 minutes, and blocking non-specific binding with 1% bovine serum albumin (BSA) in PBS for 1 hour at room temperature, the cells were stained with TRITC-phallioidin for actin cytoskeleton, anti-vinculin and FITC-labeled secondary antibody for focal adhesion and DAPI for nuclei. Fluorescent images were obtained by epifluorescence imaging (Olympus FSX100, Olympus). High resolution fluorescent images were obtained by confocal imaging (Zeiss LSM 700, Carl Zeiss) using the 60X oil immersion objective lens.

Fluorescent images taken at 42X of hMSCs seeded onto SNF and SNF_{Au} samples were analyzed with ImageJ software (National Institutes of Health) to quantitatively determine both the average adhered cell density and the area of individual cells. Images were first split into separate red, green, and blue channels such that actin-only, vinculin-only, and nucleus-only images were obtained. For determining cell density, the thresholds of nucleus-only images were adjusted so that the background noise (such as nanofibers) was eliminated, and only the nuclei

showed up as discrete black features on a white background. This allowed for the use of ImageJ's particle analysis function to count the number of nuclei, and therefore cells, within the selected field of view. The lower and upper pixel size bounds for defining what constituted a "particle" were 50 pixels and 1000 pixels, respectively. Adhered cell density was then calculated by dividing the number of cells counted by the area of the field of view for four different fields per sample, and for a total of four samples per membrane type. Cell area was determined in a similar fashion; however, in this case the actin-only channel was used for analysis. As before, the thresholds were adjusted to better differentiate cells from background. Using the wand tool, 30 representative cells from each field of view were selected as regions of interest (ROIs) and the areas of these ROIs were calculated using ImageJ's ROI manager. Four different fields of view per sample and four samples per membrane type were used to calculate the average cell areas.

Statistical analysis

The means of cell densities and areas were analyzed by one way ANOVA followed by post-hoc Tukey's test to evaluate the differences between specific groups with multiple comparisons. $P < 0.05$ was considered statistically significant. The data is presented as mean ± SD.

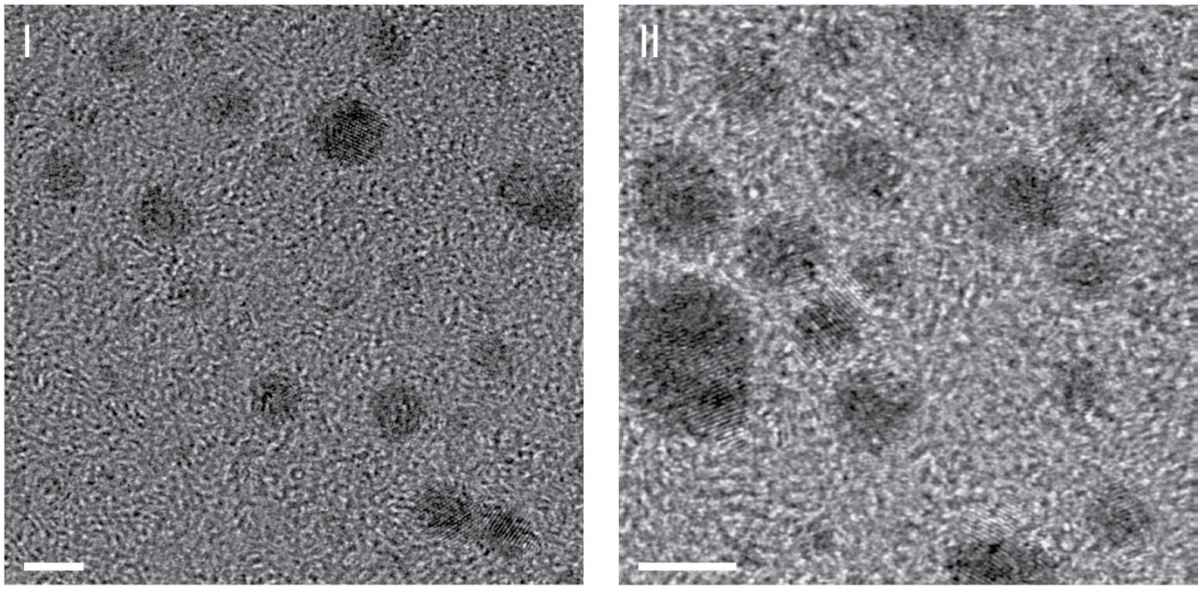

Figure S1. TEM of gold seed nanoparticles. Scale bar is 4 nm and 5 nm for I and II respectively.

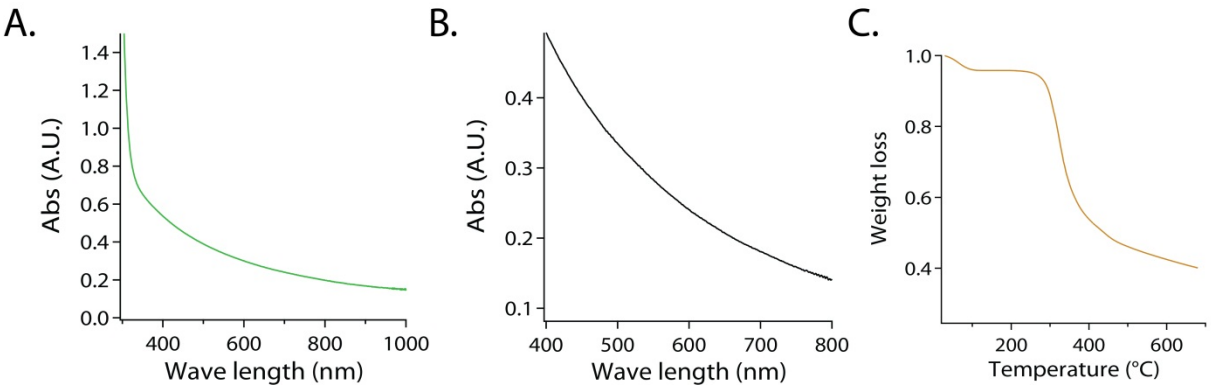

Figure S2. Characterization of SNFs A. UV/VIS spectrum of SNF with Au seed nanoparticles. B. UV/VIS spectrum of SNF after incubation for 6 days in Au^{+} solution. C. TGA of seed-doped SNFs.

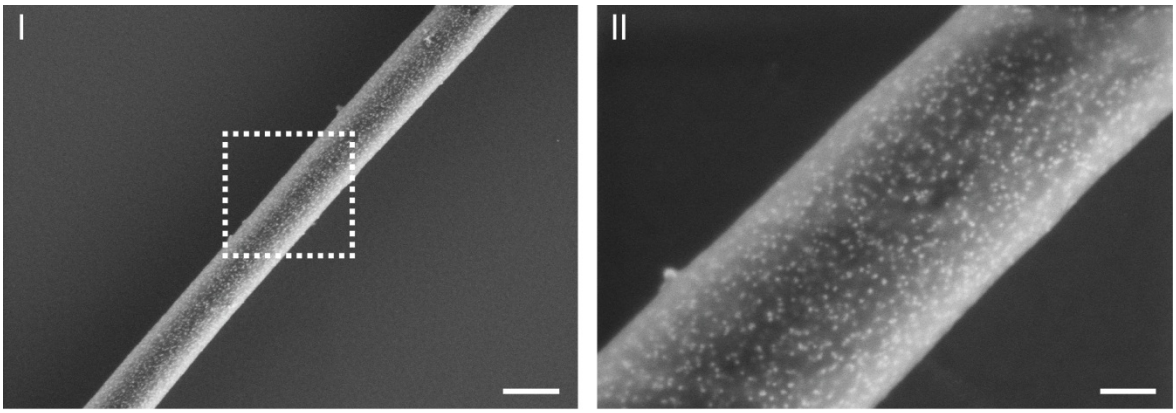

Figure S3. I. Surface morphology of a SNF_{Au}. Scale bar is 400 nm. II. Expanded view of the white dotted box in panel I. Scale bar is 100 nm.

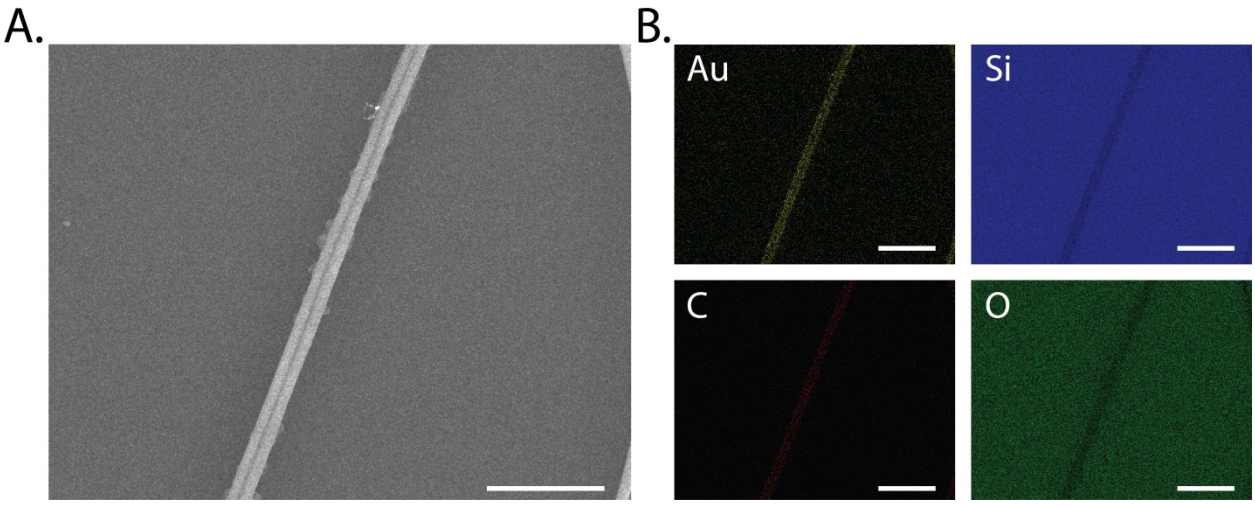

Figure S4. Elemental mapping of SNF_{Au}. A. SEM image of a SNF_{Au} fiber. B. Elemental maps of the same fiber presented in Panel A, mapping the elements gold (yellow), silicon (blue), carbon (red) and oxygen (green). Scale bar is 5 μm for all panels.

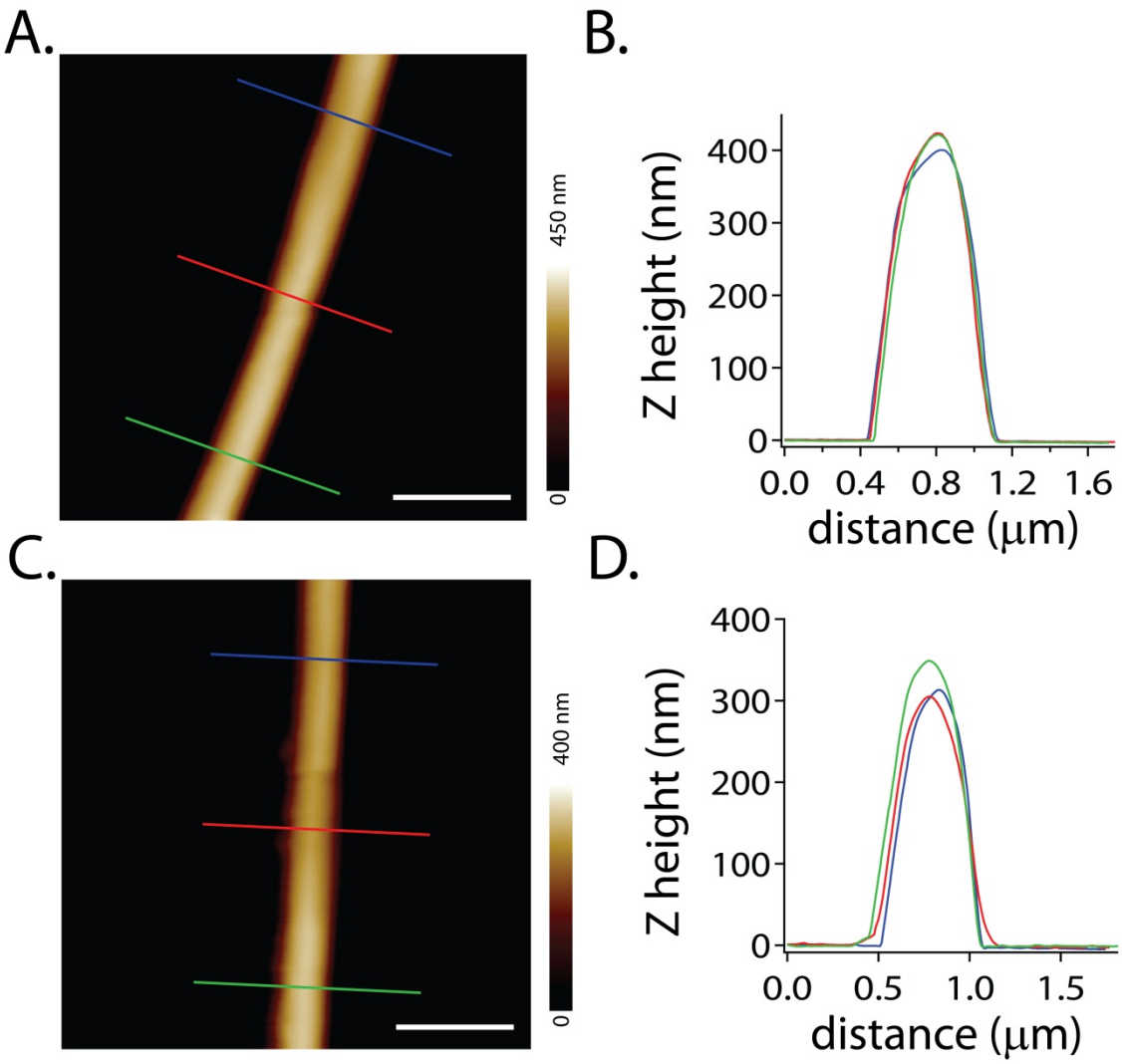

Figure S5 AFM Z height maps. A. Z height map of a SNF fiber. Scale bar is 1 μm. B Representative cross-section lines of the same fiber. C. Z height map of SNF_{Au} fibers. Scale bar is 1 μm. D. Representative cross-section lines of the same fiber.

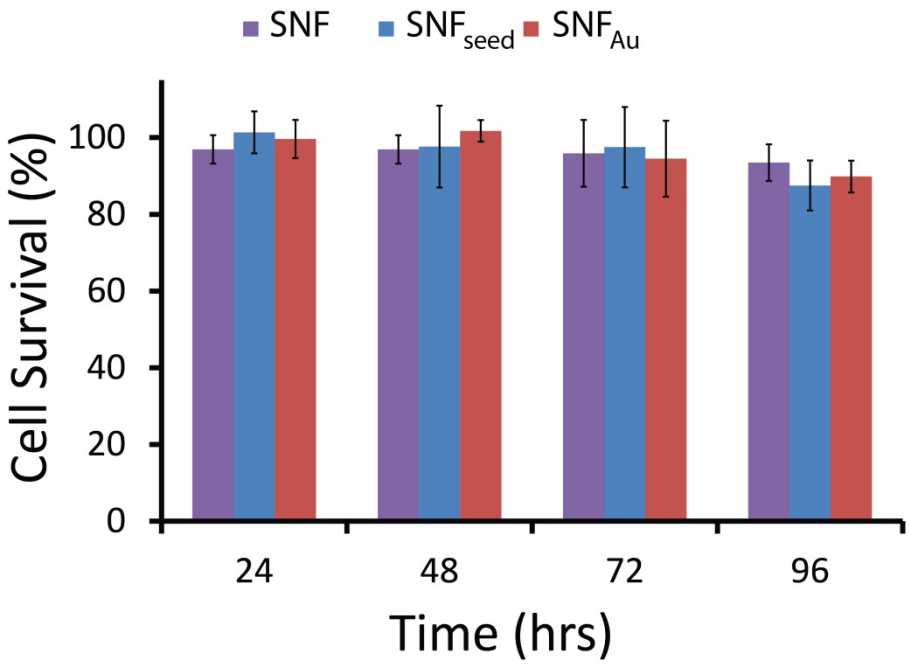

Figure S6. Cytotoxicity of SNF, SNF_{seed}, and SNF_{Au} in cultured PC12 cells. Data are mean ± SD.

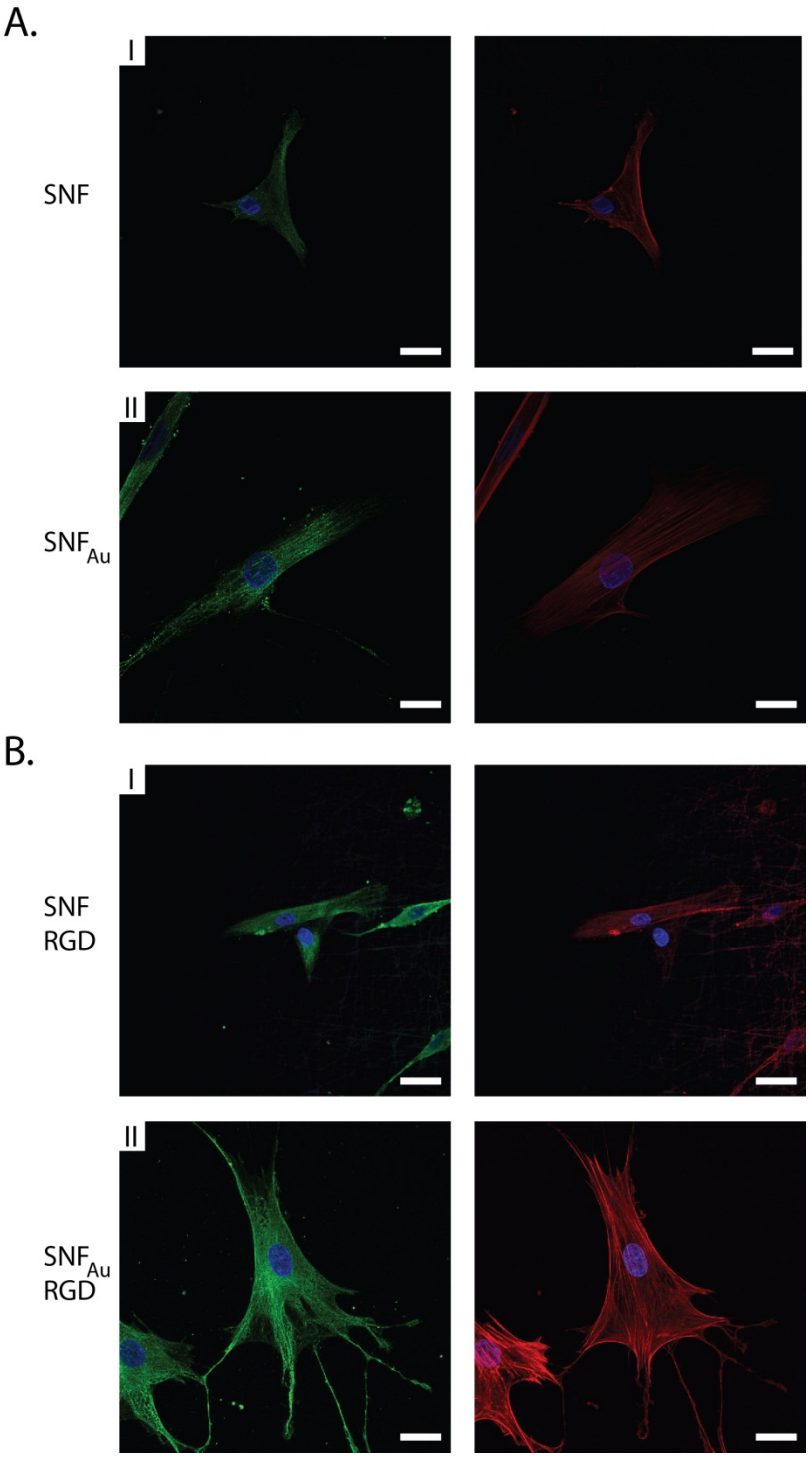

Figure S7. A. Confocal microscopy images of hMSC cultured on (I) SNF, and (II) SNF_{Au}. B. Confocal microscopy images of hMSC cultured on (I) SNF+RGD, and (II) SNF_{Au}+RGD. Stains: nucleus (blue), actin filaments (red) and vinculin (green). Scale bars are 25 μm.

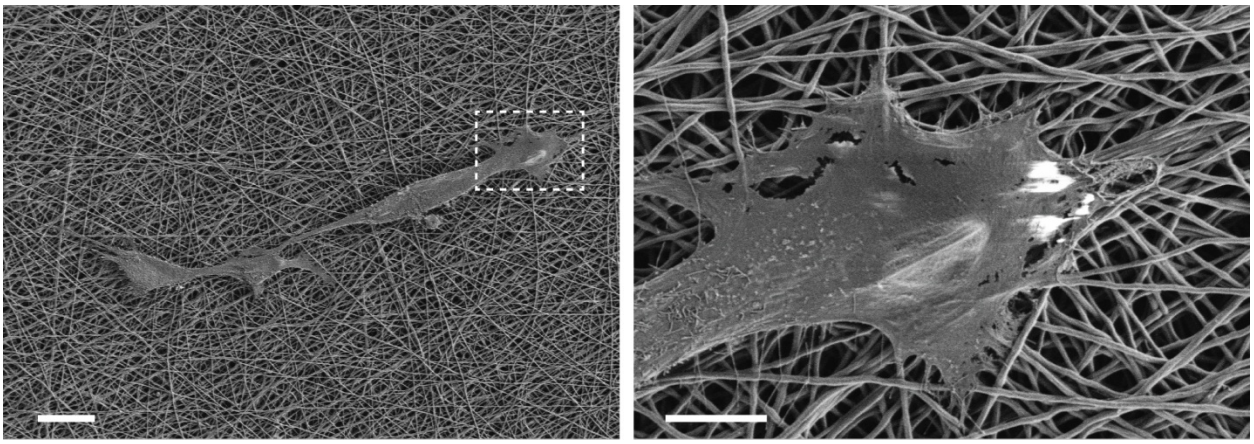

Figure S8. A. Morphology of hMSC cultured on SNF+RGD. Scale bar is 30 μm B. Expanded view of the dashed box. Scale bar is 10 μm.

References.

1. Rockwood, D. N.; Preda, R. C.; Yucel, T.; Wang, X. Q.; Lovett, M. L.; Kaplan, D. L., Materials fabrication from Bombyx mori silk fibroin. *Nat Protoc* **2011**, 6 (10), 1612-1631.
2. Derjaguin, B. V.; Muller, V. M.; Toporov, Y. P., Effect of contact deformations on adhesion of particles. *J of Colloid Interf Sci* **1975**, 53 (2), 314-326.
3. VandeVondele, S.; Voros, J.; Hubbell, J. A., RGD-Grafted poly-l-lysine-graft-(polyethylene glycol) copolymers block non-specific protein adsorption while promoting cell adhesion. *Biotechnol Bioeng* **2003**, 82 (7), 784-790.