6-7-2017

Biofabrication using recombinant spider silk proteins as a biomaterial

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Biofabrication using recombinant spider silk proteins as a biomaterial

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BioMat

Why spider silk?

Image: E. Doblhofer

- no toxicity
- no immuno-reactivity
- slow biodegradation
- good mechanical properties

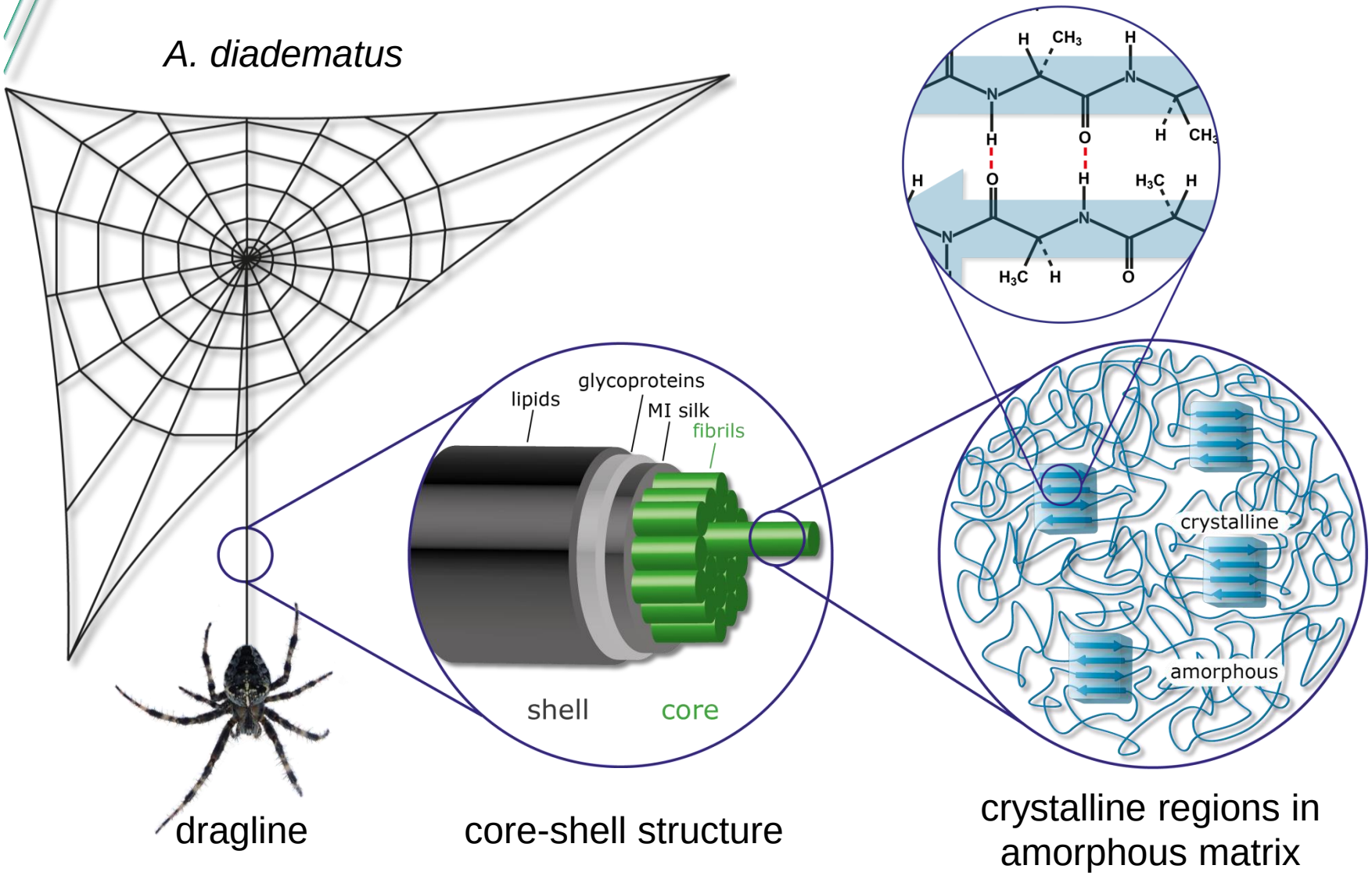
→ **interesting material for biomedical applications**

Material	Stiffness (GPa)	Strength (GPa)	Extensibility (%)	Toughness (MJm^{-3})
Araneus diadematus dragline silk	6	0.7	30	150
Bombyx mori cocoon silk	7	0.6	18	70
Nylon fiber	5	0.95	18	80
Kevlar 49 fiber	130	3.6	2.7	50
High-tensile steel	200	1.5	0.8	6

J. M. Gosline, P. A. Guerette, C. S. Ortlepp, K. N. Savage, *J Exp Biol* **1999**, *202*, 3295-3303
J. G. Hardy, T. R. Scheibel, *J Polym Sci Pol Chem* **2009**, *47*, 3957-3963

Spider silk architecture…

A. diadematus



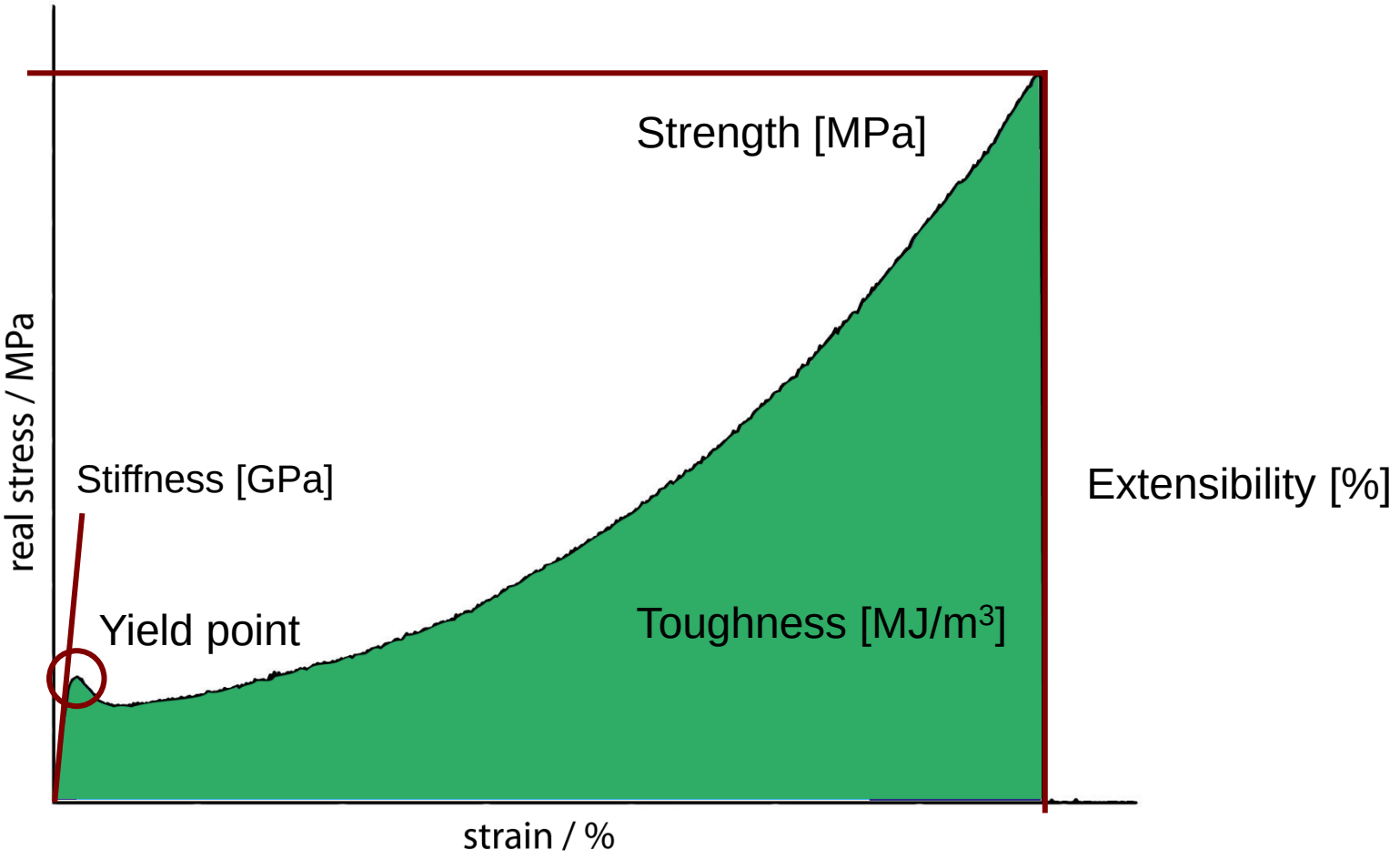
dragline

core-shell structure

crystalline regions in amorphous matrix

Heidebrecht, A. & Scheibel, T. (2013). *Adv. Appl. Microbiol.* **82,** 115-153.

…allowing outstanding toughness



Modified after: Heidebrecht, A.

Restrictions?

- spiders are cannibals
- silk quality depends on nutrition
- male spiders are hard to raise

→ farming of spiders is NOT feasible!

→ use a biotechnological approach for silk production

J. M. Gosline, P. A. Guerette, C. S. Ortlepp, K. N. Savage, *J Exp Biol* **1999**, *202*, 3295-3303

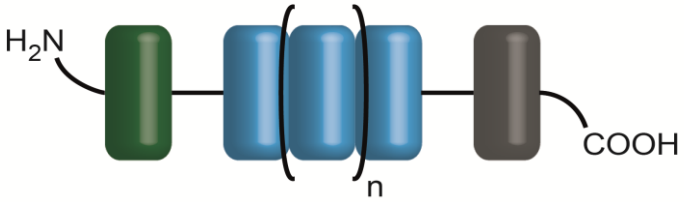
Engineered spider silk origin

major ampullate silk

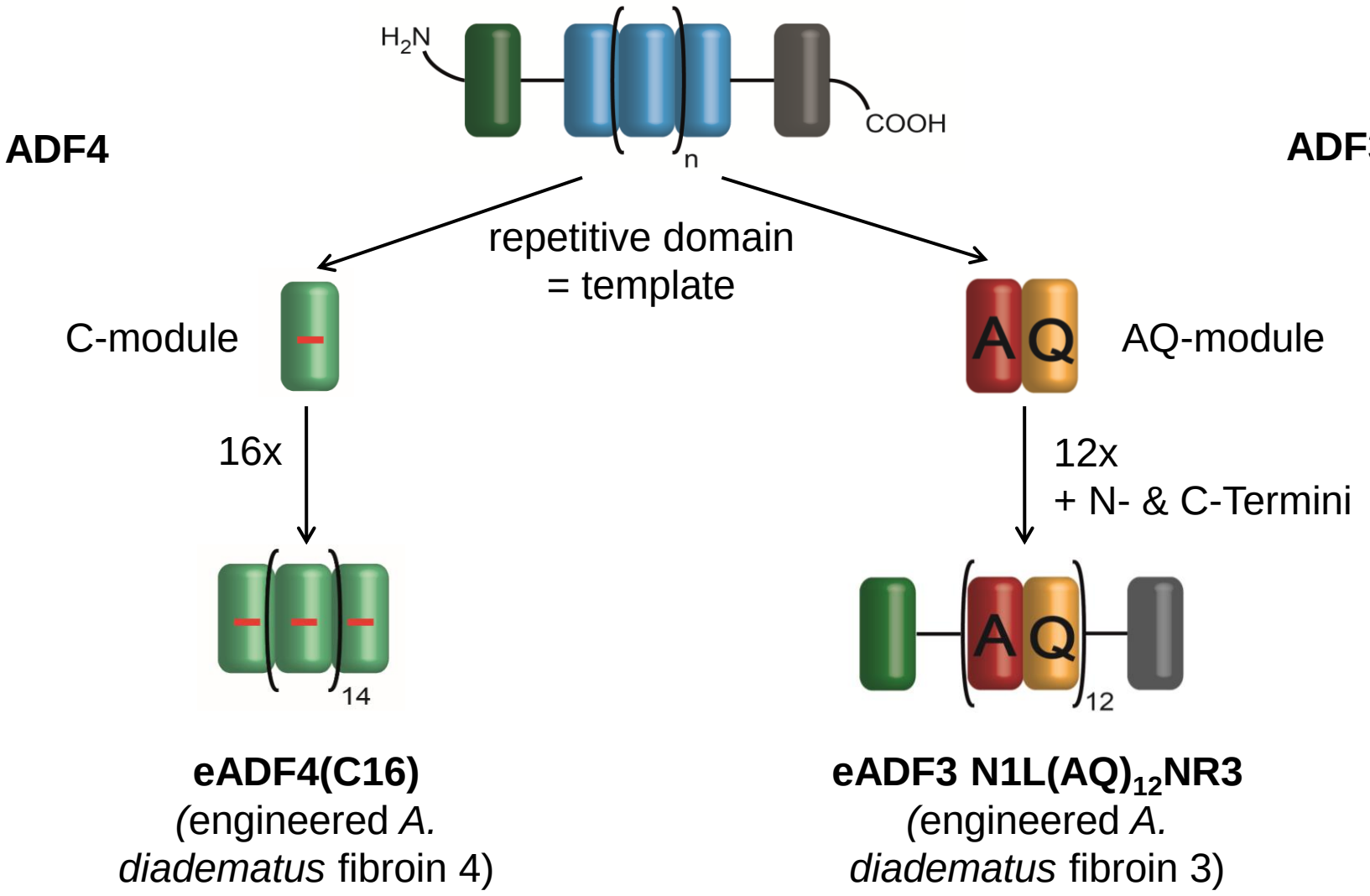
Araneus diadematus

main components

A. diadematus fibroins: ADF3 & ADF4



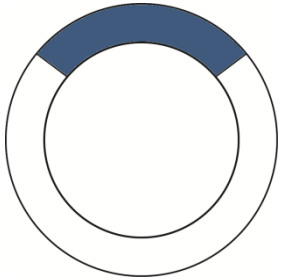
Engineered spider silk



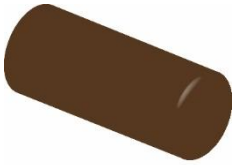
M. Heim, D. Keerl, T. Scheibel, *Angew Chem Int Edit* **2009**, *48*, 3584-3596
D. Huemmerich, C. W. Helsen, S. Quedzuweit, J. Oschmann, R. Rudolph, T. Scheibel, *Biochemistry-Us* **2004**, *43*, 13604-13612

Biotechnological silk production

genetic information



plasmid with genetic information



E. coli

fermentation

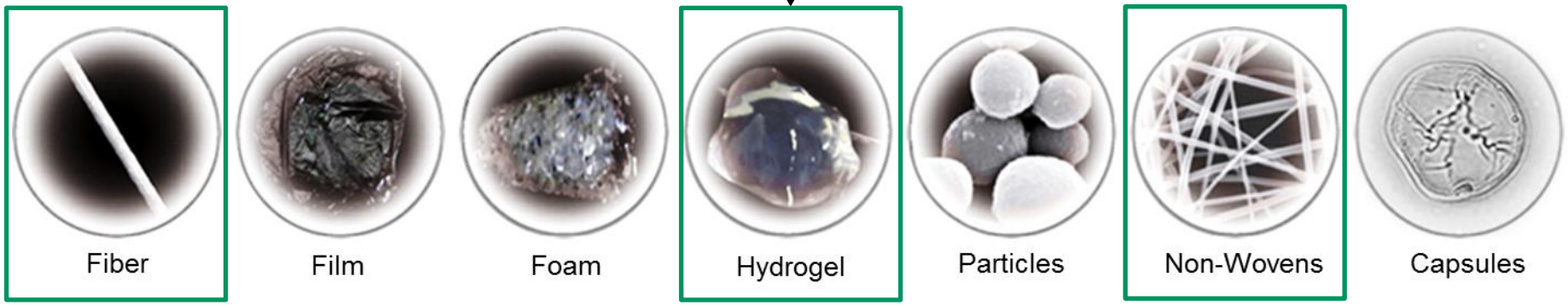
protein

D. Huemmerich, C. W. Helsen, S. Quedzuweit, J. Oschmann, R. Rudolph, T. Scheibel, *Biochemistry-Us* **2004**, *43*, 13604-13612

Engineered spider silk morphologies

Image by: K.Schacht

processing steps



Spider silk fibers

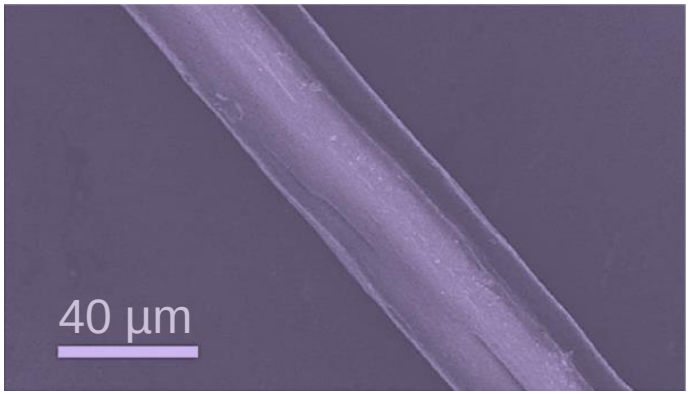
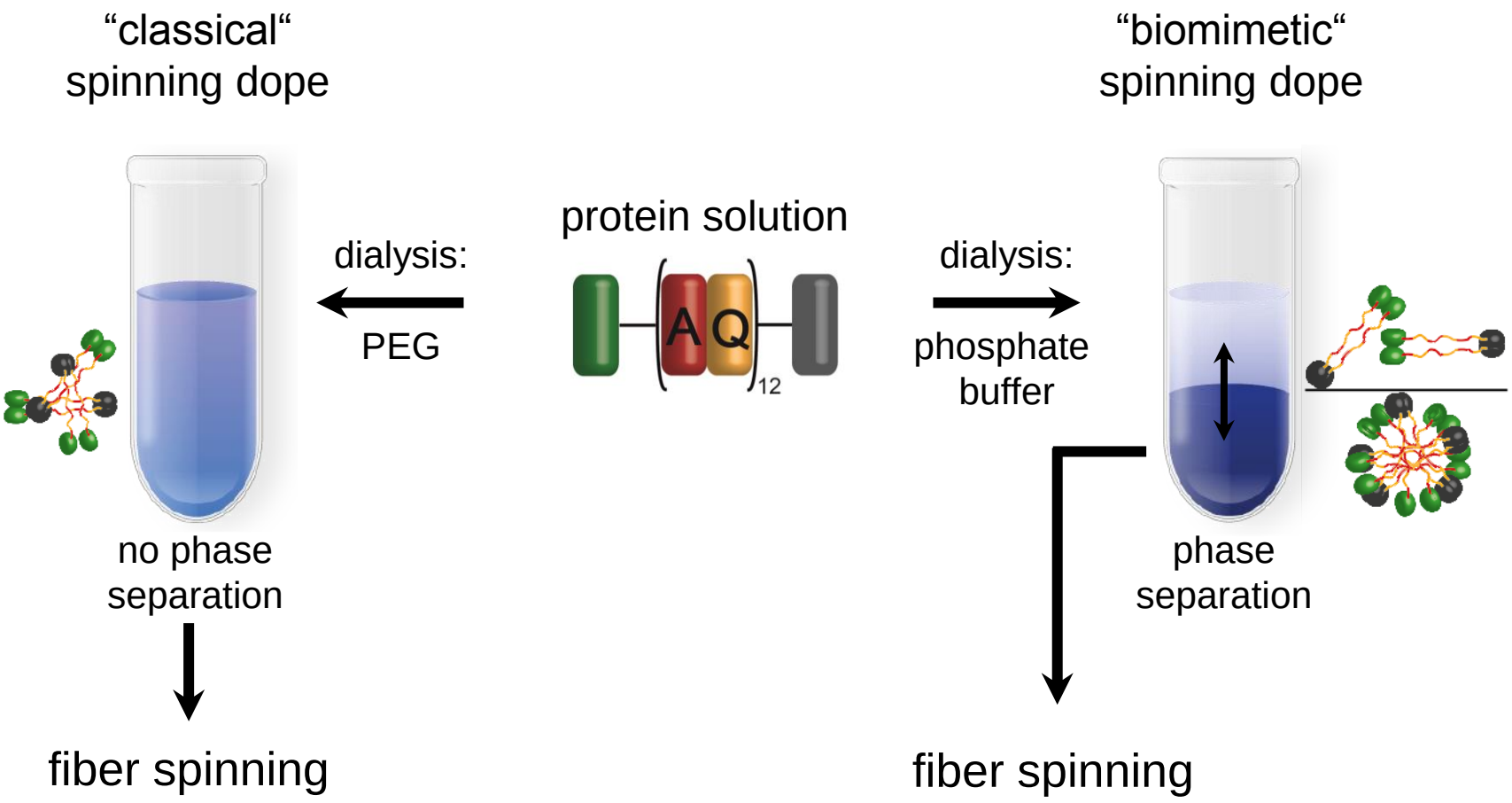


Image by: A. Heidebrecht

Preparation of spinning dope



Heidebrecht, A.; Eisoldt, L.; Diehl, J.; Schmidt, A.; Geffers, M.; Lang, G.; Scheibel, T., *Adv Mater* **2015**, *27*, 2189

Wet-spun silk fibers

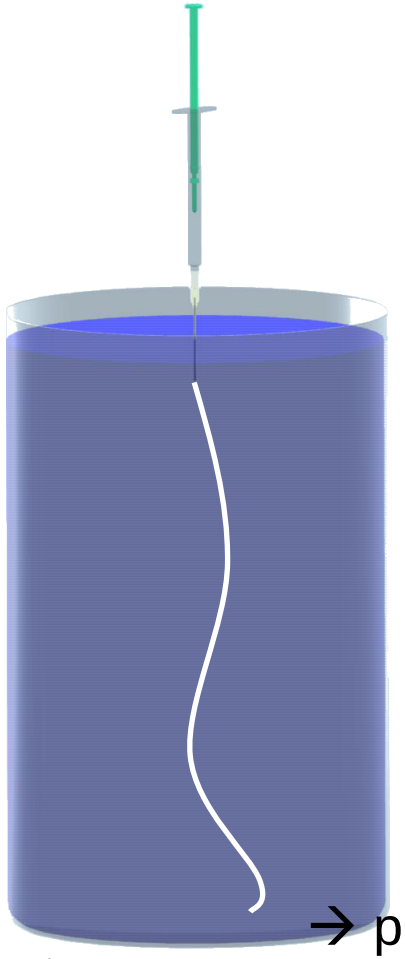
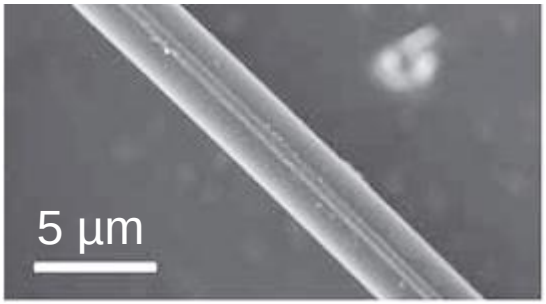


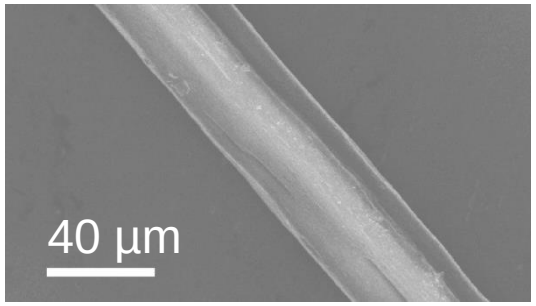
Image by: G. Lang

→ post-stretching

natural fiber



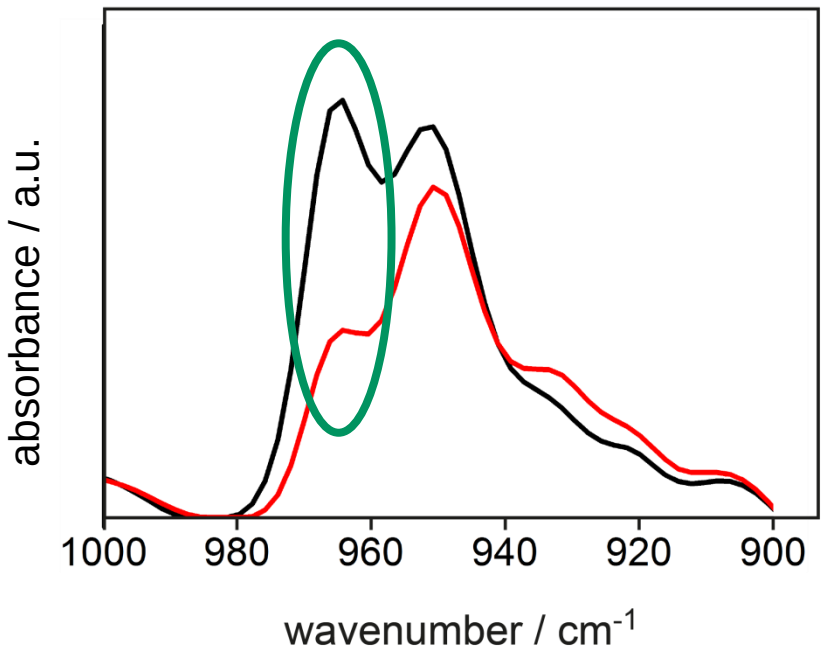
synthetic fiber

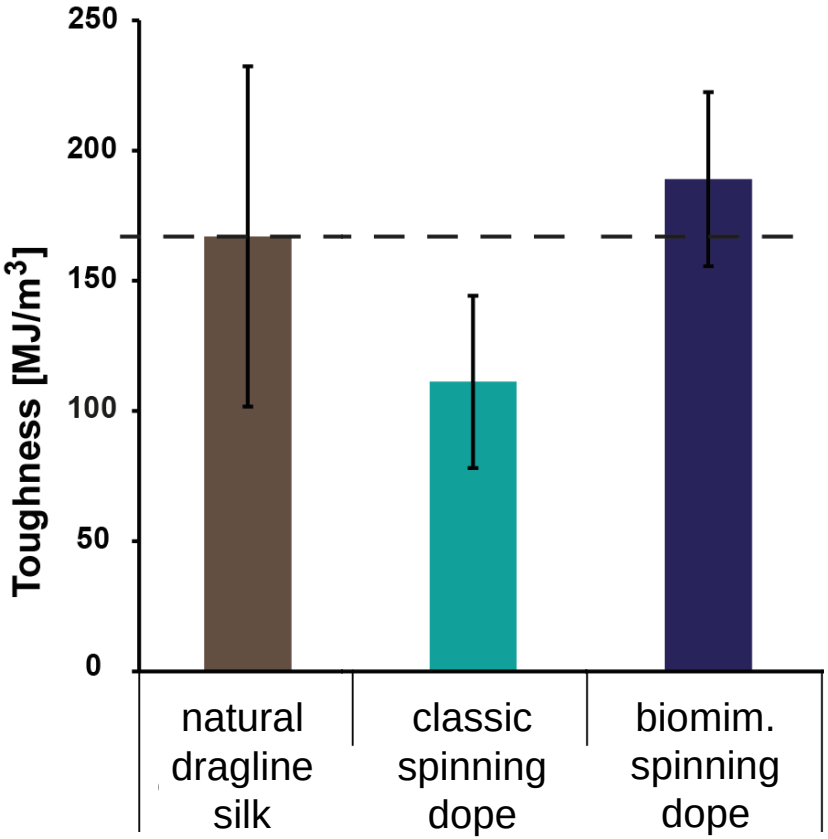


	natural fiber	synthetic fiber
filament	double	single
surface	smooth	smooth
diameter	4-8 µm	15-60 µm

Heidebrecht, A.; Eisoldt, L.; Diehl, J.; Schmidt, A.; Geffers, M.; Lang, G.; Scheibel, T., *Adv Mater* **2015**, *27*, 2189
Keerl, D. & Scheibel, T. (2012). *Bioinspired, Biomimetic Nanobiomater.* **1**, 83-94.

Polarized FTIR and tensile testing





→ **poly-Ala stretches in recombinant fibers are aligned along fiber axis**

→ **toughness of biomimetic spider silk competes with that of natural ones**

Modified after: Heidebrecht, A.; Eisoldt, L.; Diehl, J.; Schmidt, A.; Geffers, M.; Lang, G.; Scheibel, T., *Adv Mater* **2015**, *27*, 2189

Spider silk non-wovens

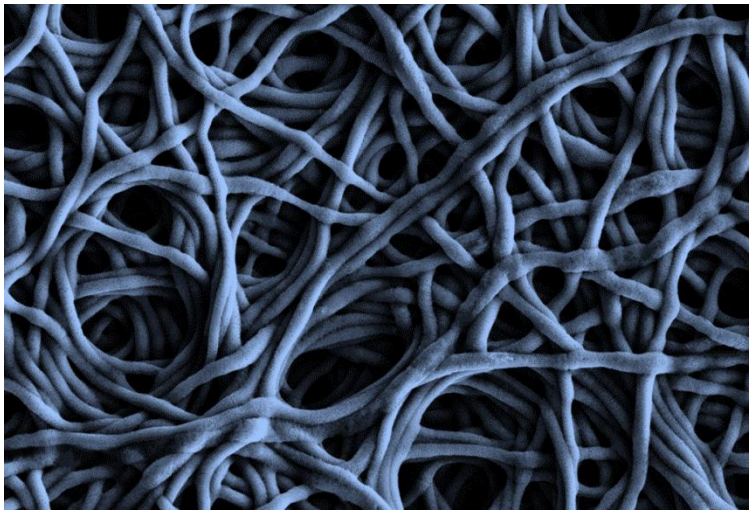
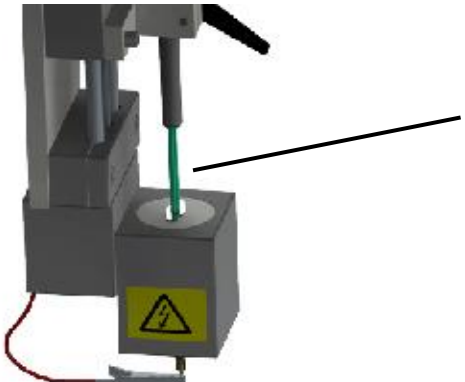
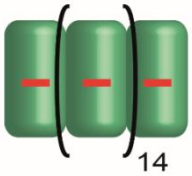


Image by: E. DeSimone

Preparation of non-wovens – e-spinning



syringe with eADF4(C16) solution



contacted canula

contacted collector plate

non-woven fiber mat

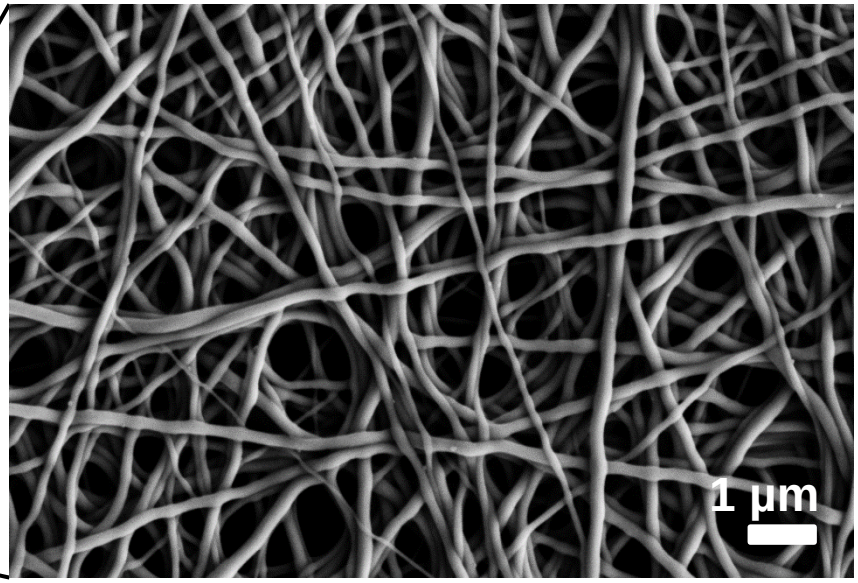


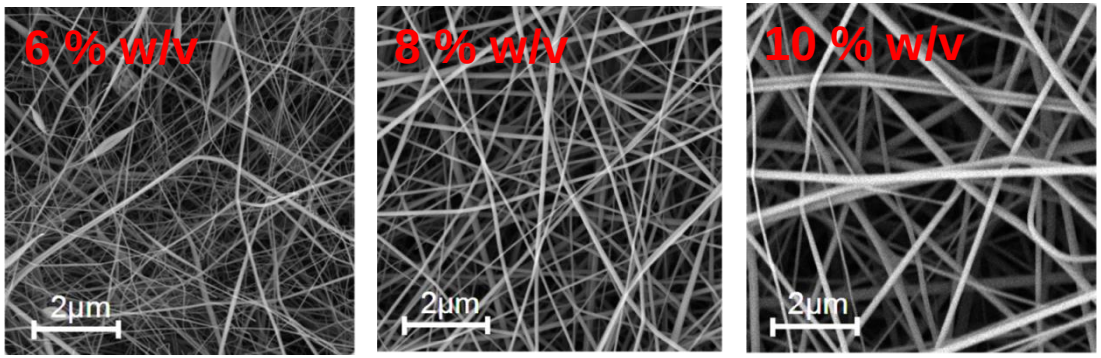
Image by: E. DeSimone

Image by: G. Lang

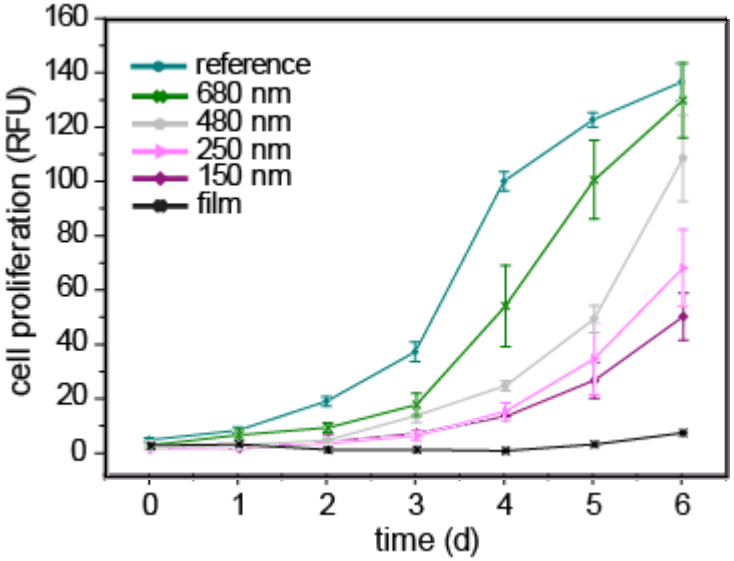
Non-woven diameter

Parameters:

- concentration
- applied voltage
- spinning distance
- needle diameter
- humidity
- temperature



Balb/3T3 fibroblasts



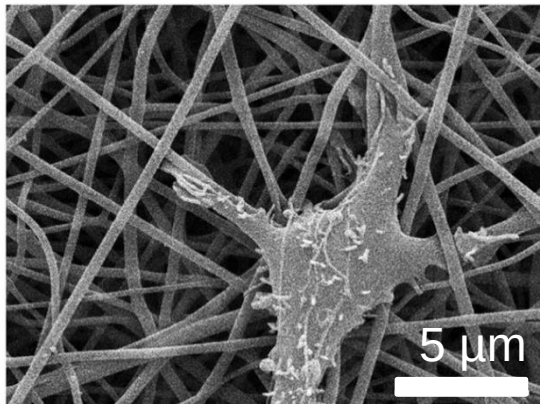
→ **Increasing protein concentration increases fiber diameter**

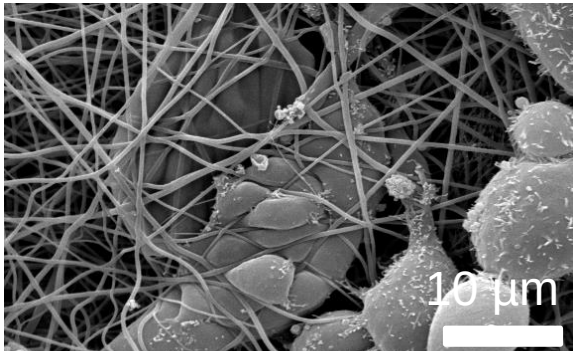
→ **larger fiber diameter promote cell proliferation**

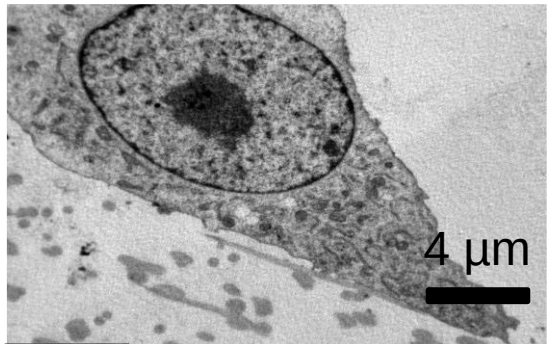
G. Lang, S. Jokisch, T. Scheibel, *Journal of visualized experiments : JoVE* **2013**, e50492
A. Leal-Egana, G. Lang, C. Mauerer, J. Wickinghoff, M. Weber, S. Geimer, T. Scheibel, *Adv Eng Mater* **2012**, *14*, B67-B75

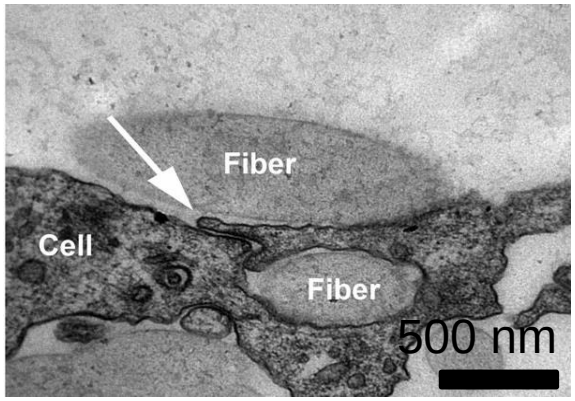
Cell interaction with non-wovens

SEM & TEM: Balb/3T3 fibroblasts









→ **fibroblasts spread on and migrate into non-woven meshes**

Spider silk hydrogels

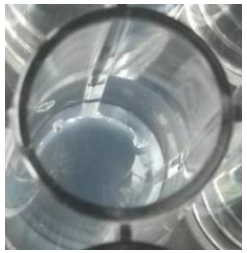
Image by: K. Schacht/T. Jüngst

Hydrogel preparation

highly concentrated eADF4(C16) solution

cast in well plates

gelation

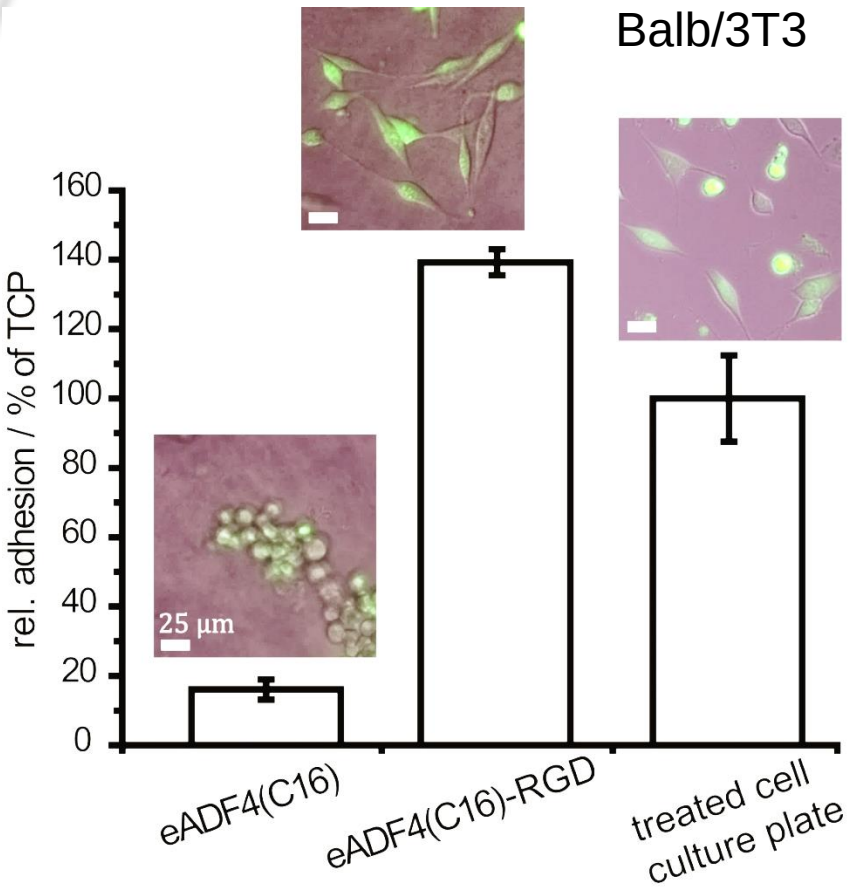


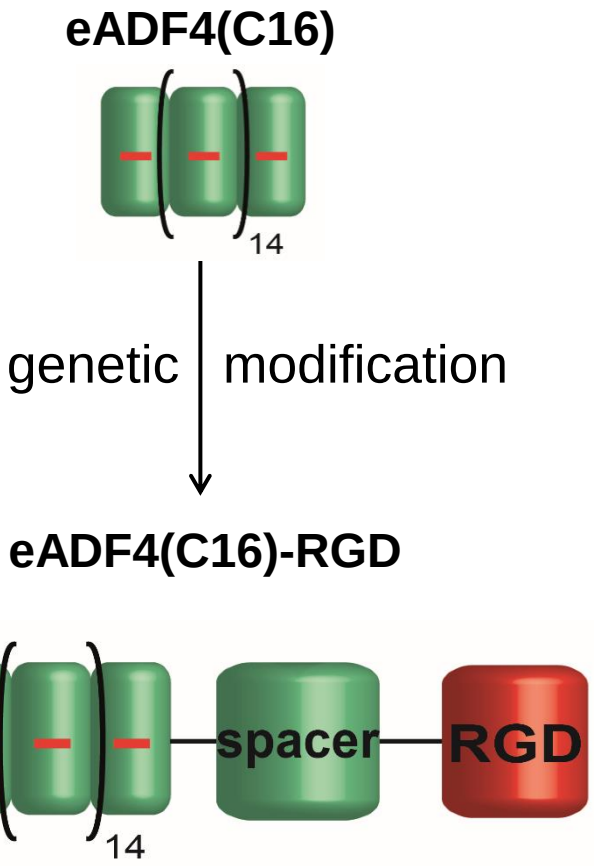
hydrogel

cell seeding

Modified after: Schacht, K.; Jüngst, T.; Schweinlin, M.; Ewald, A.; Groll, J.; Scheibel, T., *Angewandte Chemie* **2015**, *54*, 5

Spider silk variation





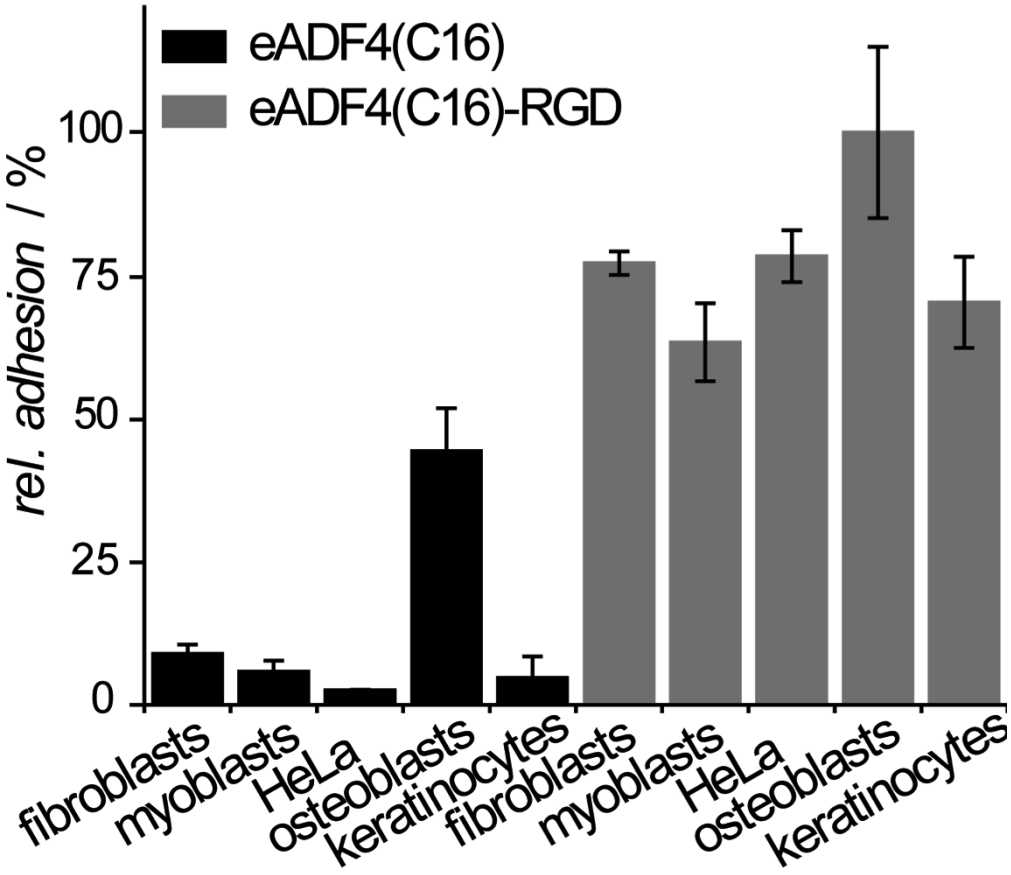
→ **weak cell adhesion on eADF4(C16) hydrogels**

→ **introduction of adhesion motif RGD leads to enhanced cell adhesion**

S. Wohlrab, S. Muller, A. Schmidt, S. Neubauer, H. Kessler, A. Leal-Egana, T. Scheibel, *Biomaterials* **2012**, *33*, 6650

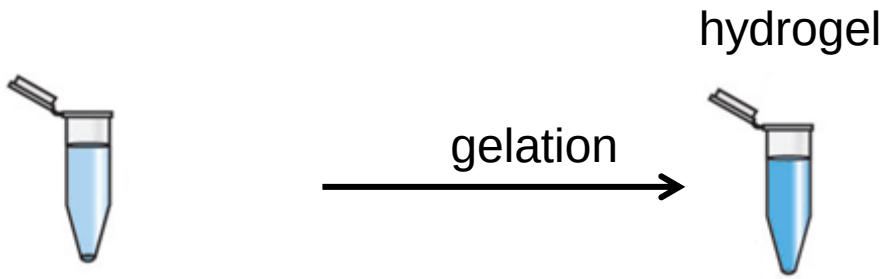
Schacht, K.; Jüngst, T.; Schweinlin, M.; Ewald, A.; Groll, J.; Scheibel, T., *Angewandte Chemie* **2015**, *54*, 5

Different cell lines on spider silk hydrogels

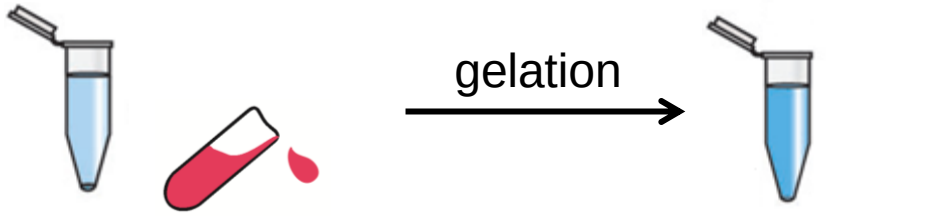


→ cell adhesion on eADF4(C16)-RGD hydrogels was significantly improved also on other cell types

Hydrogel preparation



highly concentrated eADF4(C16) solution



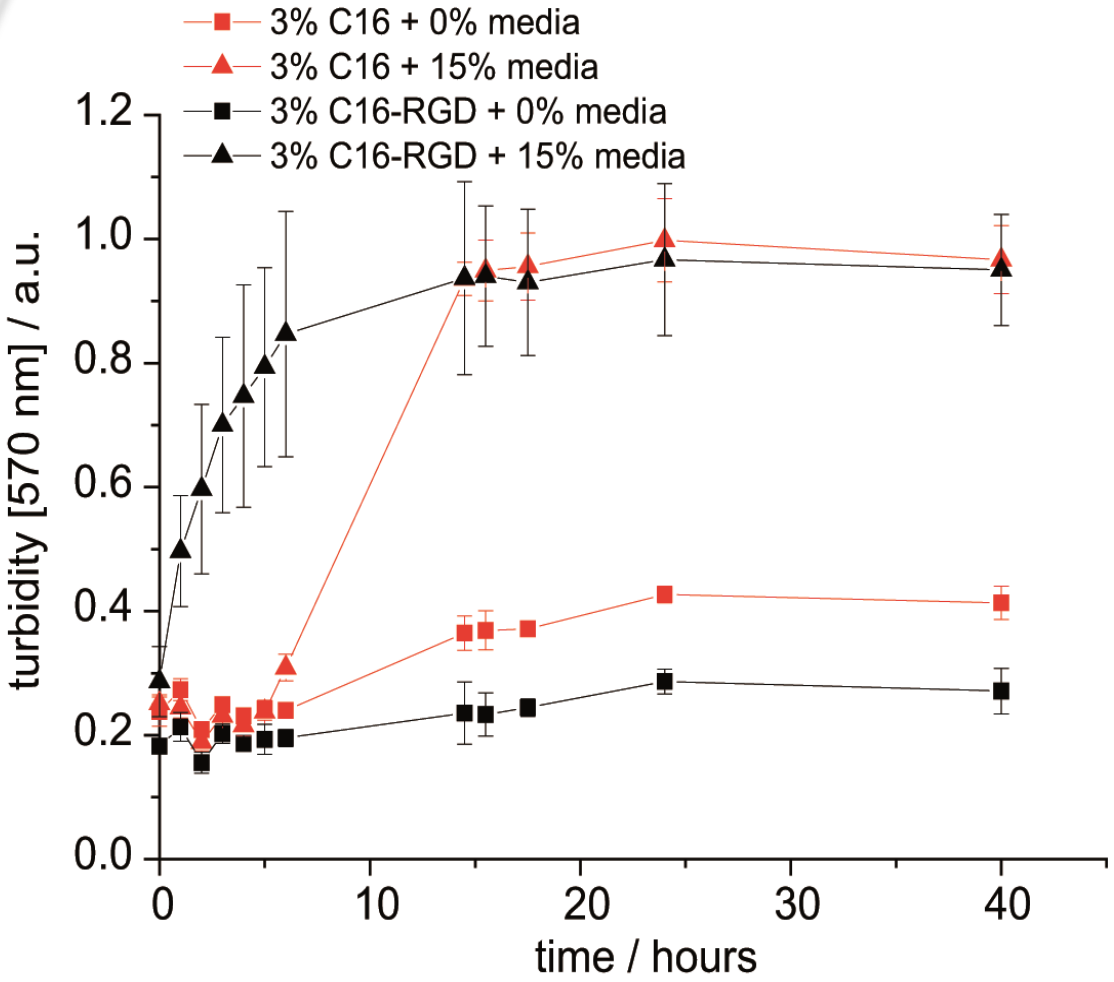
+ cell culture medium

hydrogel + cell culture medium

- gelation kinetics
- secondary protein structure
- rheology
  - stress-strain
  - viscosity

Modified after: Schacht, K.; Jüngst, T.; Schweinlin, M.; Ewald, A.; Groll, J.; Scheibel, T., *Angewandte Chemie* **2015**, *54*, 5

Gelation kinetics and secondary structure

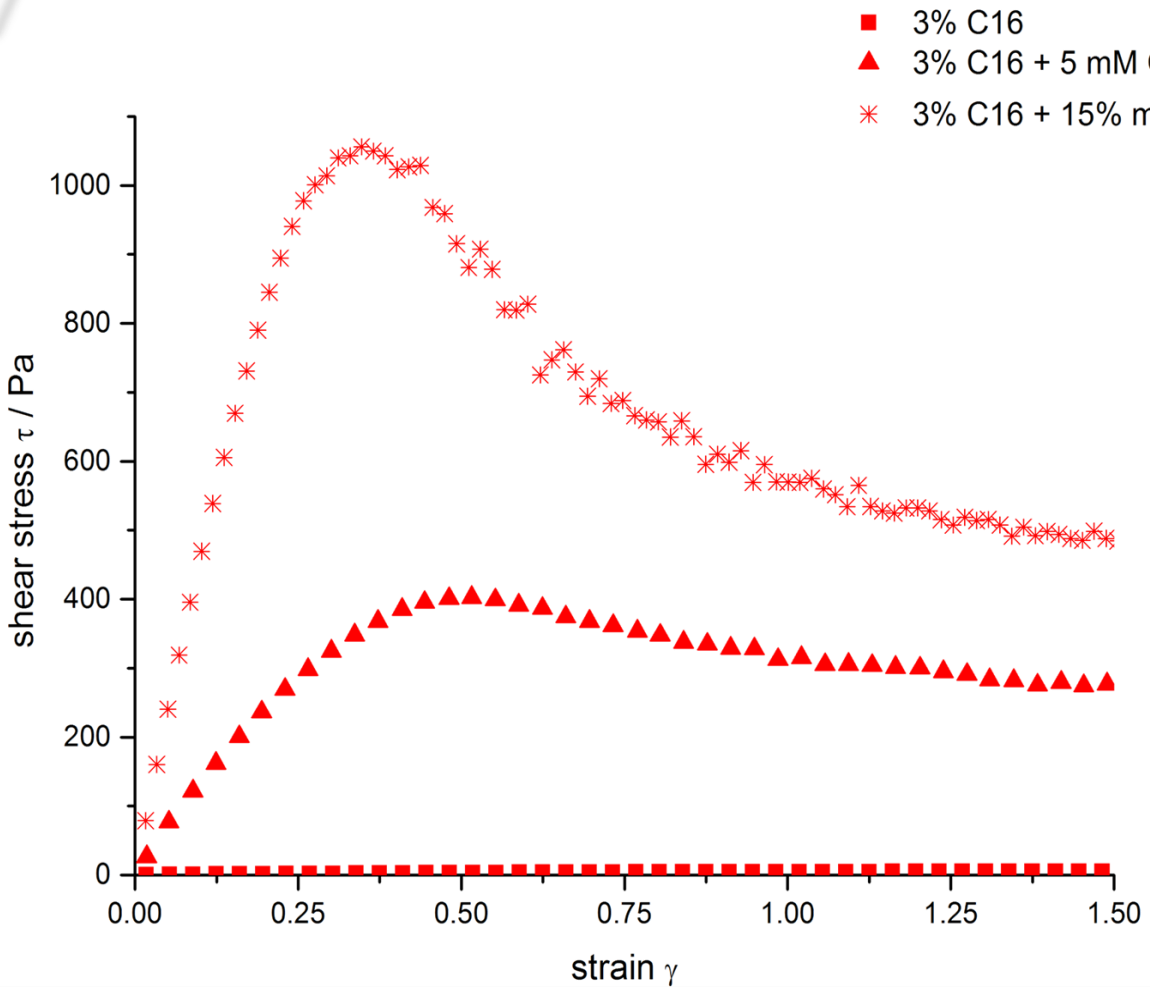


→ **Increase in the speed of nucleation and growth phase**

→ **Increase in the speed of crosslinking**

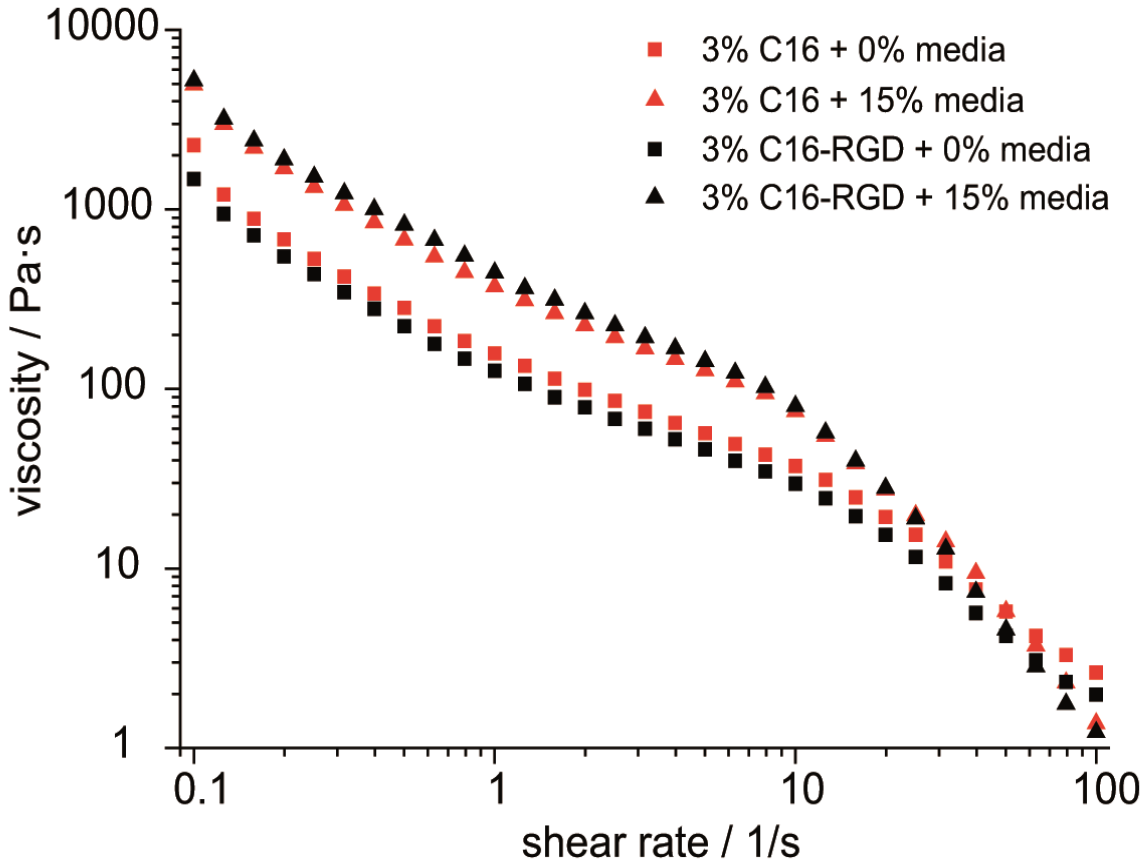
→ **FTIR measurements revealed no effects of the media on secondary structure content**

Rheology – stress-strain



→ **Bivalent cations can reduce the electrostatic repulsion between protein chains leading to an increase in stiffness**

Rheology – shear-rate dependent viscosity



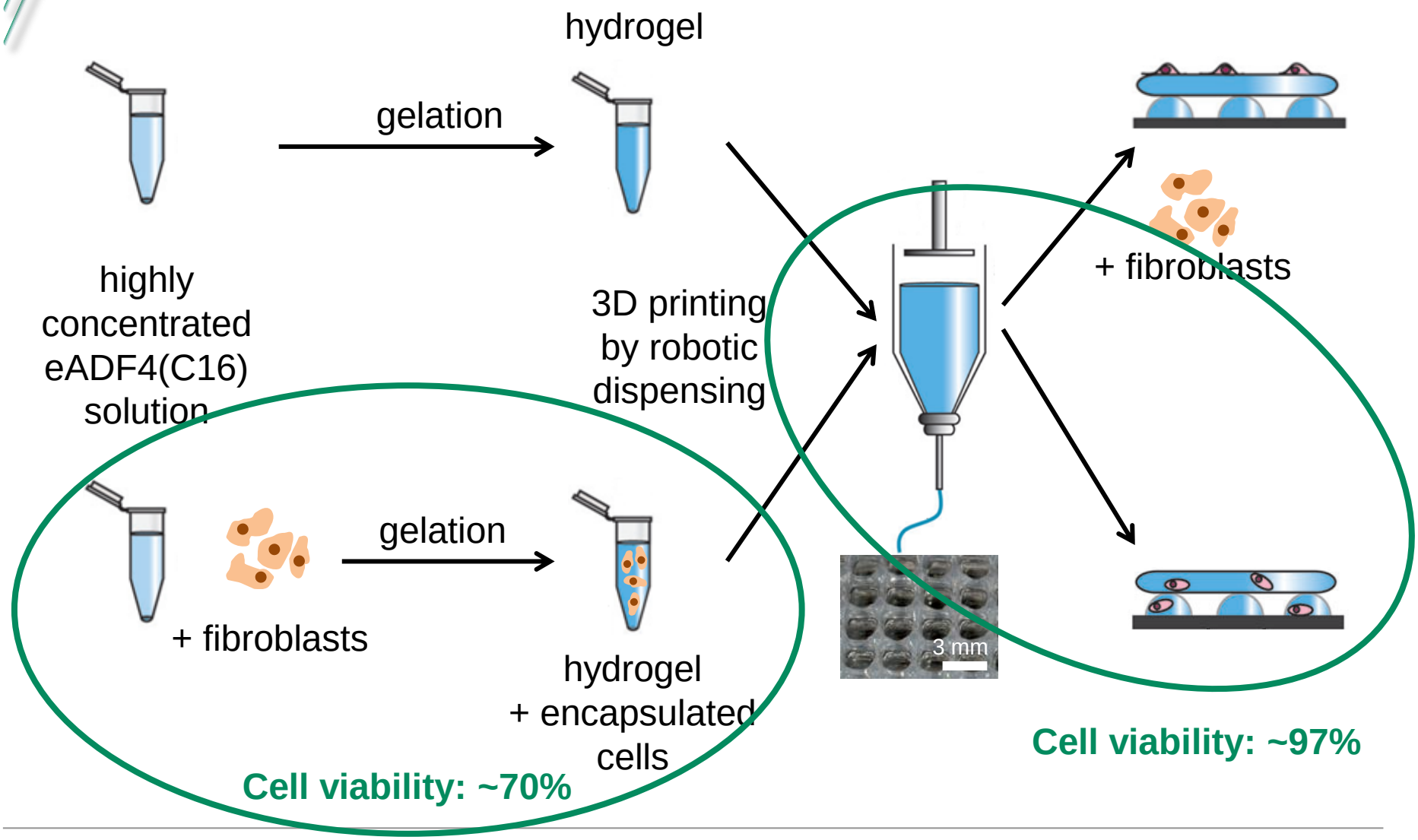
→ **High shear rates disrupt ionic binding**

→ **Shear thinning behaviour is observed**

→ **Shear thinning properties protect cells from shear forces**

DeSimone, E.; Schacht, K.; Scheibel, T.; *Mater Lett* **2016**, *183*, 101-104.

Hydrogel preparation + printing

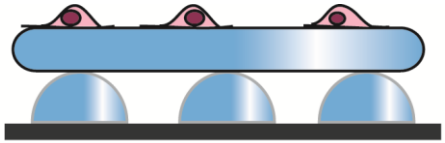


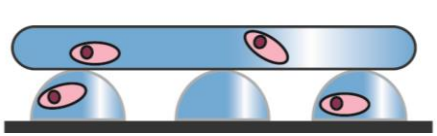
Modified after: Schacht, K.; Jüngst, T.; Schweinlin, M.; Ewald, A.; Groll, J.; Scheibel, T., *Angewandte Chemie* **2015**, *54*, 5

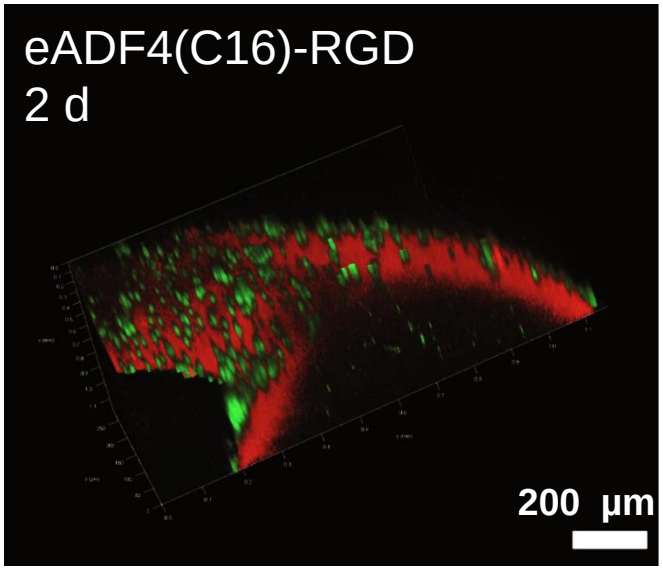
Cells on & encapsulated in printed constructs

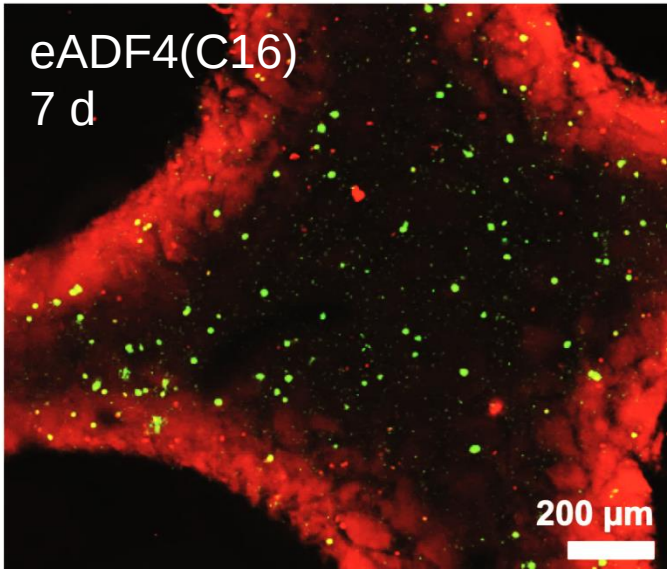
BioMat

human fibroblasts – live/dead staining









→ **fibroblasts adhere well on printed eADF4(C16)-RGD hydrogels → robotic dispensing doesn‘t disturb cell-material interaction**

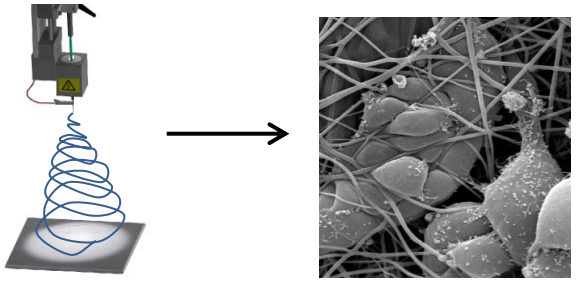
→ **fibroblasts encapsulated in eADF4(C16) hydrogels survive 7 d *in situ***

Modified after: Schacht, K.; Jüngst, T.; Schweinlin, M.; Ewald, A.; Groll, J.; Scheibel, T., *Angewandte Chemie* **2015**, *54*, 5

Summary

engineered spider silk protein

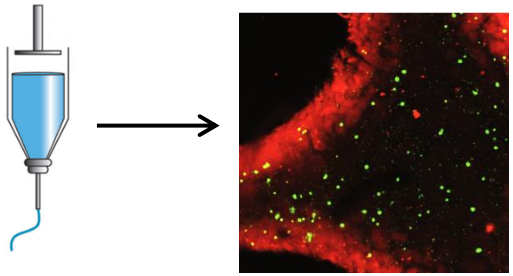
non-wovens



→ tissue engineering

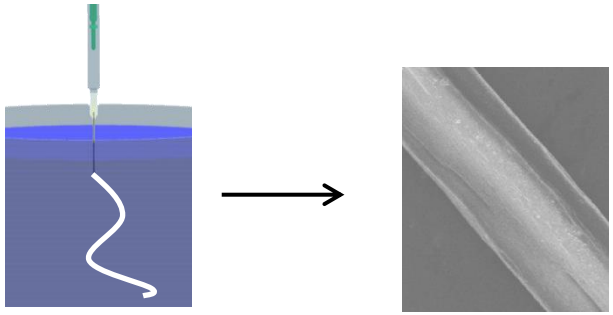
→ wound dressing

hydrogels



→ tissue engineering

fibers



→ surture material

Thank you for your attention

Deutsche Forschungsgemeinschaft DFG
SCHE 603/4-4

SFB 840

Bayerisches Staatsministerium für Umwelt und Gesundheit
U8793-2012/6-2