Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information.

Controlled Radical Polymerization of Hydrophilic and Zwitterionic Brush-Like Polymers from Silk Fibroin Surfaces

Danielle L. Heichel[a], *Ngoc Chau Vy*[a], *Shawn P. Ward*[b], *Douglas H. Adamson*[a,b], *Kelly A. Burke*[a,c,d]

[a] Polymer Program, Institute of Materials Science, University of Connecticut, 97 North Eagleville Road Unit 3136, Storrs, CT 06269-3136, United States

[b] Department of Chemistry, University of Connecticut, 55 North Eagleville Road Unit 3060, Storrs, CT 06269-3060, United States

[c] Department of Chemical and Biomolecular Engineering, University of Connecticut, 191 Auditorium Road Unit 3222, Storrs, CT 06269-3222, United States

[d] Department of Biomedical Engineering, University of Connecticut, 260 Glenbrook Road Unit 3247, Storrs, CT 06269-3247, United States

Methods.

Gel Permeation Chromatography (GPC). During the SI-ATRP reactions on the silk fibroin films, a few drops of sacrificial initiator (α-Bromoisobutyryl bromide, BIBB) were added to generate polymers in solution to approximate the molecular weights of the brush-like polymers. The polymers were isolated from the reaction mixture by dialysis (3500 MWCO regenerated cellulose tubing, Fisher Scientific, Hampton, NH) against water, and the polymers were collected by evaporating the water at 40°C overnight. The molecular weights were determined by using a Waters GPC-2 (Milford, MA) equipped with a Waters 515 HPLC pump, two Agilent PL aquagel-OH MIXED-M 8 um columns (Santa Clara, CA), and a Waters 2414 refractive index detector. The polymer samples were prepared at 0.5 mg/mL in water and filtered with a 0.45 μm PVDF filter prior to injection. Water was used as an eluent at a flow rate of 1 mL/min at 25°C, and the molecular weight was determined by a calibration curve constructed from poly(ethylene oxide) standards. A limitation of this measurement is that the molecular weight of polymers synthesized using the sacrificial initiator in solution will not be the same as the brush-like polymers polymerized from the surface.

LIVE/DEAD Imaging. Films with compositions of SF_{WVA}, SF_{OX}, SF_{OX}-zwitter, and SF_{OX}-PEG (area = 0.95 cm^2) were sterilized with ethanol for 12 h and rinsed 6 times with dH_2O to remove residual ethanol. hMSCs (Passage 4) were added at a density of 105,000 cells/film and allowed to attach for 24 h. After 24 h, the medium was aspirated and the films were rinsed with 1X PBS (300 μL) and transferred to glass bottom dishes. Attached cells were stained using the LIVE/DEAD Cell Imaging Kit (488/570) (Product R37601, Thermo Fisher, Waltham, MA) following the manufacturer's instructions. The films were imaged using a Zeiss Axiovert 200M microscope, equipped with fluorescence filters. The images were converted to 8-bit in ImageJ Fiji (NIH, Bethesda, Maryland), and a background subtraction was applied to remove artifacts. The entire image for each film composition was selected and a profile plot was generated to obtain the pixel intensity of the images.

Conjugate Reaction	Molecular Weight (g/mol)
Zwitter	11,000
PEGMA	8,000

Table S1. Molecular weight of polymers prepared using sacrificial initiator.

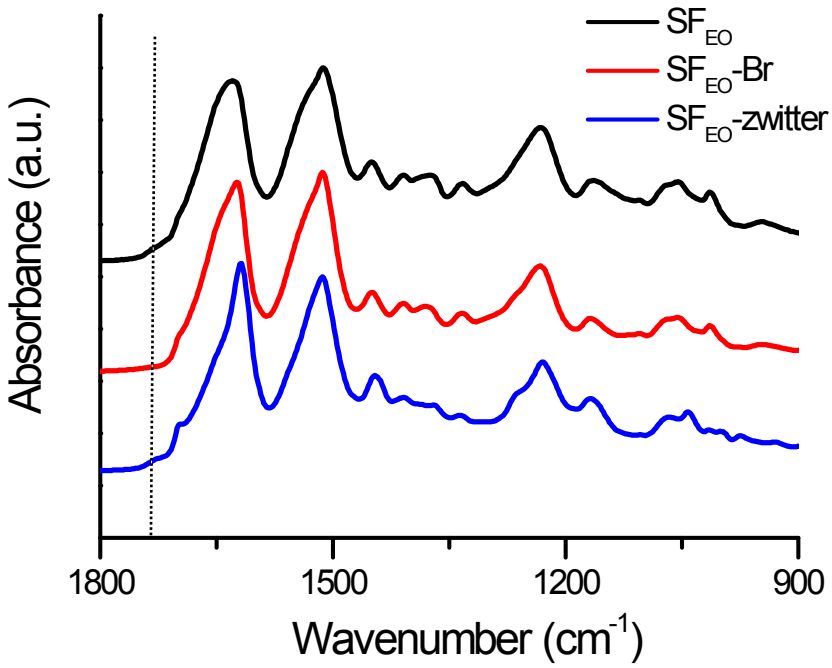

Figure S1. ATR-FTIR spectra of modified SF films that were initially treated with methanol. Reactive sites were enriched using EO treatment. Reference line shown at ~1730 cm^{-1}.

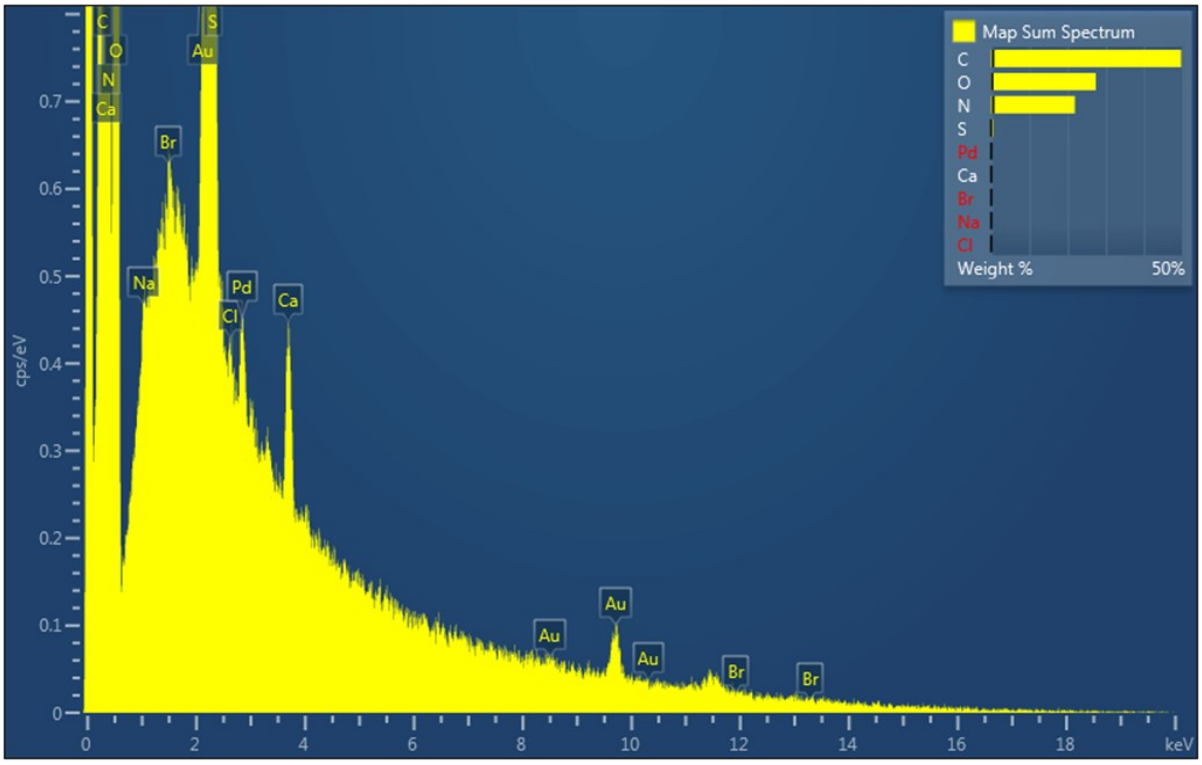

Figure S2. SEM-EDS elemental analysis of **SF_{ox}-Br**.

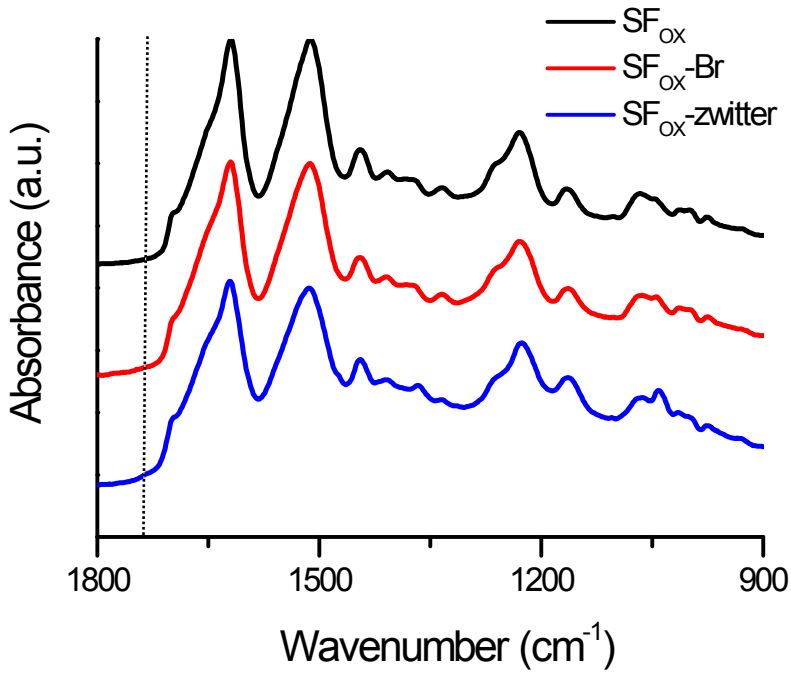

Figure S3. ATR-FTIR spectra of modified SF films that were treated with methanol using OX synthetic route. Reference line shown at ~1730 cm^{-1}.

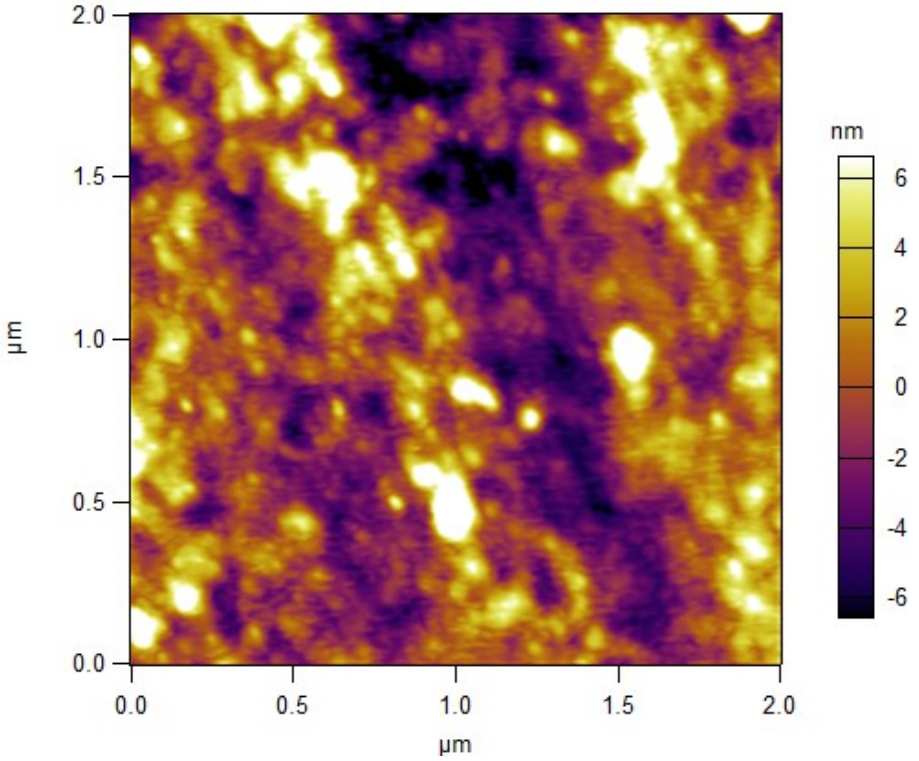

Figure S4. AFM height micrograph of **SF_{EO}-zwitter**. This film was initially treated with methanol (instead of “as cast” like in the manuscript) and reactive sites were enriched using ethylene oxide.

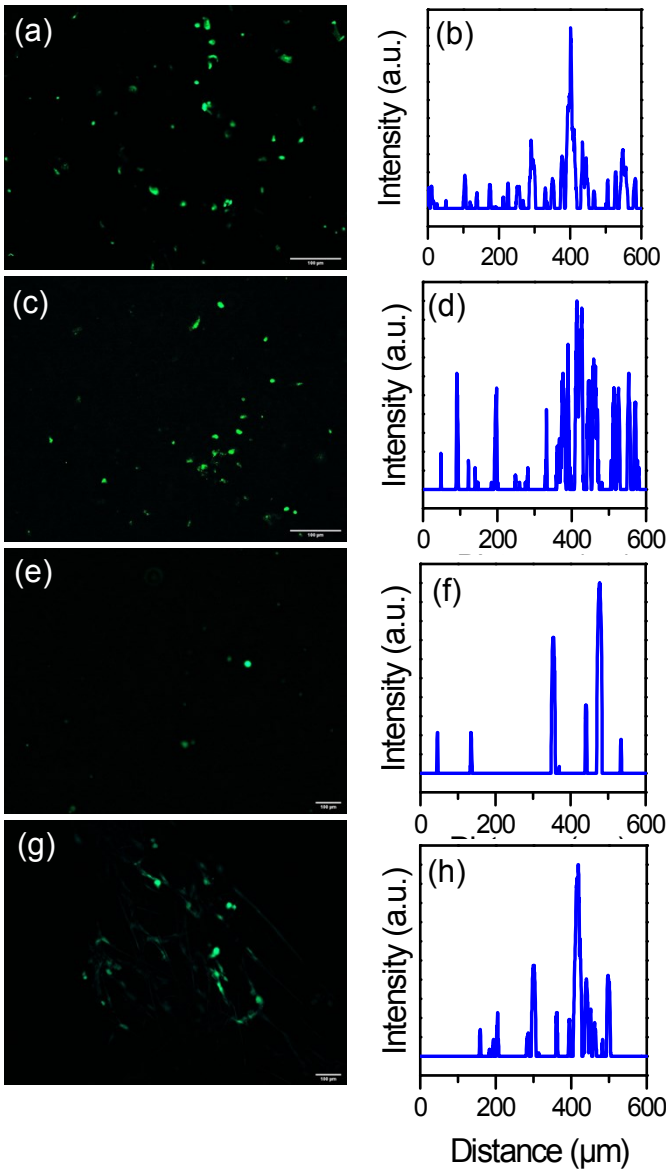

Figure S5. Images of live cells (green) and fluorescence intensity plots for the entire image area for (a,b) Unmodified **SF_{WVA}**, (c-d) **SF_{ox}**, (e-f) **SF_{ox}-zwitter**, (g-h) **SF_{ox}-PEG**. Scale bars represent 100 μm.

Safety and Hazards.

Ethylene oxide is extremely flammable and reactive. During the reaction, ethylene oxide should be used under inert conditions and the application of heat should be avoided. Ammonium persulfate is an oxidizer and an irritant; it should be used in a well-ventilated area (fume hood) and should be kept away from heat sources. α-Bromoisobutyryl bromide is corrosive and caution must be taken to avoid skin contact and inhalation of vapors. Methanol is a flammable solvent and should be kept away from heat and used in a fume hood.