Supporting Information

Transparent, nanostructured silk fibroin hydrogels with tunable mechanical properties

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Supporting Materials and Methods

Acetone detection

Salicylaldehyde was used to measure trace amounts of acetone within the hydrogel samples. 0.4 ml of 10.6 M sodium hydroxide was added to the hydrogel samples before diluting the solution with 5 ml of water. The solution was mixed and 0.12 ml of salicylaldehyde was added to the solution. 4 ml of 10.6 M sodium hydroxide was added, and the solution was incubated at room temperature for 2 hours before the absorbance was measured at 474 nm. The amount of acetone in the samples was evaluated from a standard curve prepared as previously described.[1,2]

Fourier transform infrared spectroscopy

FTIR analysis of hydrogel samples was performed in a JASCO FTIR 6200 spectrometer (JASCO, Tokyo, Japan) in attenuated total reflectance (ATR). Hydrogels were let to dry on a glass slide. For each sample, 64 scans were coded with a resolution of 1 cm^{-1}, with a wave number range from 4000-650 cm^{-1}.

Inductively coupled plasma-atomic emission spectroscopy (ICP-AES)

ICP-AES analysis of the hydrogels samples was performed on a Prodigy/Prism High Dispersion ICP (Teledyne Instruments, Hudson, NH, USA). Multi element-specific detection of Mg^{2+} (279.553 nm), Ca^{2+} (317.933 nm), and Cu^{2+} (324.754 nm) was achieved

at a flow rate of 1.4 ml/min and RF power of 1.2 kW measured in axial mode. The detector technology in the Prodigy allows for simultaneous measurements of the peak and the background emission to generate the net emission intensity. Scans were performed using the time-resolved-analysis mode (Salsa software) and data was acquired for 10 seconds in triplicate. Samples were dissolved in warm 10% nitric acid until there were no visible aggregates before measuring.

Figure S1

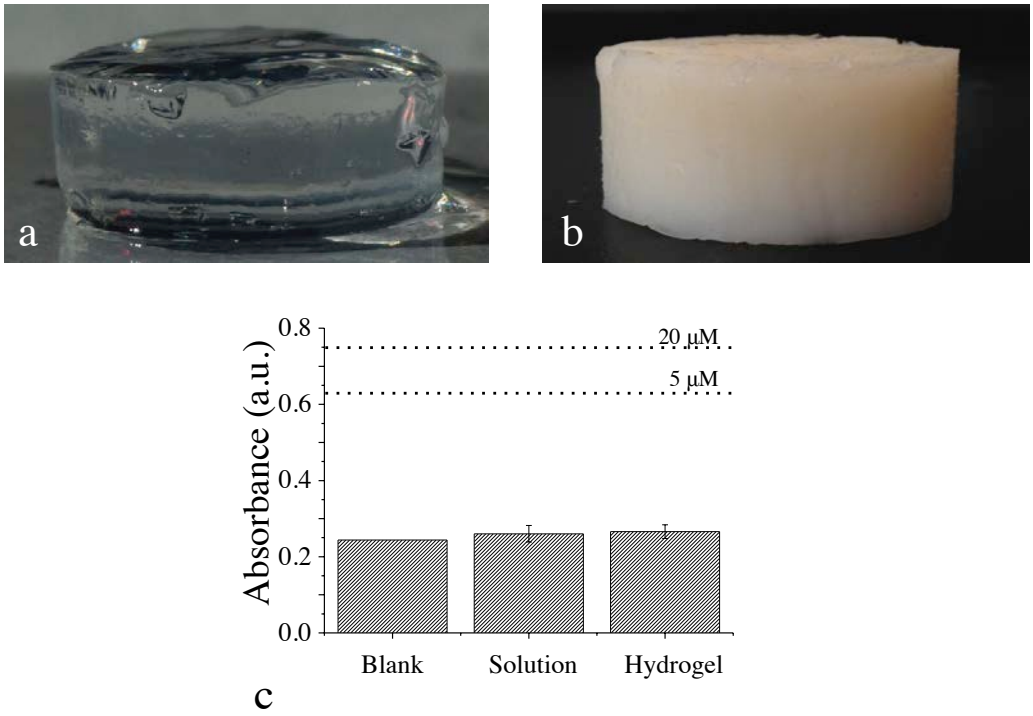

Figure S1: Macroscopic comparison between silk hydrogels obtained through organic solvent addition and through sonication. a) Image of a silk hydrogel obtained through gelation depicts the transparent nature of the hydrogel. b) Image of a silk hydrogel obtained via sonication, with the typical white color due to light scattering. For both gels, d=10 mm c) Absorbance measurements for levels of acetone in silk hydrogels compared to silk solution and a water blank. Lowest detected physiological metabolized acetone is 15 µM.[1]

Figure S2

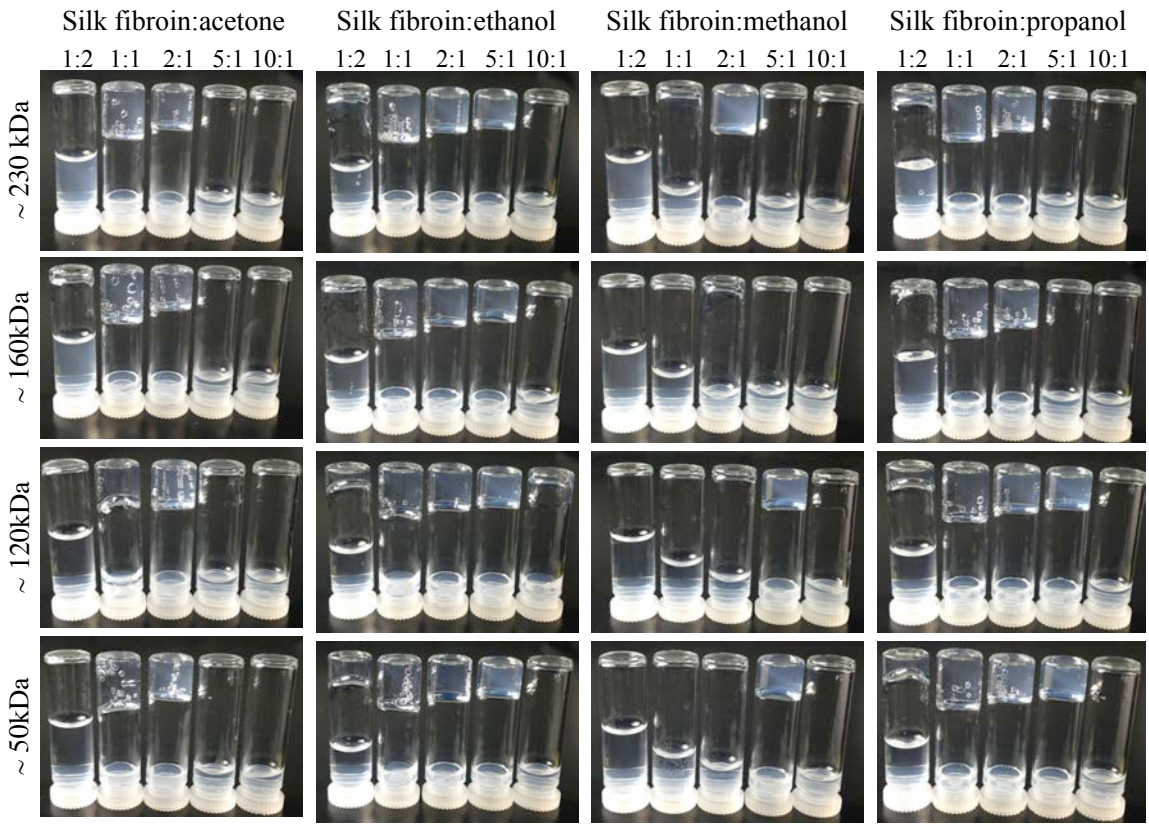

Figure S2: Effects of polar organic solvent treatments, silk fibroin molecular weight and silk fibroin:solvent ratios on gel formation. Original concentration of silk fibroin in water solution was 10 mg/ml and pictures were taken after one hour of treatment with organic solvents. The images depict the range of gelation and silk processing parameters (polar organic solvent treatments, silk fibroin molecular weight and silk fibroin:solvent ratios) within which gelation can be achieved.

Figure S3

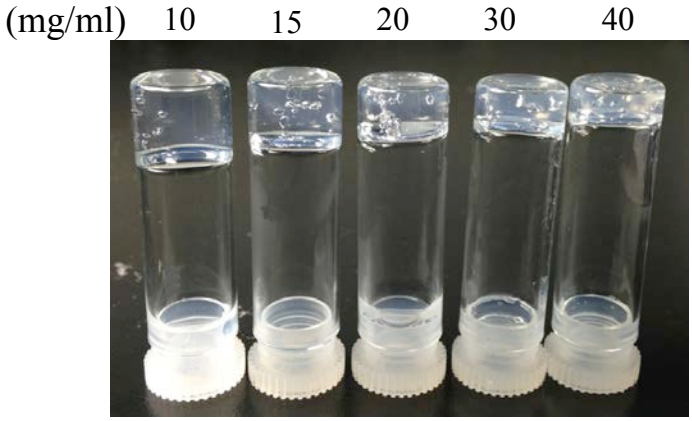

Figure S3: Gelation of silk fibroin in acetone at increasing silk fibroin concentrations. A concentration-dependent increase in light scattering was visible. The volume of silk fibroin solution was maintained constant throughout the experiment. Silk fibroin gelation was not achieved outside the indicated silk solution concentrations.

Figure S4

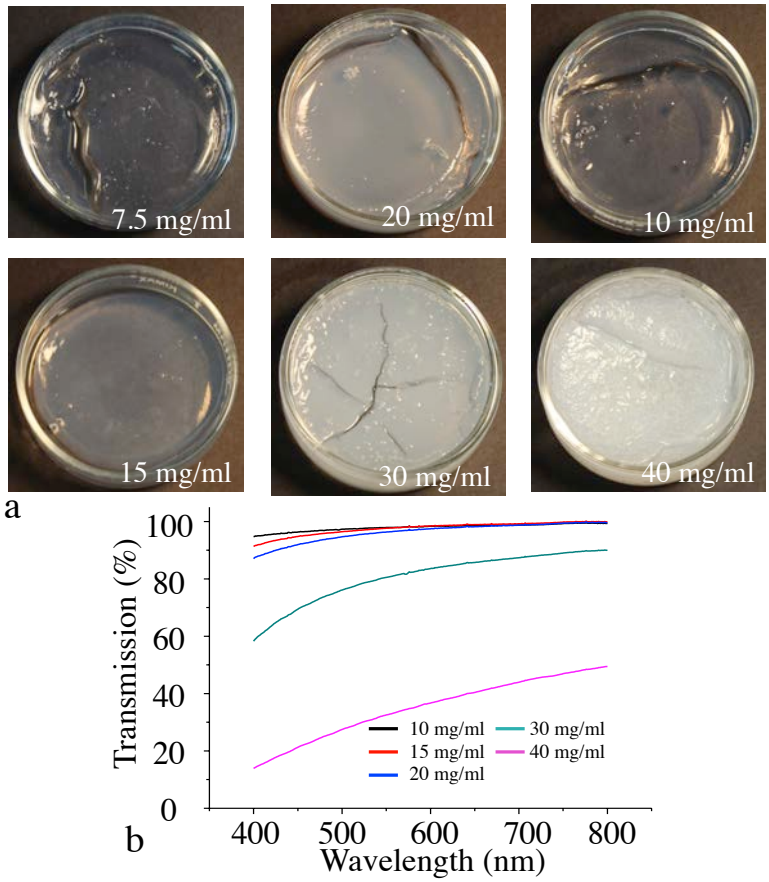

Figure S4. Effect of silk fibroin concentration on gelation of silk fibroin hydrogels. a) Photographs of silk fibroin hydrogels obtained through gelation at varying concentrations of silk fibroin solution in a 40 mm wide Petri dish. A concentration dependent increase in light scattering was visible and b) was quantified through optical transmission measurements. Optical clarity was maintained for silk fibroin concentrations < 15 mg/ml.

Figure S5

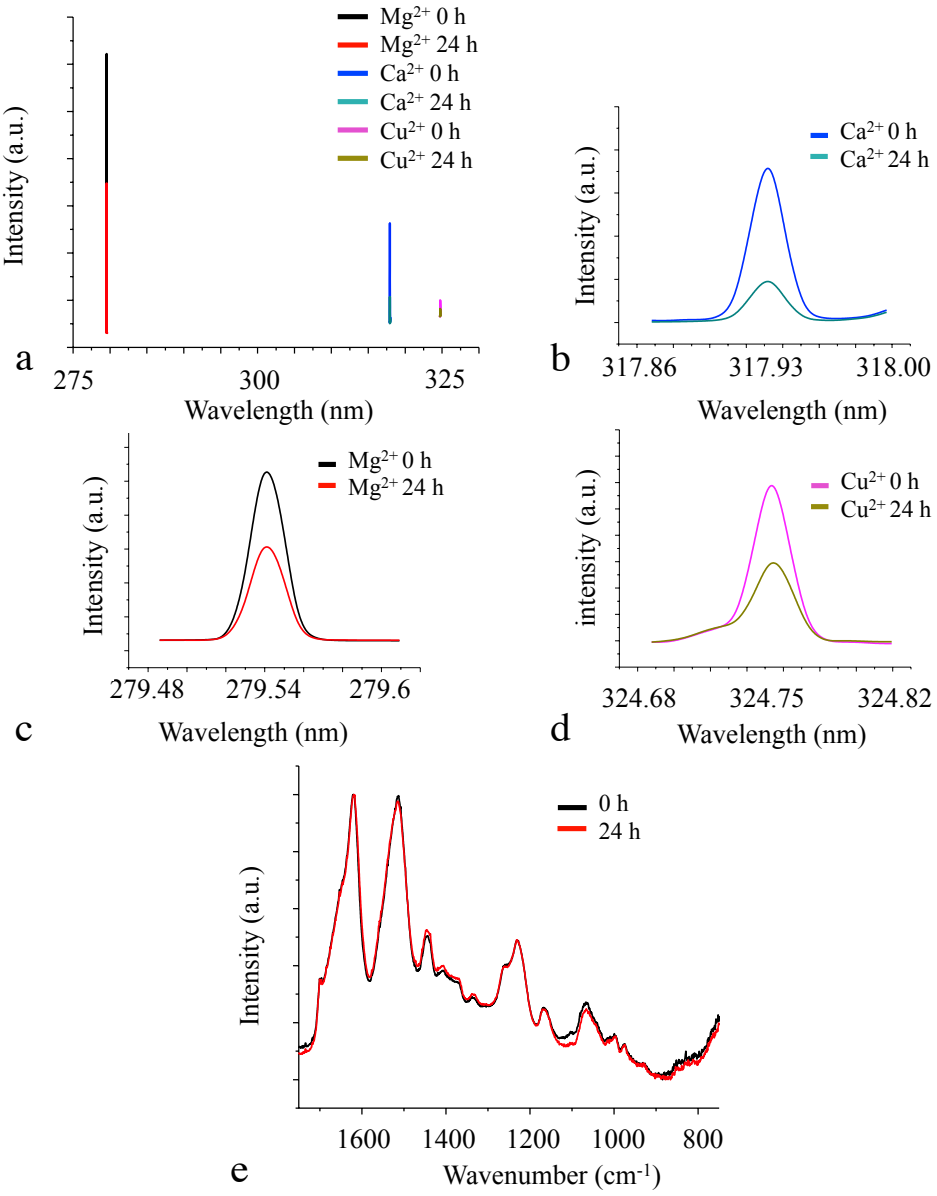

Figure S5: Effect of conditioning in Tris/EDTA solution on the properties of silk fibroin hydrogels. Gels were conditioned in 20 mM EDTA solution for 0 h and 24 h before being rinsed in deionized water. a) ICP-AES spectra of metal ions found in the silk fibroin hydrogels after conditioning in Tris/EDTA solution for 0 h and 24 h. Individual atomic emission spectra of b) Ca^{2+}, c) Mg^{2+}, and e) Cu^{2+} which relates to ion concentration found in the dissolved hydrogels before and after conditioning with EDTA solution. e) ATR-FTIR spectra of silk fibroin hydrogels as made and upon EDTA treatment.

Figure S6

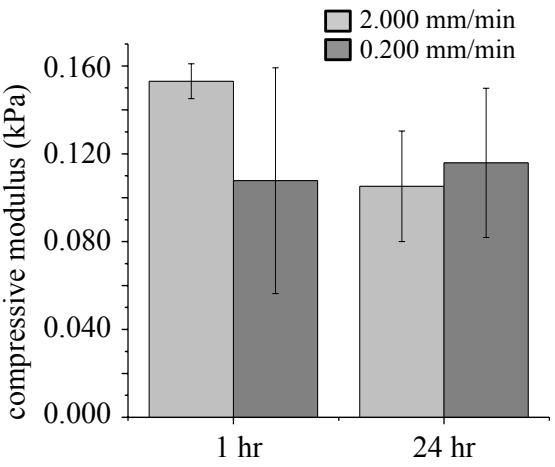

Figure S6: Effect of Tris solution on the mechanical properties of silk hydrogels. Compressive modulus of silk fibroin hydrogels at 1h and 24h of conditioning in Tris solution. Mechanical test were performed at two crosshead speeds. There was no statistically significant effect of the treatment on the mechanical properties of the hydrogels.

Figure S7

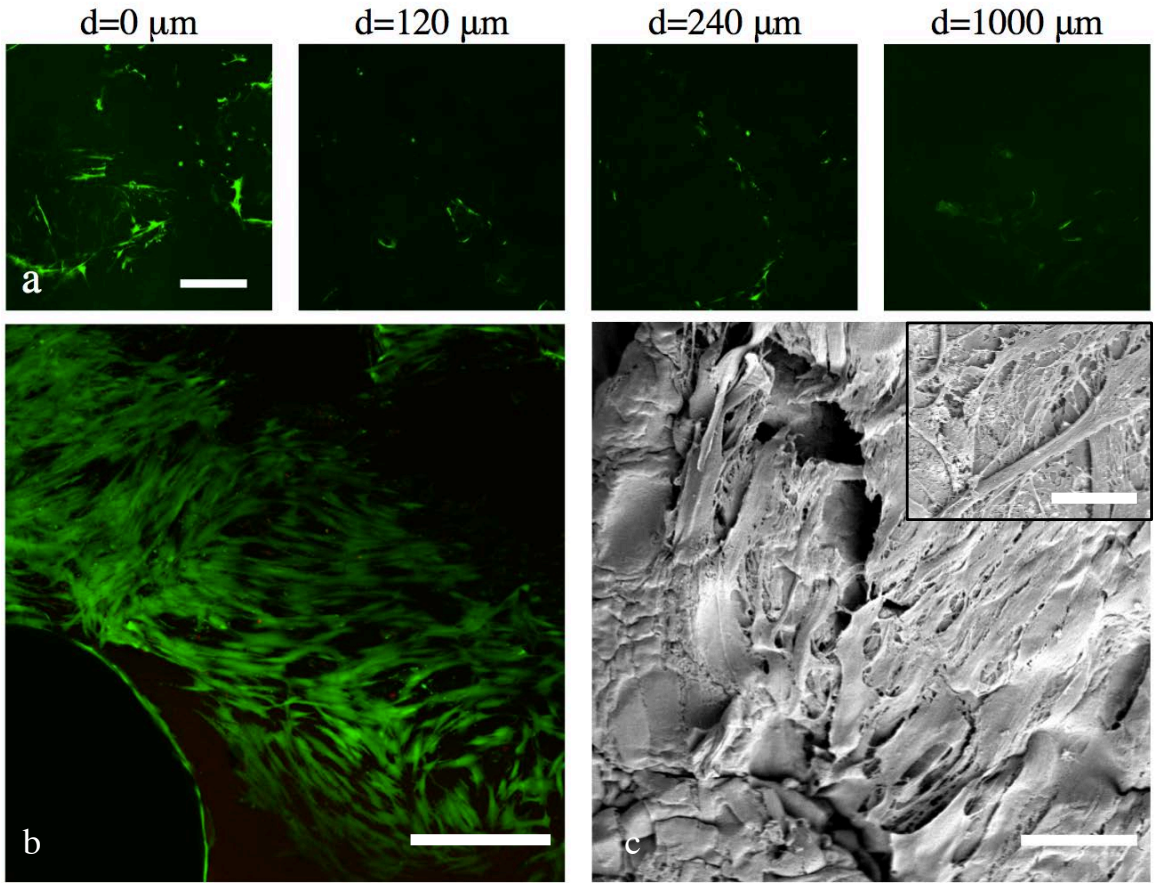

Figure S7: Cytocompatibility of silk fibroin hydrogels obtained through gelation. Human dermal fibroblasts (HDFa) were cultured on silk fibroin hydrogels. a) Confocal microscope images using live/dead assay revealed the presence of HDFa at different depths within the hydrogel at day 7 in culture. Subsequent images signify a depth scan at the surface (depth equal to 0 μm), 120 μm from the surface, 240 μm from the surface, and 1000 μm from the surface. Scale bar is 375 μm. b) Maximum intensity projection of HDFa on silk fibroin hydrogels at day 7 in culture showed that fibroblasts were well spread on the hydrogel. Scale bar is 375 μm. c) SEM micrograph of HDFa cultured on silk fibroin hydrogel at day 7 showed production of extracellular matrix by cell activity (inset image). Scale bar is 30 μm for the main image and 10 μm for the inset one.

Figure S8

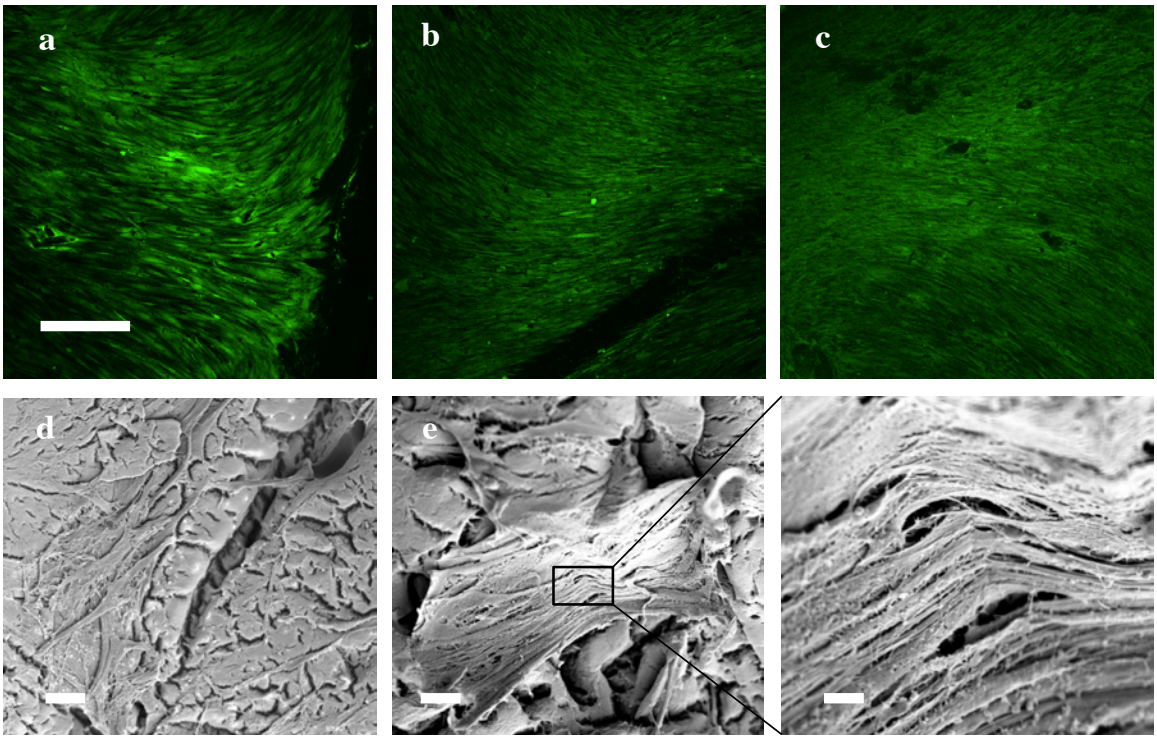

Figure S8: Cytocompatibility of silk fibroin hydrogels obtained through gelation. Human dermal fibroblast (HDFa) were cultured on silk fibroin hydrogels a-c) Maximum intensity projection of HDFa stained with live/dead assay at (a) day 7, (b) day 14, and (c) day 28 in culture showed that fibroblast were well spread on the hydrogel for all the time points considered. Scale bar is 375 μm. SEM micrographs cellular gels at (d) day 7 and (e) day 28 were collected to investigate cell morphology and production of extracellular matrix. Scale bar is 20 μm. The enlarged micrograph shows close up of extracellular matric deposition. Scale bar is 2 μm.

Figure S9

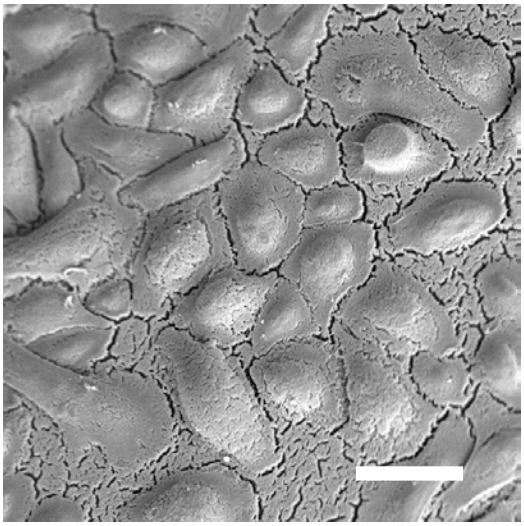

Figure S9: SEM micrograph of epithelial cornea cells cultured on the silk hydrogel at day 7 in culture. Scale bar is 40 μm.

References

(1) Kalapos, M. P. On the mammalian acetone metabolism: from chemistry to clinical implications. *Biochim. Biophys. Acta - Gen. Subj.* **2003**, *1621* (2), 122–139 DOI: 10.1016/S0304-4165(03)00051-5.

(2) Berntsson, S. Spectrophotometric determination of acetone by the salicylaldehyde method. *Anal. Chem.* **1956**, *28* (8), 1337.