Recombinant Spider Silk Forms Tough and Elastic Nanomembranes that are Protein-Permeable and Support Cell Attachment and Growth

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Biologically compatible membranes are of high interest for several biological and medical applications. Tissue engineering, for example, would greatly benefit from ultrathin, yet easy-to-handle, biodegradable membranes that are permeable to proteins and support cell growth. In this work, nanomembranes are formed by self-assembly of a recombinant spider silk protein into a nanofibrillar network at the interface of a standing aqueous solution. The membranes are cm-sized, free-standing, bioactive and as thin as 250 nm. Despite their nanoscale thickness, the membranes feature an ultimate engineering strain of over 220% and a toughness of 5.2 MPa. Moreover, they are permeable to human blood plasma proteins and promote cell adherence and proliferation. Human keratinocytes seeded on either side of the membrane form a confluent monolayer within three days. The significance of these results lies in the unique combination of nanoscale thickness, elasticity, toughness, biodegradability, protein permeability and support for cell growth, as this may enable new applications in tissue engineering including bi-layered in vitro tissue models and support for clinical transplantation of coherent cell layers.

1. Introduction

Membranes are crucial components in tissue engineering where they serve as cellular and tissue interfacial barriers to mimic the physiological microenvironment in vitro. Membranes with a sub-µm thickness have an especially great appeal thanks to their high porosity, large surface area, high flexibility, and superior mechanical strength. To be suitable for tissue engineering, the membranes must be biocompatible, biodegradable, permeable, and have a thickness and mechanical properties fit for the intended application. Previously produced nanomembranes have failed on at least one of these aspects (Table S1, Supporting Information). In short, membranes of synthetic materials (e.g., PDMS, SiO₂ or Al₂O₃) are not biodegradable, and those made of bio-derived materials (e.g., PLGA, PEI and PCFG, ferritin globules, or compressed Bombyx mori silk) are impermeable to proteins.

Thick (>3 µm) silk membranes were proven protein-permeable, degradable in vivo, and support cell proliferation. Such membranes of B. mori silk have been suggested for bone tissue grafting and as corneal implants and membranes of recombinant spider silk proteins as replacements for the Bruch’s membrane in the eye. However, their thickness makes them unsuited for applications requiring ultrathin membranes, such as tissue barrier models, coculture systems and models of the basal lamina. Whereas natural spider dragline silk is one of the materials with the highest toughness per weight, films synthesized from soluble silk proteins have lacked the same mechanical robustness. Freestanding

DOI: 10.1002/adfm.202002982
nanomembranes formed from silk have therefore required reinforcement with inorganic fillers[6] or film compression,[2] which has resulted in protein impermeability. With respect to biocompatibility and biodegradability, recombinant spider silk proteins are of specific interest, as materials made thereof trigger only a limited immune response[18,21] and are degraded within 2–4 weeks.[7,22] Recombinant spider silk proteins can also be functionalized with cell adhesion motifs from, e.g., fibronectin to promote cell attachment and proliferation, e.g., FN-4RepCT.[22] Interestingly, FN-4RepCT spider silk proteins with the introduced FN-motif can form silk structures with increased stability and bioactivity.[22]

In this work, we report on the formation of freestanding silk nanomembranes by self-assembly of a recombinantly produced spider silk protein at the liquid-air interface of a standing solution. Spider silk proteins spontaneously self-assemble at liquid–air interfaces into films with a nanofibrillar structure.[23,24] Such silk films have an overall thickness depending on their assembly time and initial protein concentration, and feature retained bioactivity.[23,25–28] However, previous attempts to remove such assembled films intact from a liquid interface have failed.[27] The membranes formed in this work can be lifted intact from the interface, which allows their practical handling as well as characterizing their thickness, surface structure, mechanical properties, and permeability. We show that a confluent layer of human keratinocytes can be established on either side of the membrane within three days. In short, we show the simplistic production of a biocompatible membrane that addresses key challenges for life science applications (e.g., organ-on-a-chip systems) as well as medical applications (e.g., support for direct delivery of cells to wound sites).

2. Results and Discussion

2.1. Membrane Formation

We studied the formation of free-standing silk nanomembranes by self-assembly of a silk protein at the liquid-air interface[23] of a standing solution. Solutions of the recombinant spider silk protein FN-4RepCT[22] were placed in open wells at ambient conditions. Within minutes, the protein formed nanofibrils at the liquid–air interface, and after a few hours, the protein had self-assembled into coherent nanofibril membranes covering the entire interface (Figure 1a–c). The membranes could be lifted from the liquid interface in two different ways: 1) a holder is inserted into the well pre-formation, causing the membrane to form inside the holder (Figure 1d), or 2) a holder is lowered down onto the membrane post-formation, causing the membrane to adhere and seal against the outer wall of the holder (Figure 1e). These two methods allowed lifting and handling of intact cm-sized free-standing membranes of pure silk as thin as 250 ± 110 nm.

During the formation of the membranes, the upper “air side” and the bottom “liquid side” are in contact with different media, giving them significantly different properties and appearances. When the amphiphilic FN-4RepCT protein forms the first layer of nanofibrils at the liquid–air interface, their hydrophobic residues likely arrange toward the air interface while hydrophilic residues face the liquid.[28] The alignment of proteins along the interface forms a smooth air side (Figure 2a,b), featuring a contact angle of 37° (Figures S1 and S2, Supporting Information). Silk proteins reaching the membrane on the liquid side continue to build up more fibrils. Some of the silk proteins in solution form assemblies, which attach to the liquid side...
of the membrane. Therefore the liquid side features a texture built up from silk assemblies (Figure 2c,d) generating a nanotopography that renders them superhydrophilic[29] (Figures S1 and S3, Supporting Information). During formation, the membranes remain in constant contact with liquid. This differs from previous approaches, in which droplets of recombinant spider silk protein were dried,[22,25] resulting in double membranes (assembled from air-water interfaces at both sides) with unordered protein globules trapped between, yielding a thickness $>$1 µm.[25]

The final thickness of the membrane could be controlled in different ways. First, the thickness depended linearly on the initial protein concentration (Figure 2e), as previously shown.[23,25–28] As typical for self-assembled layers, the membrane thickness varies across the membrane. For the membranes with an average thickness of 280 nm (sd 110 nm), the percent difference in thickness was 130%. Second, the thickness of the membrane increased with formation time (Figure 2f) until the solution was depleted after 8 h (Figure S4, Supporting Information). As such, if the FN-4RepCT solution is replenished, the growth of the membrane resumes (Figure S4, Supporting Information). We speculate that switching the type of spider silk protein solution during assembly may enable the formation of membranes consisting of multiple layers of variously functionalized silk variants. The reason to why the growth stops, is that the assemblies in the bulk solution increase in size over time, and thus decrease in diffusivity and remain in solution. After 8 h, the solution mostly consists of large silk assemblies unable to diffuse to the interface. The formation of silk assemblies remaining in solution is in line with previous findings for evaporation-driven silk film formation, where spherical aggregates were observed after all liquid had evaporated.[23] This hypothesis is supported by the membranes' structure, growth rate, liquid side topography, and the surface energy difference between the air and liquid sides. While the growth of the membrane stops upon depletion of diffusible FN-4RepCT constructs, the structural rearrangement of the silk protein continues, with a continuous increase in $\beta$-sheets for 24 h (Figure 2g).

2.2. Mechanical Characterization

We evaluated the mechanical characteristics of the membranes on 280 nm ± 110 nm thick membranes formed within cylindrical holders with 6 mm inner diameter. Force-deformation measurements using a cylindrical stylus of radius $r = 2.0$ mm resulted in a maximum vertical membrane center displacement of 3.7 mm ± 0.7 mm at a stylus force of 1.4 mN ± 0.5 mN at rupture (Figure 3a and Figure S5, Supporting Information). The limited energy losses during cyclic mechanical loading and unloading of the membranes indicate elastic material properties (Figure 3b and Figure S5, Supporting Information). The ultimate stress and strain of the membranes were evaluated with a standard bulging experiment.[30,31] In short, the holder with the membrane was placed upside down in a large beaker, which was slowly filled with water, thus capturing an air column inside the holder (Figure S6, Supporting Information). The increasing water pressure inflated the membranes to a center deflection as high as 5.3 mm before rupture (Figure 3c-e).
The bursting pressure was 86 Pa ± 9 Pa, and the resulting maximum vertical displacement 4.3 mm ± 0.7 mm.

The results of the bulge inflation experiments were used to characterize the mechanical properties of the membranes. Finite deformation membrane finite elements, loaded by a pressure follower load, was used to model the bulge inflation experiment (FEAP, University of California at Berkeley, US). A decoupled neo-Hookean hyperelastic strain energy ($\Psi(C)$, Equation (1)) per unit undeformed material volume described the membrane’s continuum properties

$$\Psi(C) = \left( K - \frac{2G}{3}\right) U(J) + \frac{G}{2}(I_1 - 3)$$

where $K = E/(3(1 - 2v))$ and the shear modulus $G = E/(2(1 + v))$ define the properties of the undeformed material, and $E$ and $v$ denote the referential Young’s modulus and the Poisson’s ratio, respectively. The non-linear function $U(j) = (j^2 - 1 - 2 \ln(j))$ depends on the volume ratio $j = detF$, and $I_1 = trC$ is the first invariant of the right Cauchy-Green strain $C = F^TF$ with $F$ denoting the deformation gradient. Given the strain energy $\Psi(C)$, the Coleman-Noll procedure specifies the second Piola-Kirchhoff stress $s(C) = \frac{2\Psi}{detF}$ and the second Piola transform yields then the Cauchy stress $\sigma(C) = J^{-1}F s(C) F^T$ in the membrane. Given the available experimental data from the bulge inflation experiment, it was not possible to identify both, the bulk modulus $K$ and shear modulus $G$ of the membranes. Fitting the measurements to the decoupled neo-Hookean hyperelastic finite element model and assuming that silk has “rubber-like” incompressible deformation, i.e., $v = 0.5$, we estimated a Young’s modulus $E = 115 ± 42$ kPa, an ultimate principle Cauchy stress $\sigma_f = 4.70 ± 4.50$ MPa, an ultimate engineering strain $\varepsilon_f = 2.23 ± 0.94$, and a toughness $\tau = 5.20$ MPa (Table S3, Supporting Information).

Despite their nanoscale thickness, which has been shown to form more fragile silk structures, the mechanical properties of our membranes are similar to those for thicker silk membranes and films (Table 1). The toughness of the membranes is amongst the highest measured for synthetic silk constructs and allows their easy handling via the holder for various applications. We speculate that the large deflection of the membranes could be of interest for their use as pneumatic actuators in MEMS microvalves or micropumps, or in organ-on-a-chip devices, where they could be used for fluid control or as cell layer actuators.

**2.3. Membrane Permeability**

Use of the membranes for cell-culture of medical applications requires a permeability to both small molecules and proteins. The 280 nm ± 110 nm thick membranes were found permeable to the small molecules Rhodamine B (Rhb, 0.5 kDa), Texas Red-Dextran of 3 and 10 kDa (TRD3 and TRD10) as well as to the protein BSA-FITC (66 kDa) and proteins in human
blood plasma, but not to 100 nm sized gold particles or 3 \( \mu \)m polystyrene beads (Figure 4a).

To evaluate permeability to small molecules, droplets of concentrated solution were placed in the center of a membrane that had adhered to a holder pre-formation (Figure 4b) and remained intact for the duration of the experiment (Figure 4c). Outliers due to leakage were excluded based on visual observation and statistical analysis (Figures S8 and S9, Supporting Information). The diffusion coefficient for the small molecules were determined by fitting Equation (2) to the measured fluorescent data

\[
c_2(t) = \frac{V_1 c_0}{V_1 + V_2} \left(1 - e^{-\frac{D^* A (1 + \frac{V_1}{V_2})}{V_1 + V_2} t}\right)
\]

where \( c_2 \) is the concentration below the membrane, \( V_1 \) the volume above the membrane, \( c_0 \) the initial concentration above the membrane, \( V_2 \) the volume below the membrane, \( D^* \) the diffusion coefficient, \( A \) the area of the membrane, \( s \) the thickness of the membrane, and \( t \) time. Equation (2) is derived from Fick’s law of diffusion, which describes the transfer of a solute between two compartments separated by a membrane, described in depth elsewhere.[41] The diffusion coefficients for the small molecules (0.5–10 kDa) through the silk membrane were two to three orders of magnitude below those in an obstacle-free medium (Table 2).[42,43]

To study permeability to proteins, the membranes were instead adhered to the holder post-formation, as illustrated in Figure 1e and visually shown in Figure 4e. The set-up was altered to allow permeation of concentrations relevant for SDS-PAGE (Figure S10, Supporting Information). A strong color dye was added in all experiments to visually verify that there was no leakage (Figures S11 and S12, Supporting Information).

Based on the above-observed permeability properties, the silk nanomembranes can be classified as ultrafiltration membranes. In comparison to silk membranes made by compression of fibrils,[3,5] no external forces but only self-assembly

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**Table 1.** Overview of stress and strain properties of wet silk films and membranes measured in previous and current work. The toughness is estimated as half the product of ultimate stress and strain.

<table>
<thead>
<tr>
<th>Type of Silk</th>
<th>Thickness [( \mu )m]</th>
<th>Strain [%]</th>
<th>Stress [MPa]</th>
<th>Toughness [MPa]</th>
<th>Method</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>FN-4RepCT</td>
<td>0.3</td>
<td>223</td>
<td>4.7</td>
<td>5.2</td>
<td>Bulging/model</td>
<td>This work</td>
</tr>
<tr>
<td>rMaSp1 and rMaSp2</td>
<td>3.4</td>
<td>28</td>
<td>8</td>
<td>0.7</td>
<td>Uniaxial</td>
<td>[18]</td>
</tr>
<tr>
<td>B. mori</td>
<td>10</td>
<td>24</td>
<td>2</td>
<td>0.2</td>
<td>Uniaxial</td>
<td>[37]</td>
</tr>
<tr>
<td>B. mori fibrils</td>
<td>30</td>
<td>136</td>
<td>2</td>
<td>1.4</td>
<td>Uniaxial</td>
<td>[38]</td>
</tr>
<tr>
<td>B. mori</td>
<td>200</td>
<td>260</td>
<td>1</td>
<td>1.3</td>
<td>Uniaxial</td>
<td>[26]</td>
</tr>
<tr>
<td>B. mori cocoons</td>
<td>500</td>
<td>40</td>
<td>28</td>
<td>5.6</td>
<td>Uniaxial</td>
<td>[40]</td>
</tr>
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**Figure 4.** Permeability properties of 280 nm thick membranes. a) SEM image (false colored) of a cracked silk membrane (edge in blue) retaining 100 nm gold particles (yellow) and 3 \( \mu \)m polystyrene beads (green). Top view photographs of membranes with a droplet of 10 kDa Dextran on top b) directly after adding the droplet and c) 40 min later. d) Fraction of RhodamineB, 3 kDa Dextran, and 10 kDa Dextran passing through the membrane over time, measured by fluorescence intensity. The dashed, dotted and solid line indicate the respective corresponding exponential fit in accordance with Equation (2). e) Side view photographs of i–iii) the membrane adhesion to the holder, iv) 200 \( \mu \)L of 10 kDa Dextran inside the holder in contact with solution and v) after lifting from the interface. Scalebars represent 2 \( \mu \)m in (a), 1 mm in (b,c), and 2 mm in (e).
was used during the formation of our membranes, which we speculate resulted in larger voids between the nanofibrils (Figure 1c). This explains the higher size cut-off and opens up for tissue engineering applications where the permeability of biomolecules and cellular crosstalk across a barrier membrane are of interest.

### Table 2. Effective mass diffusivity (in μm² s⁻¹) of Rhodamine B (RhB), 3 kDa Dextran (TRD3), and 10 kDa Dextran (TRD10) through a spider silk nanomembrane, $D^\text{*}$ (this work), and in pure water, $D$ (previously reported).

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<th>TRD10</th>
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<tr>
<td>$D^\text{*}$</td>
<td>1.4</td>
<td>0.2</td>
<td>0.1</td>
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#### 2.4. Formation of Confluent Cell Layer

Human keratinocytes, seeded on either side of 440 nm ± 170 nm thick membranes, adhered within 30 min and established a confluent layer within three days, as shown by immunofluorescence staining (Figure 5a). SEM imaging verified that the cells had flattened out and established tight connections to each other as well as to the silk membrane through protrusion-like formations (Figure 5b and Figure S13, Supporting Information). The FN-4RepCT protein used here contains a cell-binding motif derived from fibronectin which allows integrin-mediated cell binding.[22] The unforced membrane formation process preserves the bioactivity of the silk protein.[25] Moreover, the internal fibrous structure of the membranes (Figure 1c) mimics that of the natural extracellular matrix in the human body,[44] which we hypothesize further aids cell attachment and proliferation. In contrast,

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<td>$D^\text{*}$</td>
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#### Figure 5. Cell growth on spider silk nanomembranes. a) Fluorescence microscopy images of human keratinocytes after 1 and 3 days in culture on the air side (left two columns) and liquid side (right two columns). Top row: live (green)/dead (red) staining at 2x magnification. Bottom row: F-actin (green)/DAPI (blue) staining at 10x magnification. b) SEM images of a single keratinocyte (false colored green) on the air side (left) after 1 day of culture and on the liquid side (right) after 3 days of culture. Scalebars in (a) top row represent 1 mm, bottom row 100 µm, and in (b) 10 µm.
previously reported silk membranes required post-coating with either fibronectin or collagen to enhance cell attachment.[39]

Membranes with nanoscale thickness could be used in tissue engineering, serving as in vitro interfacial barriers that mimic the physiological microenvironment conditions of, e.g., the basal lamina in vivo. Previous work shows that recombinant spider silk proteins triggers only a limited immune response[20,21] and is degraded in vivo within 2-4 weeks.[22] This bio compatibility and biodegradability, combined with the herein demonstrated nanothickness, elasticity, toughness, protein permeability and cell adherence, make our silk nanomembranes uniquely suited for novel or improved tissue engineering applications. The sub-µm thickness can enable new cellular barriers and coculture systems.[30] The successful growth of cells on either side of the nanomembranes, in combination with the protein permeability, allows for biomolecular communication across the membranes and can enable new in vitro models of, for example, the endothelium and mural cells, lung epithelia, the brain–blood barrier and the gastrointestinal tract. Culturing of cells on the membranes is also of interest for surgical transplanta-

3. Conclusion

We demonstrated a simple method to form cm-sized, free-standing, protein-permeable spider silk nanomembranes at the liquid-air interface of a standing solution. The membranes can easily be lifted from the interface for further characterization. The unforced formation of the membrane generates an internal nanofibrillar structure which supports an engineering strain over 220% and makes the membrane permeable to human blood plasma proteins. The functionalization of the recombinantly produced spider silk proteins enables the rapid formation of a monolayer of keratinocytes on either side of the membrane. The importance of these results lays within the unique combination of all these properties: nanoscale thickness, elasticity, toughness, biodegradability, protein permeability, and support of cell growth. We foresee that this may enable new applications in tissue engineering, including bi-layered in vitro tissue models and support for clinical transplantation of coherent cell layers.

4. Experimental Section

Preparation of FN-4RepCT: Frozen aliquots of 3 mg mL⁻¹ FN-4RepCT in phosphate-buffered saline (PBS, pH 7.4) were kindly provided by Spiber Technologies AB (Stockholm, Sweden). The FN-4RepCT solution was thawed, centrifuged for 30 s (13,000 rf), and diluted to the desired concentration in PBS buffer (National Veterinary Institute, Uppsala, Sweden).

Membrane Formation: The prepared FN-4RepCT solution was placed in an open well of either a hydrophobic 24-well plate (Sarstedt, Germany) or a homemade PMMA well, 16 mm in diameter. The well was left with the lid on at room temperature for 24 h. The cylindrical holders used to lift the membranes were transwells (Greiner, Germany) from which the PET membrane was removed. For the small molecule permeation and cell growth experiments described below, a circular hole with an inner diameter of 4 mm made out of off-stoichiometric thiol-ene (OSTE 322, Mercere Labs, Sweden) was attached to the bottom of the transwell to prevent leakage around the edges (Figure S7, Supporting Information).

Membrane Characterization: The thickness of the membranes was characterized using a Scanning Electron Microscope (SEM) (Gemini Ultra 55, Zeiss, Germany). The thickness was determined using pixel counting in Matlab (R2017a) (n = 3). For close up surface visualization (Figure 1d, 2b,d) a 18 h membrane was coated with gold through metal evaporation (Prova PAK 600 Coating System, Germany). Early time point characterization was done using Atomic Force Microscopy (AFM). Samples were prepared by bringing a glass cover slide (Thickness No. 1, hydrophobic, Marienfeld-Superior, Germany) into contact with a 1 mg mL⁻¹ FN-4RepCT solution, which had been standing for 10 min (n = 2). The slide was rinsed 3 times with 20 × 10⁻³ m Tris(hydroxymethyl)aminomethane (Tris) buffer (pH 8.0) and kept wet. The samples were imaged in a droplet of 20 × 10⁻³ m Tris buffer in a Dimension FastScan instrument (Bruker), using ScanAsyst Fluid+ tips and PeakForce Tapping. All the AFM images were flattened using the Gwyddion 2.43 program to remove the tilts had been standing for 10 min (n = 2). The slide was rinsed 3 times with 20 × 10⁻³ m Tris buffer in Dimension FastScan instrument (Bruker), using ScanAsyst Fluid+ tips and PeakForce Tapping. All the AFM images were flattened using the Gwyddion 2.43 program to remove the tilts.
Permeability to 3 μm microbeads (microparticles based on melanine resin, carboxylate modified, FITC-labeled, Sigma-Aldrich, Sweden) and to 100 nm gold nanoparticles (A11-100-CIT-DIH-1-15, Nanopartz, USA) was studied using SEM.

To characterize the permeability for proteins, FITC labeled BSA (n = 2) (Sigma-Aldrich, Sweden) and human blood plasma (n = 2) (Uppsala Akademiska Hospital, Uppsala, Sweden; two times diluted in PBS) were used to allow SDS-gel analysis. In short, a linear stage motor was used to lower the holder onto the membrane and the solution of interest was added on top of the membrane. After 2 hours, the holder was lifted from the interface, permeate was collected and the membrane was re-inserted on top of PBS, the solution inside the holder was increased, and the procedure was repeated. The permeation was evaluated using fluorescence and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The solution below the membrane was mixed with denaturing and reducing gel loading dye. The samples were boiled for 5 min at 95 °C and then run on SDS-PAGE (NuPAGE, 4–12% Bis-Tris, Invitrogen), followed by staining using Coomassie Brilliant Blue R-250 (Sigma-Aldrich, Sweden).

Cell Cultures: Human keratinocytes (HaCaT) (Cell Lines Service, Heidelberg, Germany) were cultured in Dulbecco’s Modified Eagle Medium/Nutrient Mixture F-12 (Gibco, Waltham, MA, USA) supplemented with 5% fetal bovine serum and 1% Penicillin-Streptomycin. HaCaT cells were used at passage 35. Growth medium was changed every second day.

Cell Seeding: HaCaT cells were harvested at 80% confluency. Cells were washed once with pre-warmed PBS, enzymatically detached with TrypLE Express (Life Technologies, Waltham, MA, USA), and diluted to 10^5 cells mL^-1. Cells were seeded onto the liquid- or air-side of the membrane in a final density of 3 × 10^4 cells per 10 μL and 3 × 10^3 cells per 20 μL respectively and incubated for 30 min at 37 °C, 5% CO₂, and 95% humidity. Non-adherent cells were removed with pre-warmed PBS. Adherent cells were kept in fresh growth medium and further analyzed as described below.

Cell Viability Assay: Live/dead viability assay (Molecular Probes, Waltham, MA, USA) was performed at days 1, 2, and 3 of culture.

Cell Fixation and Immunostaining: HaCaT cells were washed twice with pre-warmed PBS and fixed with 4% paraformaldehyde (PFA) for 10 min at room temperature. Cells were then washed twice with PBS, permeabilized with 0.2% Triton X-100 in PBS, washed twice with 0.05% Tween in PBS for 5 min and finally, blocked with 1% bovine serum albumin (BSA) in PBS for 60 min. Alexa Fluor 488 Phalloidin (Thermo Fisher Scientific, Waltham, MA, USA) 1:80 in 1% BSA in PBS was used to stain the actin filaments for 2 h at room temperature, before nuclear staining with DAPI for 10 min. Stained cells were washed twice with 0.05% Tween in PBS for 5 min and documented using fluorescence microscopy. Images captured using the NIS elements BR software were subtracted blurriness using the Unsharp Mask command (radius 2.0 pixels and mask weight 0.60) in ImageJ.

Sample Preparation for SEM: After fixation with 4% PFA, samples were washed 3 times in PBS and then the membranes were transferred to a metal mesh placed on top of a tissue with the cell side up. The membranes were then serially dehydrated in 30%, 70%, and 95% ethanol in Milli-Q for 10 min, two times each and, 99.5% ethanol in Milli-Q for 15 min, three times each, with agitation. Samples were then chemically dried in 2 parts 99.5% ethanol in Milli-Q and 1 part hexamethyldisilazane (HMDS, Sigma Aldrich, St. Louis, MO, USA) for 15 min, 1 part 99.5% ethanol in Milli-Q and 1 part HMDS for 15 min, 1 part 99.5% ethanol in Milli-Q and 2 parts HMDS for 15 min and finally, 3 times in HMDS alone for 15 min each. The membranes were lifted from the metal mesh, placed on top of conductive carbon tape and coated with a 12 nm thick layer of gold using thin film deposition as above.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements
The authors would like to thank Spiber Technologies AB for providing soluble FN-silk protein. The authors would also like to thank Mikael Bergqvist for constructing the force-deformation set-up, Andreas Barth for his assistance with the FTIR measurements and interpretation of the results, Fredrik Lundell for kindly giving access to the OCT, Cecilia Aronsson for coating the SEM samples with gold, and Anna Herland for supplying the Dextran.M.H. and Wv.d.W. share last authorship and are both corresponding authors.

Conflict of Interest
MK works for and MH has shares in Spiber Technologies AB, a company that aims to commercialize recombinant spider silk.

Keywords
elasticity, nanomembranes, permeability, recombinant spider silk, tissue engineering

Received: April 3, 2020
Revised: May 25, 2020
Published online: