Fiber-reinforced spider silk hydrogels

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INTRODUCTION

Spider silk has remarkable mechanical and biological properties. Inspired by *Araneus diadematus* fibroin 4, the engineered silk protein eADF4(C16) has been biotechnologically produced [1]. This enables engineering of the primary sequence for controlled adjustment of physicochemical properties like charge [2] as well as modification with e.g. peptides for specific cellular interactions, like the integrin-binding motif RGD [3]. The eADF4 variants can assemble into crystalline domains, yielding structures like hydrogels and fibers which are well suited for tissue engineering applications [4, 5]. By creating composite materials, the scaffold properties can be customized to meet cellular needs and to better resemble the natural extracellular matrix [6].

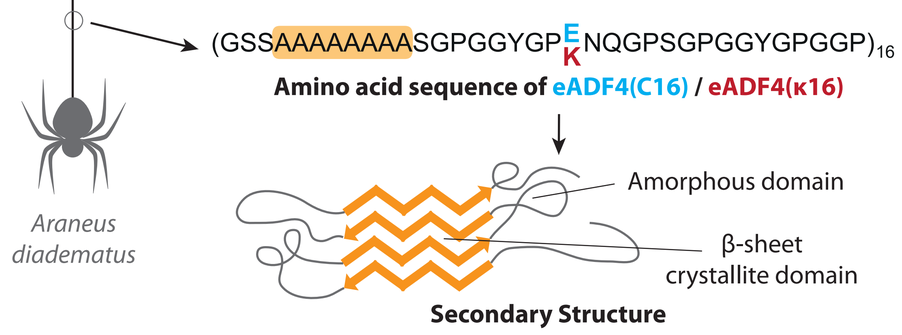


Figure 1. Genetic engineereing of spider silk proteins yield the negatively charged eADF4(C16) and the positively charged eADF4(κ16) by exchange of one amino acid. The proteins assemble into β-sheet rich crystalline and amorphous domains [6].

1. HYDROGELS

Hydrogels of the recombinant spider silk proteins eADF4(C16) and eADF4(C16)-RGD are obtained by self-assembly of protein monomers into nanofibrils yielding a dense physically entangled network, in which cells can be encapsulated.

Protein concentration is a key factor:

- Higher concentrations yield stiff but formstable and printable gels, with the dense network compromising cell activity [7].
- Low concentrated (1% w/v) hydrogels are soft and hard to handle, but showed a higher cell proliferation.

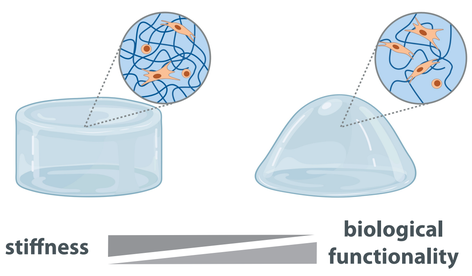


Figure 2. Variation of protein concentration leads to stiff form-stable and printable (left) and soft (right) hydrogels. Created with BioRender.com

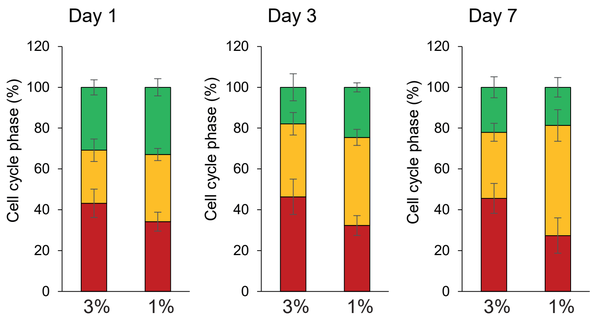
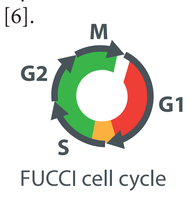


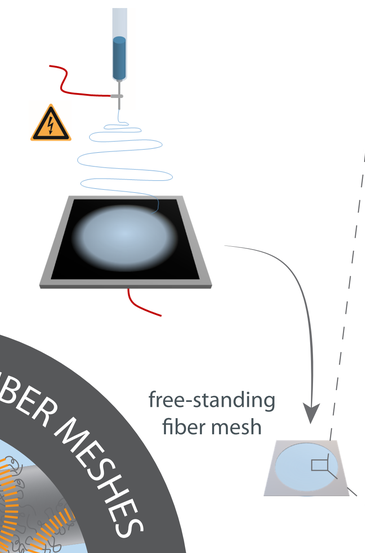
Figure 3. Cultivation of murine NIH/3T3 cells expressing the FUCCI proliferation reporter system in 3% vs. 1% (w/v) eADF4(C16)-RGD hydrogels indicated a higher proliferative activity of cells in the lower concentrated gels [6].



2. FIBERS

eADF4(C16) and eADF4(κ16) can be spun into free-standing fiber meshes with fiber diameters and cross-sections of a few hundred nanometers to microns.

Figure 4. Spider silk proteins are processed using electrospinning to create non-woven fiber meshes. Free-standing meshes were obtained in 3D- printed PLA frames [6].



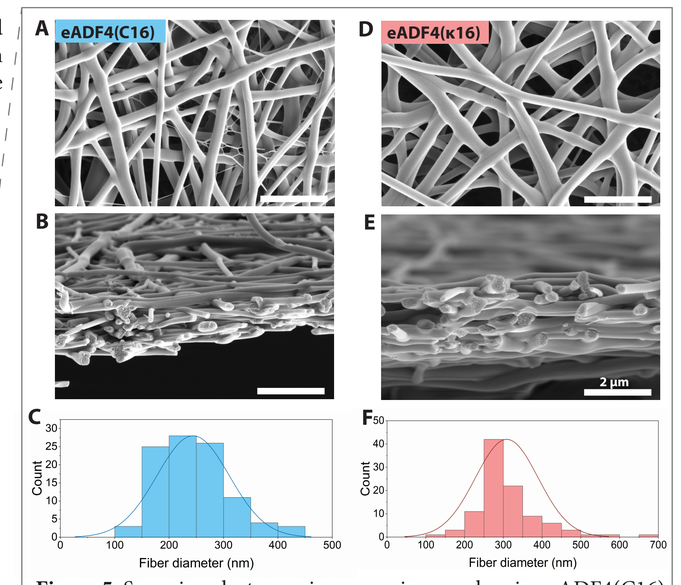
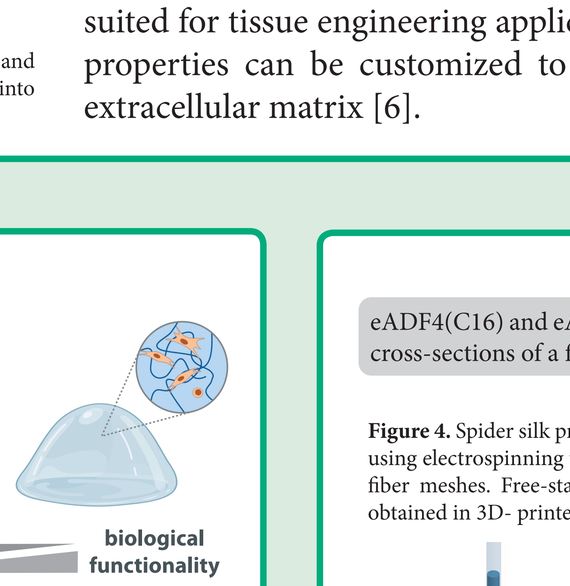


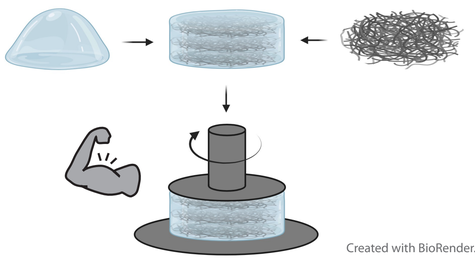
Figure 5. Scanning electron microscopy images showing eADF4(C16) (A, B) and eADF4(κ16) fiber meshes (D, E) with a mean fiber diameter of 244 ± 67 nm (C) and 309 ± 81 nm (F), respectively [6].



CONCLUSION

- Low concentrated spider silk-based hydrogels supported higher proliferative activity of cells
- Incorporation of fiber meshes into soft hydrogels improved structural integrity and mechanical performance
- No detectable influence of the silk proteins net charge
- Structure resembles „grafting-from“ approach, where fibers presumably act as nucleation sites for fibril growth

We demonstrated a promising strategy for the development of advanced composite biomateris with tunable mechanical properties and enhanced functionality for tissue engineering applications.



3. COMPOSITES

Combination of fiber meshes and aqueous spider silk solutions in a layer-by-layer fabrication process yielded fiber mesh-hydrogel composites (fmhc).

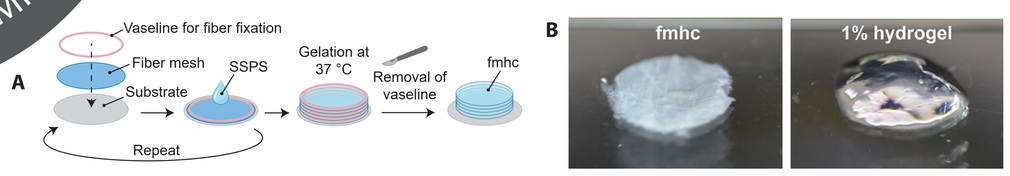


Figure 6. (A) Manufacturing process to create multilayered fiber mesh-hydrogel composites (fmhc), combining fiber meshes and 1% (w/v) eADF4(C16) silk solutions (SSPS). (B) Images after complete gelation of the composites vs. pure hydrogel [6].

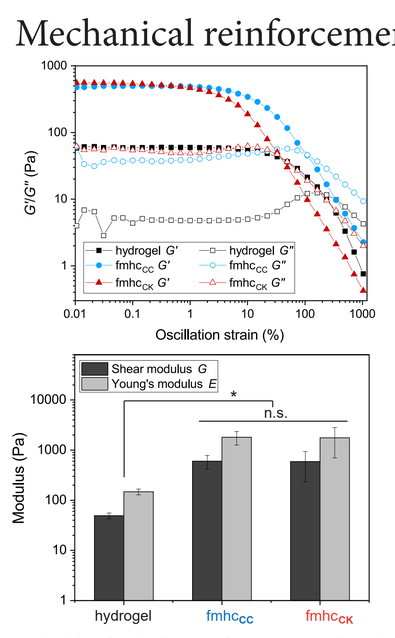


Figure 7. Rheological tests demonstrated an increase of the Young's modulus by the factor 10 for both types of composites [6].

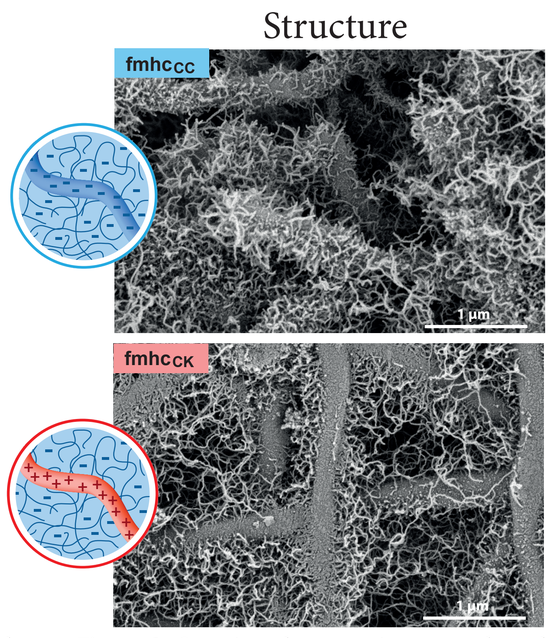


Figure 8. Scanning electron microscopy showed fibers embedded in a fibril network. On the fiber surfaces, a brush-like morphology was observed [6].

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