Supporting Information to: Facile Photochemical Modification of Silk Protein-Based Biomaterials

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Experimental

Materials

Unless otherwise stated, all chemicals were of ACS grade, purchased from Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany) and used as supplied. Naturally spun Bombyx mori silk fibers were purchased from ebay.com (supplied in the form of cocoons from a supplier based in the USA), and purified (degummed) as described previously.[34] The dragline silk produced by *A. diadematus* spiders is predominantly composed of two proteins, *A. diadematus* fibroin 3 and 4, ADF3 and ADF4 respectively. Engineered silk protein analogues of these proteins (eADF3 and eADF4 respectively) were optimized for production in *Escherichia coli* bacteria.[33] The recombinant variant eADF4(C16) used herein is composed of sixteen repeats of the polypeptide module C (amino acid sequence: GSSAAAAAAAASGPGGYGPENQGPSGPGGYGPGGP).[33]

Preparation of Silk Protein-Based Materials

B. mori silk fibers were used after degumming with Na_2CO_3, followed by vacuum drying, yielding *B. mori* fibroin (BMF) fibers.[34] Films of eADF4(C16) were prepared by spin casting by adapting our previously described methodology[35]. Films of eADF4(C16) were prepared by spin casting by adapting our previously described methodology. A glass slide was fixed on the hollow axis of the rotor of a spin-coater (WS-400-6NPP/LITE, Laurell Technologies, North Wales, USA) by a vacuum. In a control program at first the rotation rate was automatically adjusted to 1750 rpm for 50 s and then to 2250 rpm for 25 s. Upon starting the program 600 µL of the silk protein solution was added drop wise onto the middle point of the rotating target. The solvent was allowed to evaporate over a period of 24 hours in a fume hood, and the films were then immersed in anhydrous methanol for 1 hour prior to drying for a further 48 hours under high vacuum, followed by washing the films with ethanol/water 70:30 (v/v) and drying in a sterile fume hood for 48 hours.

Photochemical Modification of Silk Protein-Based Biomaterials

The light source used for photochemical modification of materials was a 150W mercury medium pressure lamp (Heraeus TQ 150). Irradiation occurred through the borosilicate glass (Duran) of the reaction vessel, filtering off the high-energy UV light with $\lambda < \sim 300$ nm. Degummed BMF fibers (1g) and films made of eADF4(C16) on glass cover slips (2.5 cm x 2.5 cm) were immersed in an aqueous solution of monomer (acrylic acid, methacrylic acid or

allylamine) at a concentration of 2 mg/mL, that had been degassed twice. The container was put under an inert argon atmosphere and exposed to UV light, sample-to-lamp distance: 10-20 cm, for 48 hours. The acrylic acid/methacrylic acid modified samples were washed with HEPES buffer (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 1L, pH 8.0), then ethanol (50 mL), then ultrapure water (1.5L, 18 MΩ); the allylamine modified samples were washed with sodium acetate buffered water (1L, pH 5.5), ethanol (50 mL), ultrapure water (1.5L, 18 MΩ). All samples were subsequently washed with water and methanol, dried under high vacuum for 48 hours, and then washed with ethanol/water 70:30 (v/v) and dried under high vacuum for 48 hours.

Mineralization of Materials with Calcium Carbonate

Silk-based materials (BMF fibers or eADF4(C16) films, either unmodified or modified with PAA or PMAA), were incubated in an aqueous solution (1 mL) of calcium chloride (10 mM). The films were placed in an airtight container with a beaker containing ammonium carbonate for 24 hours. The samples were subsequently washed with water until the pH was neutral, and then rinsed with ethanol/water 70:30 (v/v) and dried under high vacuum for 48 hours, after which they were imaged by SEM.[26]

Mineralization of Materials with Silica

Silk-based materials (eADF4(C16) films) modified with PAAm were incubated in phosphate buffer (0.2 mL, pH 5.5). Tetraethylorthosilicate (2.33 mL), water (3.85 mL), ethanol (3.85 mL) and 1N HCl (0.1 mL) were mixed and incubated for 10 minutes at room temperature, and aliquots of this solution (20 µL) were added to the phosphate buffer covering the PAAm modified silk films. The reaction mixture was incubated for 1 hour after which it was removed and the films were washed thoroughly with Millipore water, rinsed with aqueous ethanol (70%) and dried under high vacuum for 48 hours, after which they were imaged by SEM.

Stem Cell Culture

Commercially available Nunclon® Δ surface tissue culture plates were used for control experiments. Silk films with thicknesses of ca. 100 µm (as determined with high precision digital calipers (Bochem, Weilberg, Germany)) were prepared by casting in Nunclon® Δ surface tissue culture plates, and subsequently photochemically modified, and mineralized as described above. The films were sterilized by incubation in 70% ethanol solution in a laminar flow cabinet followed by exposure to UV for 60 minutes in a laminar flow cabinet. After sterilization, the samples were incubated for 30 minutes under 3 mm of HMSC growth medium in a laminar flow cabinet. HMSC growth medium was composed of: high glucose Dulbecco's Modified Eagle Medium (DMEM, 440 mL); fetal bovine serum (50 mL); antibiotic-antimycotic (5 mL); non-essential amino acids (5 mL), and 2 ng/mL basic fibroblast growth factor. Medium was aspirated and replaced prior to HMSC seeding. Cell viability before starting the experiment was determined by the Trypan Blue exclusion method, and the

measured viability exceeded 95% in all cases. HMSCs were seeded at 10,000 cells/cm^2 under 3 mm of medium, and incubated at 37°C, 95% humidity, and a CO_2 content of 5%. After 3 days the medium was aspirated in a laminar flow cabinet, the films were washed gently with PBS and replaced with osteogenic medium. Osteogenic medium was composed of: high glucose Dulbecco's Modified Eagle Medium (DMEM, 425 mL); fetal bovine serum (50 mL); antibiotic-antimycotic (5 mL); non-essential amino acids (5 mL), dexamethasone (100 nM), β-glycerol phosphate (10 mM) and ascorbic acid (50 μM). Thereafter the osteogenic medium was aspirated in a laminar flow cabinet and replaced every 2 days until the samples were analysed. Alkaline Phosphatase (ALP) activity was visualized with a Leukocyte Alkaline Phosphatase Kit using the manufacturer's protocol. Images of stained cells were obtained using a camera AxioCam MRm attached to a Zeiss Axio Observer Z1 equipped with an ApoTome unit. Images are representative of 3 samples.

Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS)

Samples were mounted on metal stubs, coated with Pt/Pd or Carbon using a Cressington 208 benchtop sputter coater before being observed with a Hitachi S5500 SEM equipped with an EDS probe.

Water contact angle measurements

Measurements were carried out with a high-speed contact angle measurement device (OCAH 230 video-based semi-automatic contact angle measurement device supplied by Dataphysics GmbH, Germany). Images of a drop of deionized water (2 μL) laid on the surface of the samples were recorded at a frame rate of 360 frames per second, and the contact angles for the droplets were recorded after 3 seconds of contact with the film. Prior to measurement, the films were stored as described in the tensile testing section. The reported values are the average of at least 3 measurements at different positions on a film.

Fourier Transform infrared (FTIR) spectroscopy

FTIR spectra of films were recorded by attenuated total reflection (ATR) on a Bruker Tensor 27 spectrometer equipped with a Ge crystal (Bruker, Germany) using the average of 60 spectra recorded at a resolution of 1 cm^{-1}.

X-ray photoelectron spectroscopy (XPS)

XPS was performed on a Kratos Axis X-ray photoelectron spectrometer (Kratos Analytical Ltd., Manchester, UK). All XPS spectra were collected at 90° take-off angle. The binding energy was calibrated using the C 1s photoelectron peak at 284.6 eV as a reference. The CasaXPS computer program was used for peak fitting of the C 1s and O 1s peaks in the XPS spectra. The reported spectra are representative of two measurements at different positions on a film.

Thermogravimetric analysis (TGA)

Analyses were carried out with a Mettler Toledo TGA/SDTA 851E thermobalance (Mettler Toledo GmbH, Giessen, Germany). Films were precisely weighed into ceramic crucibles (VWR, Germany), and analyses were carried out under a nitrogen atmosphere (flow rate 100 mL per minute), over a temperature range between 25 and 600 °C, at a heating rate of 10 °C per minute. The TGA mass loss profiles are representative of at least 2 samples.

Differential Scanning Calorimetry (DSC)

Analyses were carried out with a DSC Q1000 (TA Instruments, Hüllhorst, Germany), using aluminum pans. Samples were precisely weighed into aluminum pans (TA Instruments, Hüllhorst, Germany), and analyses were carried out under a nitrogen atmosphere (flow rate of 100 mL per minute). The samples were treated as follows: heated from room temperature to 110 °C (10 °C per minute), cooled to -40 °C (10 °C per minute), and finally heated from -40 °C to 400 °C (10 °C per minute). The DSC thermographs are representative of at least 2 samples.

Tensile Testing

The Young's modulus, tensile strength and elongation at break were carried out with an Instron Universal Testing Machine (model 5565, Instron Deutschland GmbH, Germany) equipped with a 10 N load cell, at a drawing rate of 0.6 mm/min. Prior to testing, the fibers were stored at 21 °C in an air tight Teflon container (Hagebaumarkt, Germany) in the presence of a glass beaker containing a saturated aqueous solution of calcium chloride (in order to obtain an atmosphere with a relative humidity of 31%) for 1 week. Fibers with lengths of 10 mm were cut with a razor blade; their diameters were determined with high precision digital calipers (Bochem, Germany) and were the average of at least 5 positions on the strip. The initial grip separation was set at 2 mm, and the experiments were carried out at 21 °C in a laboratory with a relative humidity of ca. 50%, and the tensile properties reported are the average of at least 5 measurements. When testing under tension, the fibers did not fail in the center as would be desirable, consequently it was not possible to obtain accurate yield strengths, extension at break etc., and therefore the only data presented is Young's modulus.

Supplementary Data

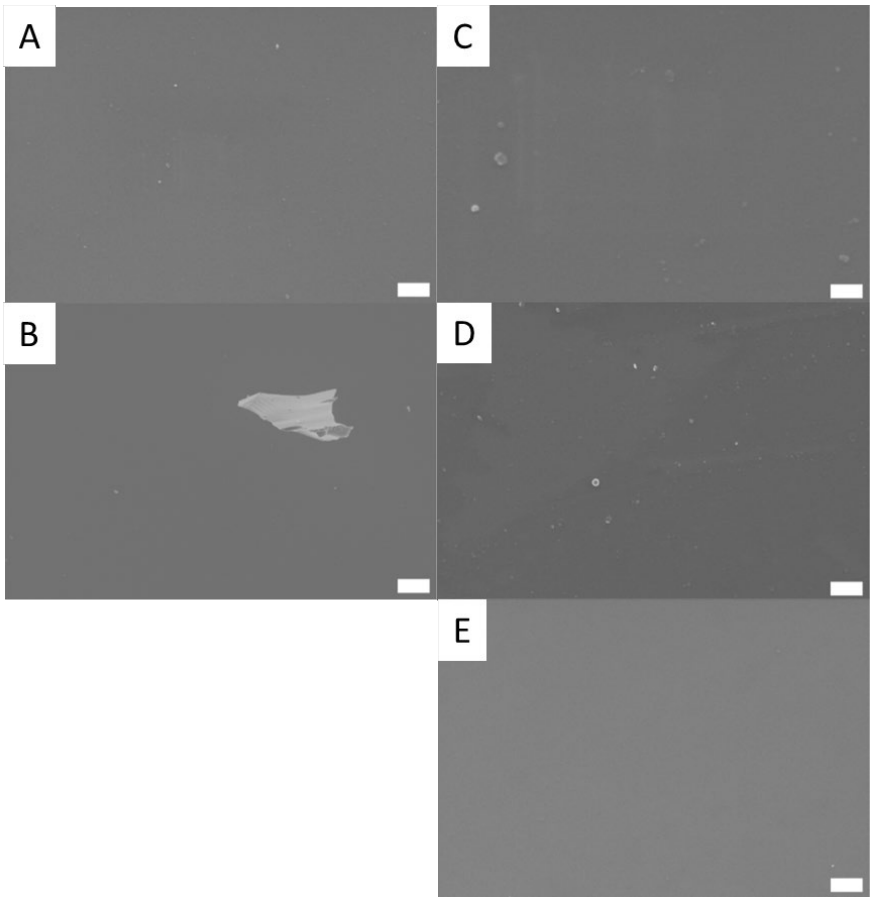

Figure S1. SEM images of silk-based films. A) Spin-coated film of eADF4(C16). B) Spin-coated film of eADF4(C16) exposed to UV light in the dry state. C) Spin-coated film of eADF4(C16) exposed to UV light in acrylic acid. D) Spin-coated film of eADF4(C16) exposed to UV light in methacrylic acid. E) Spin-coated film of eADF4(C16) exposed to UV light in allylamine. Scale bars represent 4 μm.

Table S1. Water contact angle analysis of unmodified and polymer modified silk-based films.

Material	Water Contact Angle (°)
eADF4(C16) alone	52.7 ± 5.4
eADF4(C16)-Acrylic acid	51.5 ± 5.3
eADF4(C16)-Methacrylic acid	55.4 ± 2.2
eADF4(C16)-Allylamine	56.4 ± 3.7

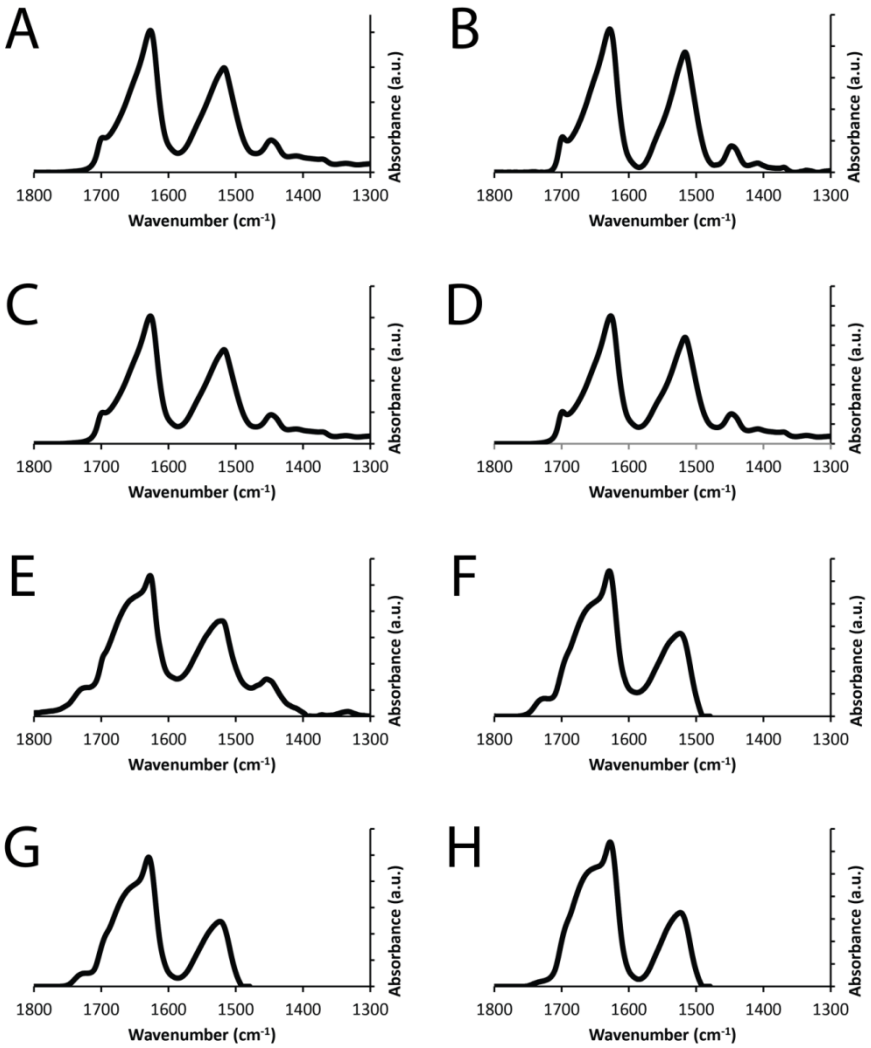

Figure S2. Fourier transform infrared (FTIR) analysis of silk-based materials. A) Degummed BMF fibers. B) Degummed BMF fibers exposed to UV light in acrylic acid. C) Degummed BMF fibers exposed to UV light in methacrylic acid. D) Degummed BMF fibers exposed to UV light in allylamine. E) Spin-coated film of eADF4(C16). F) Spin-coated film of eADF4(C16) exposed to UV light in acrylic acid. G) Spin-coated film of eADF4(C16) exposed to UV light in methacrylic acid. H) Spin-coated film of eADF4(C16) exposed to UV light in allylamine.

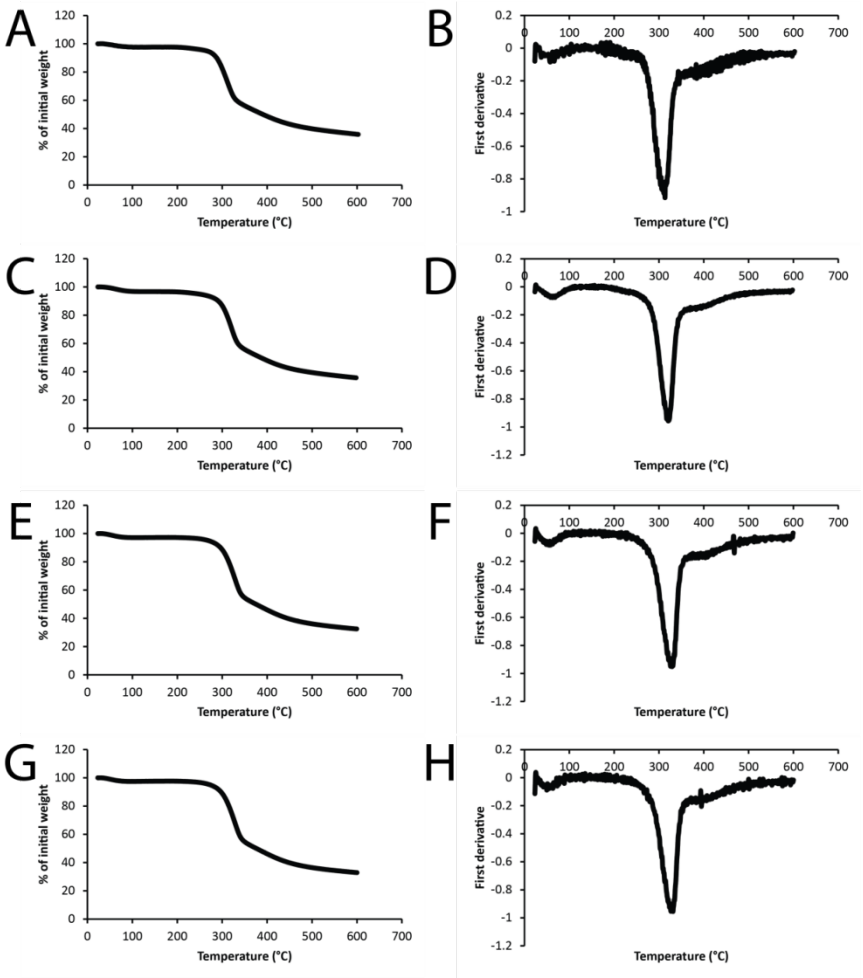

Figure S3. Thermogravimetric analysis of BMF-based materials. A) TGA profile of degummed BMF fibers exposed to UV light. B) First derivative of the TGA profile of degummed BMF fibers exposed to UV light. C) TGA profile of degummed BMF fibers exposed to UV light in acrylic acid. D) First derivative of the TGA profile of degummed BMF fibers exposed to UV light in acrylic acid. E) TGA profile of degummed BMF fibers exposed to UV light in methacrylic acid. F) First derivative of the TGA profile of degummed BMF fibers exposed to UV light in methacrylic acid. G) TGA profile of degummed BMF fibers exposed to UV light in allylamine. H) First derivative of the TGA profile of degummed BMF fibers exposed to UV light in allylamine.

Table S2. Thermal (TGA analysis) and Mechanical (Tensile) Properties of unmodified and polymer modified BMF silk fibers.

Material	Degradation Temperature (°C)	Young's Modulus (GPa)
BMF alone	311	5.7 ± 1.9
BMF-Acrylic acid	317	6.3 ± 1.2
BMF-Methacrylic acid	326	6.6 ± 1.6
BMF-Allylamine	326	5.5 ± 0.6

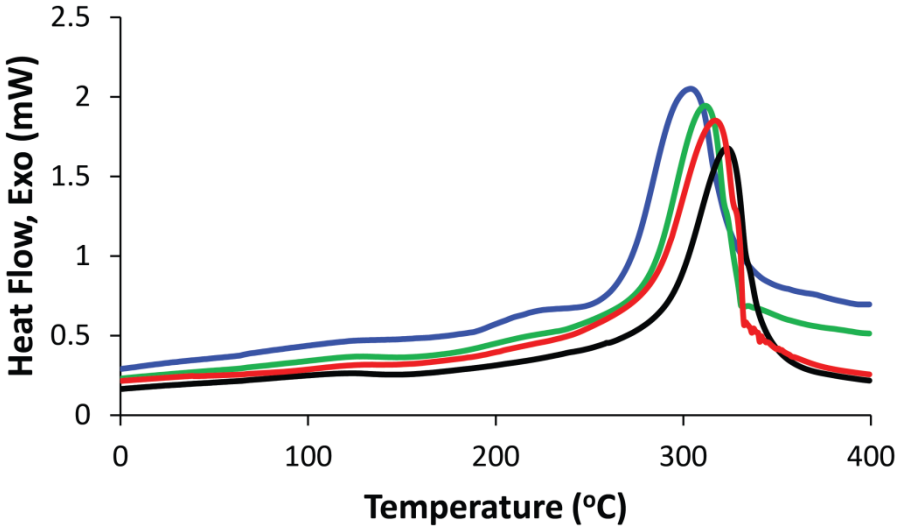

Figure S4. DSC thermographs of the second heating cycle of BMF-based materials. Blue) Degummed BMF fibers exposed to UV light. Green) Degummed BMF fibers exposed to UV light in acrylic acid. Black) Degummed BMF fibers exposed to UV light in methacrylic acid. Red) Degummed BMF fibers exposed to UV light in allylamine.

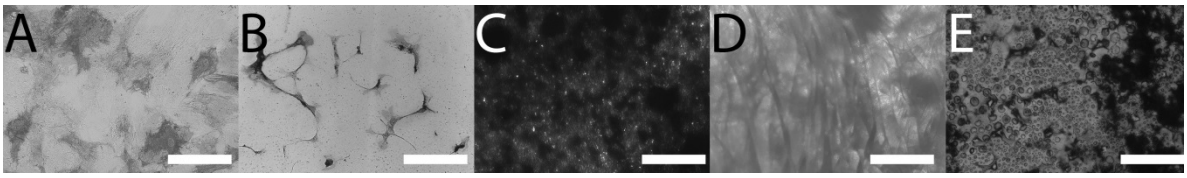

Figure S5. Qualitative analysis of ALP activity of stem cells using bright field microscopy after ALP live staining. A) Nunclon® Δ. B) eADF4(C16). C) eADF4(C16)-PAA-$CaCO_3$. D) eADF4(C16)-PMAA-$CaCO_3$. E) eADF4(C16)-PAAm-Silica. Scale bars represent 150 µm.