Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

Multi-Interpenetrating Network (IPN) Hydrogel by Gelatin and Silk Fibroin

Shiwha Park[a], Seth Edwards[a], Ryann Boudreau[a], Shujie Hou[a], Rachel Yee[b] and Kyung Jae Jeong[a]*

[a]Department of Chemical Engineering, University of New Hampshire, Durham, NH 03824

[b]Department of Molecular, Cellular and Biomedical Sciences, University of New Hampshire, Durham, NH 03824

*Corresponding Author: Kyungjae.Jeong@unh.edu

Materials and methods

1. Materials

Gelatin from bovine and porcine bones was purchased from Sigma Aldrich (St. Louis, MO, USA), and Silk fibroin 5% solution from Advanced Biomatrix (Carlsbad, CA, USA). Microbial transglutaminase (mTG) was purchased from Ajinomoto North America., Inc. (Fort Lee, NJ, USA). Ethyl alcohol 200 proof was obtained from Pharmco-Aaper (Brookfield, CT, USA). Normal human dermal fibroblasts were purchased from Lonza Biologics (Portsmouth, NH).

2. Synthesis of hydrogels

1-IPN hydrogel: Gelatin, mTG and silk fibroin were mixed in 1X PBS with a final concentration 2.5%, 1% and 2.5% (w/v), respectively, and the mixture was incubated at 37 °C for 2 hours for the initial covalent crosslinking of gelatin. This weak hydrogel was incubated at 50ºC for 4 hours to allow the physical crosslinking of silk fibroin. Subsequently, the hydrogel was immersed in 20% mTG solution and stirred overnight at 37ºC. The hydrogel was washed with 1x PBS three times followed by overnight incubation in 90% ethanol at room temperature with a constant agitation. Afterwards, the hydrogels were washed with deionized (DI) water and lyophilized.

2-IPN hydrogel: 1-IPN hydrogel was swelled in the gelatin, mTG and silk fibroin mixture (concentration = 2.5%, 1% and 2.5%, respectively). The same covalent and physical crosslinking methods were repeated. The resulting hydrogel was washed with DI water three times and lyophilized.

Silk hydrogel: 5% (w/v) silk solution in PBS (without gelatin) was treated the same way as 1-IPN hydrogel.

Gelatin hydrogel: 5% (w/v) gelatin solution in PBS (without silk fibroin) was treated the same way as 1-IPN hydrogel.

3. Rheology

Viscoelastic properties of the hydrogels were characterized using a rheometer (AR 550, TA Instruments, New Castle, DE) with a 20mm stainless steel plane geometry. The stage was heated to 37°C and the gelatin-silk mixture with or without mTG (4% w/v) was pipetted onto the center of the stage. A small amount of mineral oil was added around the perimeter of each sample to prevent water evaporation. Prior to the measurements, the linear viscoelastic regime was determined in the stress-strain mode. The time sweep was performed for 1 hour with a shear stress of 2 Pa and angular frequency of 10 rad/s. Frequency sweep was performed at 37°C, with the angular frequency range from 0.1-100 rad/s with a stress of 2Pa. The temperature sweep was performed at 10 rad/s with the temperature range of 5 -45 °C with the interval of 2.5 °C.

4. Fourier Transform Infrared (FT-IR)

FT-IR was performed using Nicolet iS10 FT-IR (Thermo Fisher Scientific, Waltham, MA). Each spectrum was collected with 128 scans and a resolution of 4cm^{-1} from 500 to 4000 cm^{-1}.

5. Scanning electron microscopy (SEM)

The hydrogels were frozen in -80ºC and lyophilized prior to the imaging by field-emission SEM (Lyra3 GMU, Tescan, Czech Republic). Fragmented hydrogels were mounted and attached on 25.4mm aluminum stub using double-sided carbon tape. The samples were sputter-coated with gold/palladium. Images were obtained with the acceleration voltage at 6-10 kV.

6. Enzymatic degradation of hydrogels.

Enzymatic degradation of hydrogels was evaluated by incubating the hydrogels in collagenase type II (0.5U/mL) at 37 °C. At each time point (0, 12, 24, 48, 72, 96 h), the hydrogels were collected, frozen at -80 °C, and lyophilized. The mass of each lyophilized hydrogels was weighed to determine % weight remaining.

7. Compressive mechanical properties of crosslinked hydrogel

Mechanical tests were performed using eXpert 2600 (ADMET, Norwood, MA). A disc-shaped hydrogel was placed between two flat surfaces, and the compression of the hydrogel was achieved at 5 mm/min until the force reached 100 N. From the force - displacement curve, stress-strain curve was obtained, from which compressive moduli was calculated at different strains from the slope of the curve.

8. *In vitro* biocompatibility

Cell culture and proliferation assay: Normal human dermal fibroblasts (hDFs) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and

1% penicillin/streptomycin at 37 °C in a humidified chamber (5% CO_2). Prior to seeding, the hydrogels were sterilized in 70% ethanol overnight and re-swelled in sterile 1X PBS. hDFs were added to the hydrogels at 2 x 10^4/mL. The media was changed every other day. The proliferation of hDFs was measured by alamarBlue on days 1, 3 and 7.

SEM: For visualization, the hydrogels (after day 3) were fixed in 4% formaldehyde in 1X PBS overnight. The hydrogels were then dried by critical point drying to minimize the deformation of cells by dehydration. SEM images were obtained using Lyra3 GMU field-emission SEM.

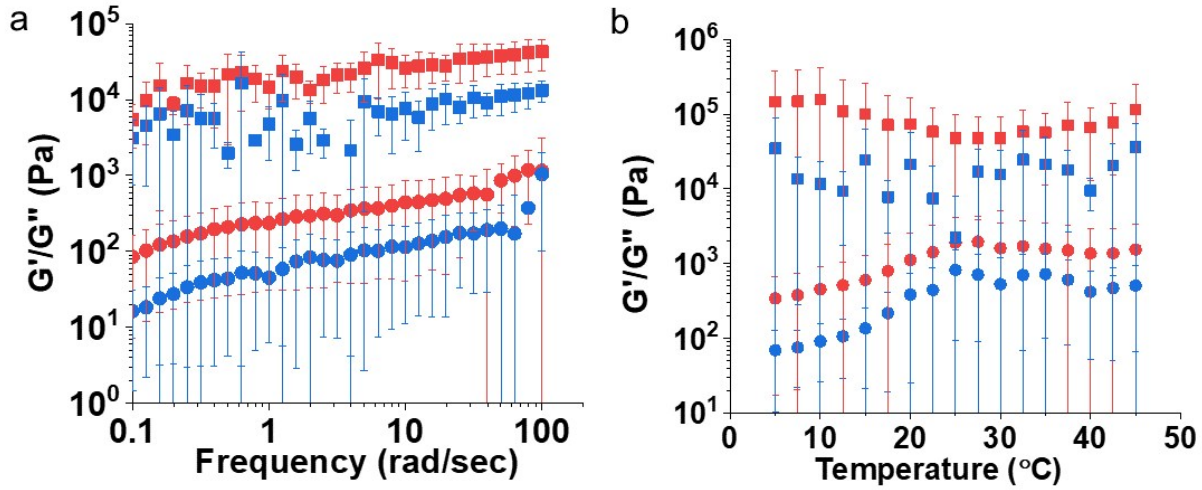

Figure S1. (a) Frequency and (b) temperature sweep of gelatin (2.5% w/v)-silk fibroin (2.5%) mixture in the presence (squares) and in the absence (circles) of mTG (4% w/v). Red and blue markers are G' (storage modulus) and G'' (loss modulus), respectively. The data are means and standard deviations (n = 3). Note that the error bars are exaggerated for low values in log scales.

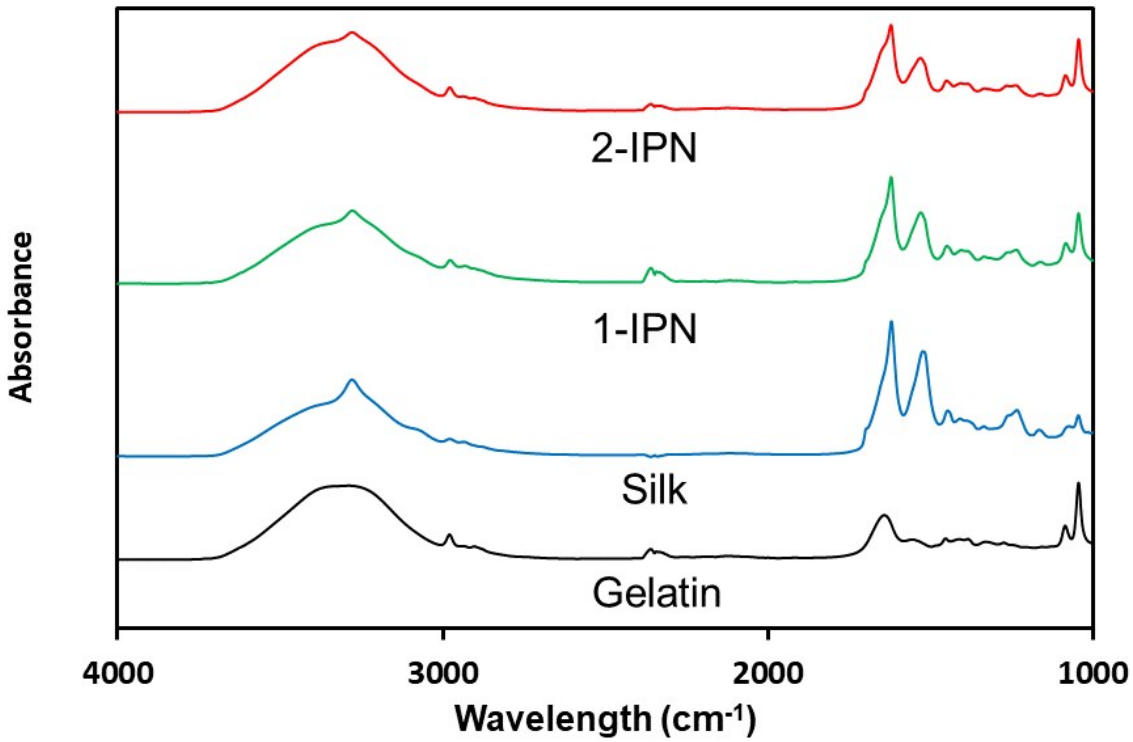

Figure S2. Full FT-IR spectra of hydrogels.

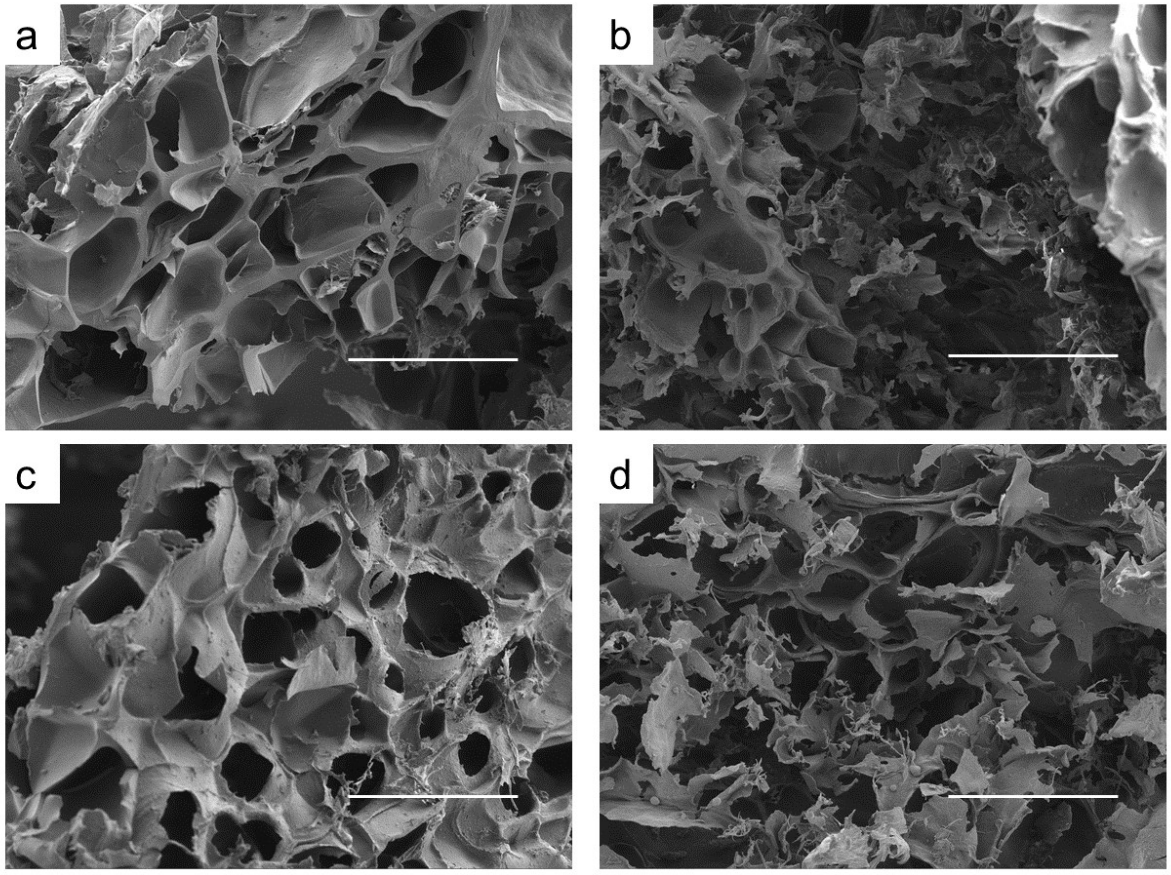

Figure S3. SEM images of (a) gelatin hydrogel, (b) silk hydrogel, (c) 1-IPN and (d) 2-IPN. Scale bar = 200 μm.

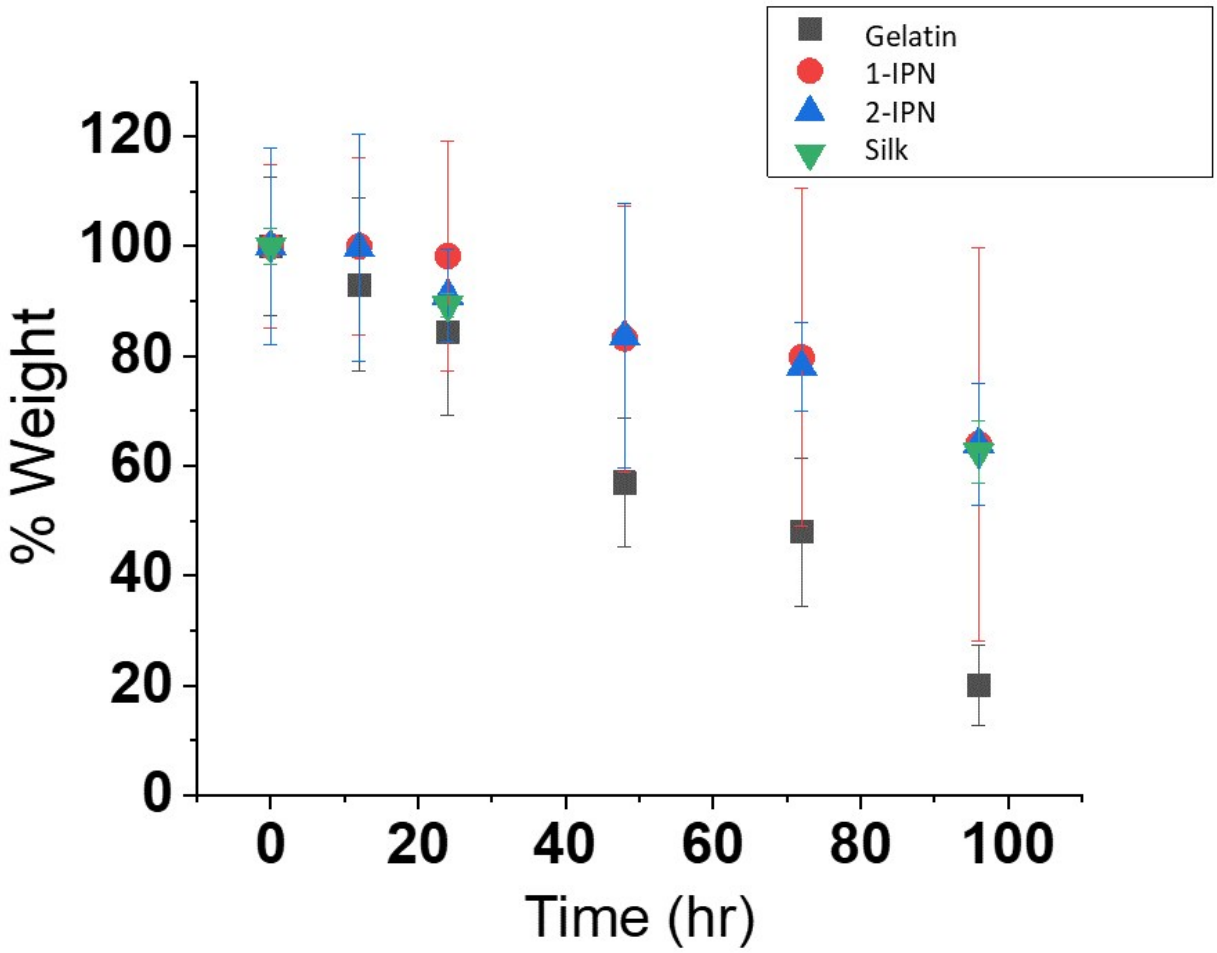

Figure S4. Enzymatic degradation of hydrogels by 0.15% collagenase type II.

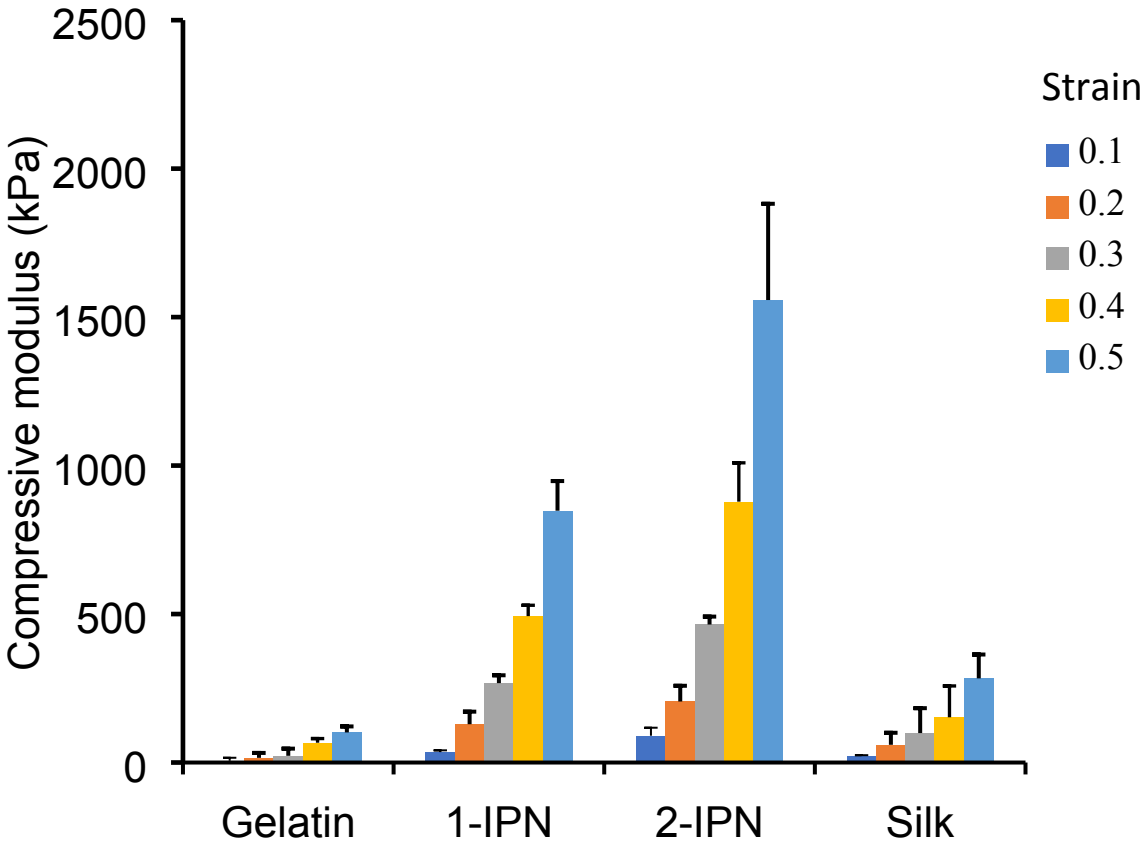

Figure S5. Compressive modulus at different strains.

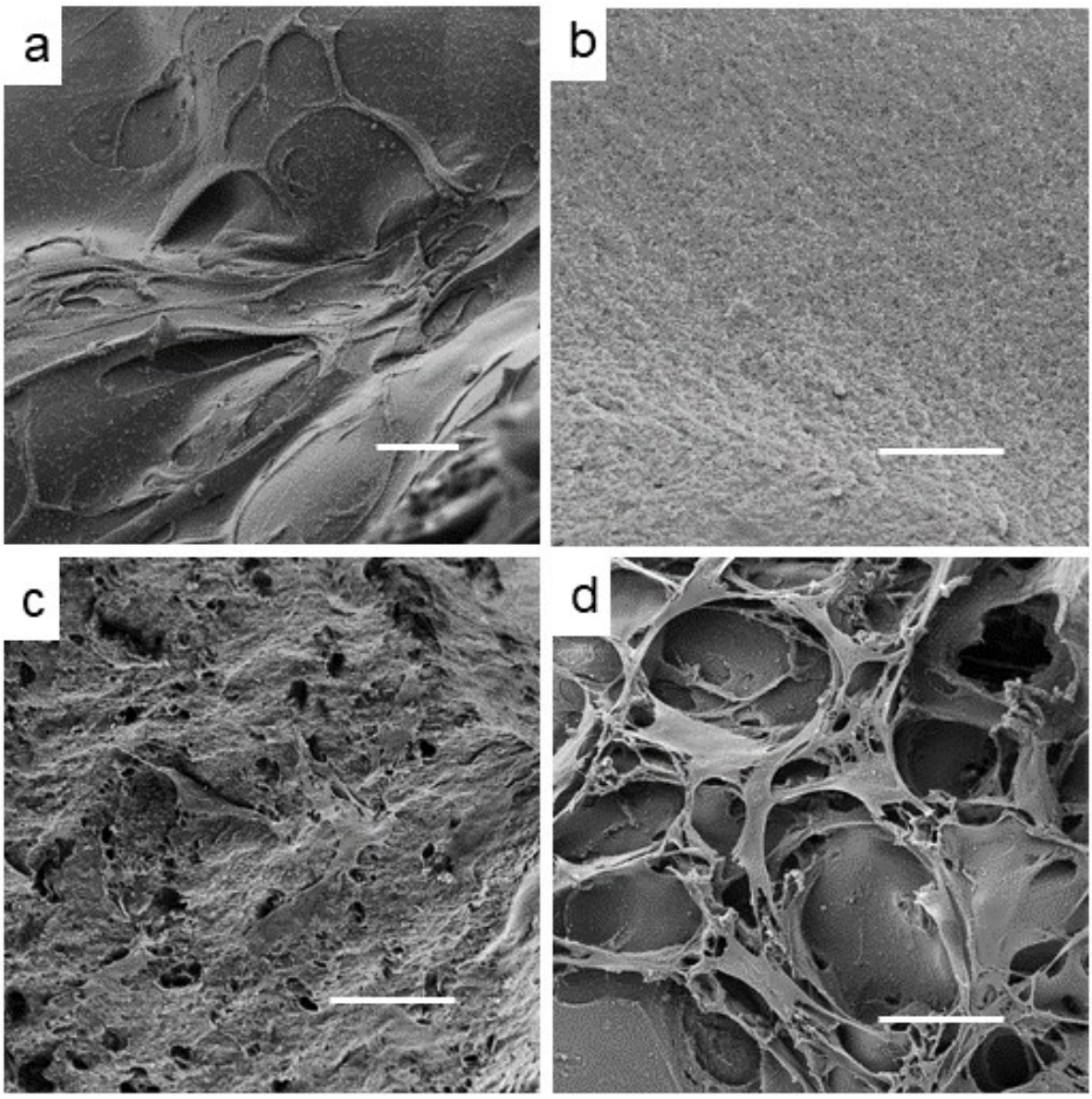

Figure S6. SEM images of hDFs on (a) gelatin hydrogel, (b) silk hydrogel, (c) 1-IPN and (d) 2-IPN.