Supporting Information

Site-Specific Functionalization of Recombinant Spider Silk Janus Fibers

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Experimental Procedures

Protein Solutions
The genes encoding recombinant spider silk proteins eADF4(C16) and ntag\textsuperscript{Cys}eADF4(κ16) were cloned, expressed, and the proteins were purified as previously described.\textsuperscript{[1]} Subsequently, spider silk proteins were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, abcr GmbH, Germany) and mixed in an overhead shaker overnight. The respective protein concentrations were 20\% (w/v) for wet spinning and 10\% (w/v) for electrospinning.

Wet Spinning
For wet spinning, spinning dopes (20\% w/v in HFIP) were extruded through a syringe with an inner needle diameter of 0.8 mm (B. Braun Sterican\textsuperscript{®} 21 G) at a rate of 0.1 ml/min into a 500 ml beaker filled with a mixture of acetone and ethanol (80\%/20\%) as a coagulation bath. After fiber formation the fibers were taken out of the bath and wound up on a roll. Rehydration series were carried out by storing the silk fibers in 100\%, 70\%, 30\% ethanol at 21°C for 2h each and subsequent washing steps (2x) in water at 21°C for 1-2 h. The fibers were then stored in water at 21°C overnight.

Electrospinning
Sub-micrometer fiber nonwoven meshes based on recombinant spider silk proteins eADF4(C16) and ntag\textsuperscript{Cys}eADF4(κ16) were produced by electrospinning spinning dopes at a concentration of 10\% (w/v). The custom-built spinning setup was composed of two needles (B. Braun Sterican\textsuperscript{®} 21 G) that were bended together to combine the flow of the two respective spinning dopes. Furthermore, a ring electrode (diameter 1.3 cm) displaying the same charge as the needles was applied to focus the jet towards the collector. The fibers were spun at a total flow rate of 700 μL/h, voltage of 22 kV (needles) vs. -4 kV (collector), and a distance of 14 cm between the needle tips and the collector plate.

Fiber production rate calculation
The effective fiber production rate $v_{\text{fiber}}$ upon electrospinning Janus fibers was calculated based on the flow rate $V$, the spinning dope concentration $c$, the silk density $\delta_{\text{silk}}$ (~1.34 g/cm$^3$) and the mean resulting fiber radius $r_{\text{Janus}}$:

$$v_{\text{fiber}} = \frac{V c}{2\pi r_{\text{Janus}}^2 \delta_{\text{silk}}}$$

Post-Treatment
Post-treatment of Janus fibers was carried out to induce crystalline structures and stabilize the fiber structure to render the samples water insoluble. Therefore, Janus nonwovens were glued on a frame with double-sided sticky tape and pulled of the collector surface to achieve free hanging samples. The samples were placed in a closed glass vessel and preheated to 60°C in an oven chamber for
30 min. Subsequently, the post-treatment agent was injected to the vessel via a tube and post-treatment was performed for 4 h. Tree different post-treatment agents were tested: 100% ethanol (Carl Roth, Germany), 70% ethanol and 100% MilliQ water.

Secondary Structure Analysis

Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) Spectroscopy was performed to examine secondary structure changes as induced by post-treatment. Therefore, Janus fiber nonwoven mats were analyzed before and after post-treatment with 100% ethanol, 70% ethanol or pure MilliQ water vapor. FTIR spectra were recorded in the range of 4000–800 cm⁻¹ on a Ge-crystal with a Bruker Tensor 27 spectrometer (Bruker, Germany). Each spectrum results from 100 scans at a resolution of 4 cm⁻¹. The secondary structure composition was determined applying Opus software (Bruker, Germany) to perform Fourier-self-deconvolution and peak fitting of the amide I region (1705–1705 cm⁻¹) according to Hu et al.[2] (supplement S3). Of each specimen, 3 samples were measured, and the spectra were analyzed via FSD 3 times each to ensure data reliability.

Gold Coupling

For coupling AuNPs, thiol-bearing ntag²⁰⁰eADF4(x16) fibers were first incubated for 1 h in 3,3',3'-phosphinetryltripropanoic acid (TCEP) buffer (1 mM TCEP, 2 mM phosphate buffer, 15 mM sodium chloride (NaCl), 0.1 mM 2,2',2''-(ethane-1,2-diyldinitrilo)tetracetic acid (EDTA), pH 6.5) to reduce the thiol groups of the cysteine tag. A Monomaleimido Nanogold aliquot (1.4 nm colloidal, citrate stabilized AuNPs, Nanoprobes, USA) was suspended in water and incubated with the fibers. After 30 min shaking, the fibers were washed three times with water. Then, gold toning was performed by first applying Li Silver solution (prepared as recommended by the user protocol, Nanoprobes, USA) for 10 min. The fibers were washed twice with water before LM Gold Enhance solution (prepared as recommended by the user protocol, Nanoprobes, USA) was applied twice for 5 min. Finally, the fibers were washed twice with MilliQ water. The gold toning steps were performed three times resulting in AuNPs with diameters of 50 ± 14 nm.

Fourier-self-deconvolution (FSD)

FSD was performed applying Opus software (Bruker, Germany). Therefore, a baseline correction was first carried out, then the curves were smoothed over 5 points and a noise reduction of 0.3 (manufacturer-specific unit) was conducted. Then, the frequency range from 1705 - 1590 cm⁻¹ was cut out and a further baseline correction was made. In the next step, the wave numbers of the vibration bands to be expected were determined using the method of Hu et al.[2] and the curves on which the Amid I band is based were interpolated according to the local least square method with Gauss and Levenberg-Marquardt algorithms (resulting in the curve fittings as shown in Figure S1). In the last step, the determined individual spectra were integrated, and the areas of the associated structures were added. The secondary structure components result from the quotient of the added integrals and the sum of all curve integrals involved.

Microscopy

To examine structural integrity of Janus fibers after post-treatment as well as after chemical GNP binding, scanning electron microscopy (SEM) was performed using a Gemini Sigma 300 VP (Zeiss, Deutschland) at an Electron High Tension (EHT) voltage of 3 kV and a working distance of 7 mm. SEM-samples were prepared using carbon glue pads to fix the samples on SEM stubs and sputtering was performed with a 208HR gold sputter (Cressington Scientific, England).

Resistivity calculation

To calculate the resistivity of wet spun fibers based on potentiostatic measurements, the following equations were applied:

\[ R = \frac{U_{\text{max}}}{I_{\text{max}}} \quad \text{and} \quad \rho = \frac{RA}{L} \]

with \( R \)=resistance, \( U_{\text{max}} \)=maximal applied voltage, \( I_{\text{max}} \)=measured current at \( U_{\text{max}} \), \( \rho \)=resistivity, \( A \)=cross-sectional fiber area, and \( L \)=fiber length

The resistivity of the gold layer alone was estimated with the simplification that the gold layer thickness \( h_{\text{gold}} \) is 50 nm (mean diameter of gold nanoparticles) and has no porosity. Furthermore, from the potentiostatic data, it was concluded, that silk alone is an isolator and AuNPs are solely responsible for changes in the electrical properties. Consequently, the conductive cross-sectional area \( A_{\text{gold}} \) of the gold layer and the respective resistivity \( \rho_{\text{gold}} \) was calculated as follows:

\[ A_{\text{gold}} = 2\pi r_{\text{fiber}} h_{\text{gold}} \quad \text{and} \quad \rho_{\text{gold}} = \frac{RA_{\text{gold}}}{L} \]

Based on this and together with the assumption, that only half of the Janus fibers is covered with AuNPs, the effective resistivity of electrospun Janus fibers \( \rho_{\text{Janus}} \) could be calculated as:

\[ \rho_{\text{Janus}} = \frac{A_{\text{Janus}}}{A_{\text{gold}}} \rho_{\text{gold}} = \frac{\pi r_{\text{Janus}}^2}{2(2\pi r_{\text{Janus}} h_{\text{gold}})} \rho_{\text{gold}} = \frac{r_{\text{Janus}}}{h_{\text{gold}}} \rho_{\text{gold}} \]
Mechanical Testing

Considering that delamination of Janus fibers could potentially occur under axial load, particularly when the elastic modulus of the two phases differs too much generating internal stress at the interface, mechanical properties of films were measured. Therefore, films were cast from silk solutions (100 mg/ml protein in HFIP) and dried before post-treatment as described in the experimental section. Besides the pure materials, also a bilayered film was produced upon coating of a post-treated eADF4(C16) film with another layer of ntagCys eADF4(κ16). The films resulted in a thickness of 30-40 µm and were cut into strips of 2-4 mm width and 2 cm length. The specimens were clamped in a Bose Electroforce tensile testing machine with a gap size of 4 mm. A 22 N load cell was used to measure the mechanical properties of such films at a deformation rate of 0.1%/s. The results are shown in Figure S2.

Figures

Figure S1. Representative FT-IR spectra after FSD and peak fitting. The curves show the Amid I bands of untreated silk Janus fibers (A), and post-treated samples. For post-treatment, samples were placed in a closed glass vessel and preheated to 60°C in an oven chamber for 30 min. Subsequently, the post-treatment agent was injected to the vessel via a tube and post-treatment was performed for 4 h. Three different post-treatment agents were tested: 100% ethanol (B), 70% ethanol (C) and 100% MilliQ water (D). The underlying peaks represent signals that can be assigned to specific secondary structures as labelled in (C).
Figure S2. Representative stress-strain curves of cast films made of eADF4(C16) and ntag\textsuperscript{Cys}eADF4(κ16) as well as eADF4(C16) combined with ntag\textsuperscript{Cys}eADF4(κ16) in a bilayer film (A). All films were cast from 100 mg/ml protein in HFIP and the bilayer film was produced by casting, drying and post-treatment of eADF4(C16) and subsequent casting of ntag\textsuperscript{Cys}eADF4(κ16) on top with subsequent post-treatment after drying. The resulting mechanical data display high similarities between the different materials (B) implying that delamination of the two phases under load will not happen. SEM-images of the respective breaking edges of pure materials in comparison with a bilayer film confirm this presumption, as the films display only one continuous breaking edge (C). Considering the strong connection of ntag\textsuperscript{Cys}eADF4(κ16) and eADF4(C16) in films, it can be concluded that electrospun Janus fibers thereof will not undergo delamination upon mechanical impact.

References

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Author Contributions

G.L and C.G contributed equally to this work. G.L. planned the Janus fiber processing set-up, performed experiments, supported the analysis and validated the results and wrote the manuscript; C.G. planned and performed most of the experiments and analyzed the results; T.S. acquired funding, planned the experiments, supervised the studies and edited the manuscript.