Supplementary Materials

Factors Influencing Properties of Spider Silk Coatings and Their Interactions within a Biological Environment

Vanessa T. Trossmann [1], Sarah Lentz [1] and Thomas Scheibel [1,2,3,4,5,6,*]

[1] Chair of Biomaterials, Faculty of Engineering Science, University of Bayreuth, Prof.-Rüdiger-Bormann-Straße 1, 95447 Bayreuth, Germany; vanessa.trossmann@uni-bayreuth.de (V.T.T.); sarah1.lentz@uni-bayreuth.de (S.L.)
[2] Bayreuth Center for Colloids and Interfaces (BZKG), University of Bayreuth, Universitätsstraße 30, 95440 Bayreuth, Germany
[3] Bavarian Polymer Institute (BPI), University of Bayreuth, Universitätsstraße 30, 95440 Bayreuth, Germany
[4] Bayreuth Center for molecular Biosciences (BZMB), University of Bayreuth, Universitätsstraße 30, 95440 Bayreuth, Germany
[5] Bayreuth Materials Center (BayMAT), University of Bayreuth, Universitätsstraße 30, 95440 Bayreuth, Germany
[6] Faculty of Medicine, University of Würzburg, Pleicherwall 2, 97070 Würzburg, Germany
* Correspondence: thomas.scheibel@uni-bayreuth.de

Table S1. Summary of recombinant spider silk proteins, spider species, production host and respective amino acid sequence.

Protein	Spider species	Production host	Amino acid sequence	Reference
eADF4(C16) eADF4(C32)	*Araneus diadematus*	*E. coli*	C- module repeated 16 or 32 times GSSAAAAAAAASGPGGYGPENQGPSGPGGYGPGGP + cell adhesion peptides (e.g. RGD, IKVAV) + biomineralization peptides + cystein-containing peptide (e.g. ntagCys)	[1-7]
eADF4(κ16)	*Araneus diadematus*	*E. coli*	κ-module repeated 16 times GSSAAAAAAAASGPGGYGPKNQGPSGPGGYGPGGP + cell adhesion peptides (e.g. RGD, IKVAV) + cystein-containing peptide (e.g. ntagCys)	[1,3-6]
eADF4(Ω16)	*Araneus diadematus*	*E. coli*	Ω-module repeated 16 times GSSAAAAAAAASGPGGYGPQNQGPSGPGGYGPGGP + cell adhesion peptides (e.g. RGD, IKVAV)	[1-4]
eADF3(AQ)$_{12}$ eADF3(AQ)$_{24}$	*Araneus diadematus*	*E. coli*	A and Q module repeated 12 or 24 times (A) GPYGPGASAAAAAAGGYGPGSGQQ (Q) GPGQQGPGQQGPGQQGPGQQ	[1,2]
eADF3 with function- nal domains			Domains: cellulose-binding domains (CBM), peptide interacting domains (SPY_C), gamma-crystalline D domain (Crys), fibronectin III domain (FN)	[8-10]

4RepCT	*Euprosthenops australis*	*E. coli*	GGSGNSGIQGQGGYGGLGQGGYGQGAGSSAAAAAAAAAAA AGGQGGQGQGGYGQGSGGSAAAAAAAAAAAAAAAGRGQG GYGQGSGGNAAAAAAAAAAAAAAAGQGGQGGYGRQSQGA GSAAAAAAAAAAAAAAGSGQGGYGGQGQGGYGQSSASASA AASAASTVANSVSRLSSPSAVSRVSSAVSSLVSNGQVNMAALP NIISNISSSVSASAPGASGCEVIVQALLEVITALVQIVSSSSVGYIN PSAVNQITNVVANAMAQVMG (contains C-terminal domain) + cell adhesion peptides (e.g. RGD, IKVAV) + antimicrobial peptides (e.g. Lac and Mag) + specific ligand-interaction peptides (e.g. IgG and albumin) + entire proteins (e.g. FGF)	[11-36]
2RepCT			GNSGRGQGGYGQGSGGNAAAAAAAAAAAAAAAGQGGQGG YGRQSQGAGSAAAAAAAAAAAAAAGSGQGGYGGQGQGGY GQSGNSVTSGGYGYGTSAAAGAGVAAGSYAGAVNRLSSAEAA SRVSSNIAAIASGGASALPSVISNIYSGVVASGVSSNEALIQALLE LLSALVHVLSSASIGNVSSVGVDSTLNVVQDSVGQYVG + N-terminal domain	[37-41]
rMaSp1	*Trichonephila clavipes*	*Transgenic goats*	QGAGAAAAAAGGAGQGGYGGLGGQGAGQGGYGGLGGQGA GQGAGAAAAAAAGGAGQGGYGGLGSQGAGRGGQGAGAAA AAAGGAGQGGYGGLGSQGAGRGGLGGQGAGAAAAAAAAGG AGQGGYGGLGNQGAGRGGQGAAAAAAGGAGQGGYGGLGS QGAGRGGLGGQGAGAAAAAAGGAGQGGYGGLGGQGAGQG GYGGLGSQGAGRGGLGGQGAGAAAAAAAGGAGQGGLGGQ GAGQGAGASAAAAGGAGQGGYGGLGSQGAGRGGEGAGAA AAAAGGAGQGGYGGLGGQGAGQGGYGGLGSQGAGRGGLG GQGAGAAAAGGAGQGGLGGQGAGQGAGAAAAAAGGAGQ GGYGGLGSQGAGRGGLGGQGAGAVAAAAAGGAGQGGYGG LGSQGAGRGGQGAGAAAAAAGGAGQRGYGGLGNQGAGRG GLGGQGAGAAAAAAAGGAGQGGYGGLGNQGAGRGGQGAA AAAGGAGQGGYGGLGSQGAGRGGQGAGAAAAAAVGAGQE GIRGQGAGQGGYGGLGSQGSGRGGLGGQGAGAAAAAAGGA GQGGLGGQGAGQGAGAAAAAAGGVRQGGYGGLGSQGAGR GGQGAGAAAAAAGGAGQGGYGGLGGQGVGRGGLGGQGAG AAAAGGAGQGGYGGVGSGASAASAAASRLSSPQASSRLSSAVS NLVATGPTNSAALSSTISNVVSQIGASNPGLSGCDVLIQALLEV VSALIQILGSSSIGQVNYGSAGQATQIVGQSVYQALG	[42-48]
rMaSp2			PGGYGPGQQGPGGYGPGQQGPSGPGSAAAAAAAAAAGPGGY GPGQQGPGGYGPGQQGPGRYGPGQQGPSGPGSAAAAAAGSG QQGPGGYGPRQQGPGGYGQGQQGPSGPGSAAAASAAASAES GQQGPGGYGPGQQGPGGYGPGQQGPGGYGPGQQGPSGPGSA AAAAAAASGPGQQGPGGYGPGQQGPGGYGPGQQGPSGPGSA AAAAAAASGPGQQGPGGYGPGQQGPGGYGPGQQGLSGPGSA AAAAAAGPGQQGPGGYGPGQQGPSGPGSAAAAAAAAAGPG	

			GYGPGQQGPGGYGPGQQGPSGAGSAAAAAAAGPGQQGLGG YGPGQQGPGGYGPGQQGPGGYGPGSASAAAAAAGPGQQGPG GYGPGQQGPSGPGSASAAAAAAAAGPGGYGPGQQGPGGYAP GQQGPSGPGSASAAAAAAAAGPGGYGPGQQGPGGYAPGQQ GPSGPGSAAAAAAAAAGPGGYGPAQQGPSGPGIAASAASAGP GGYGPAQQGPAGYGPGSAVAASAGAGSAGYGPGSQASAAAS RLASPDSGARVASAVSNLVSSGPTSSAALSSVISNAVSQIGASNP GLSGCDVLIQALLEIVSACVTILSSSSIGQVNYGAASQFAQVVGQ SVLSAF	
FLYS, FLYS$_3$ FLYS$_4$	*Trichonephila clavipes*	*E. coli*	Module repeated up to 4 times GPGGPGGYGPGGSGPGGYGPGGSGPGGYGPGGSGPGGYGPGG SGPSGPGSAAAAAAAAGPGGYGPGGSGPGGYGPGGSGPGGYG PGGSGPGGYGPGGSGPGGYGPGGSGPSGPGSAAAAAAAAGPG GYGPGGSGPGGYGPGGSGPGGYGPGGSGPSGPGSAAAAAAAA GPGGYGPGGSGPGGYGPGGSGPGGYGPGGSGPGGYGPGGSGP GGYGPGGSGPGGYGPGGSGPSGPGSAAAAAAAAGPGGYGPG GSGPGGYGPGGSGPGGYGPGGSGPGGYGPGGSGPGGYGPGGS GPGGYGPGGSGPGGYGPGGSGPGGYGPGGSGPSGPGSAAAAA AAA	[44,47]
1-mer until 192-mer	*Trichonephila clavipes*	*E. coli*	1 mer module repeated up to 96mer SGRGGLGGQGAGAAAAAGGAGQGGYGGLGSQGT + cell adhesion peptides (e.g. RGD) + antimicrobial peptides + biomineralization peptides + cancer treating peptides	[49-72]

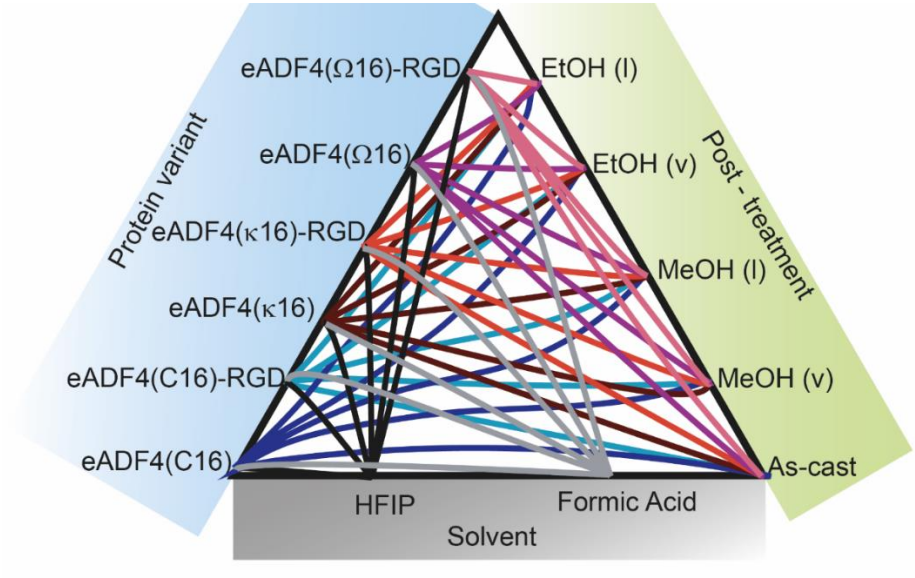

Figure S1. Scheme of the sample matrix of experimental settings used to analyze how the spider silk protein sequence, the casting solvent and the post-treatment influence cell adhesion. The recombinant ADF4 variants (eADF4(C16), eADF4(κ16), eADF4(Ω16), eADF4(C16)-RGD, eADF4(κ16)-RGD and eADF4(Ω16)-RGD) were either solved in FA or HFIP and cast on ozone-pre-treated PS surfaces. Furthermore, coatings were differently post-treated (MeOH vapor or liquid, EtOH vapor or liquid) and compared to non-post-treated films regarding adhesion of human BJ skin fibroblasts and human MG63 bone fibroblasts. The results thereof are summarized in Figure 6, S2 and S3.

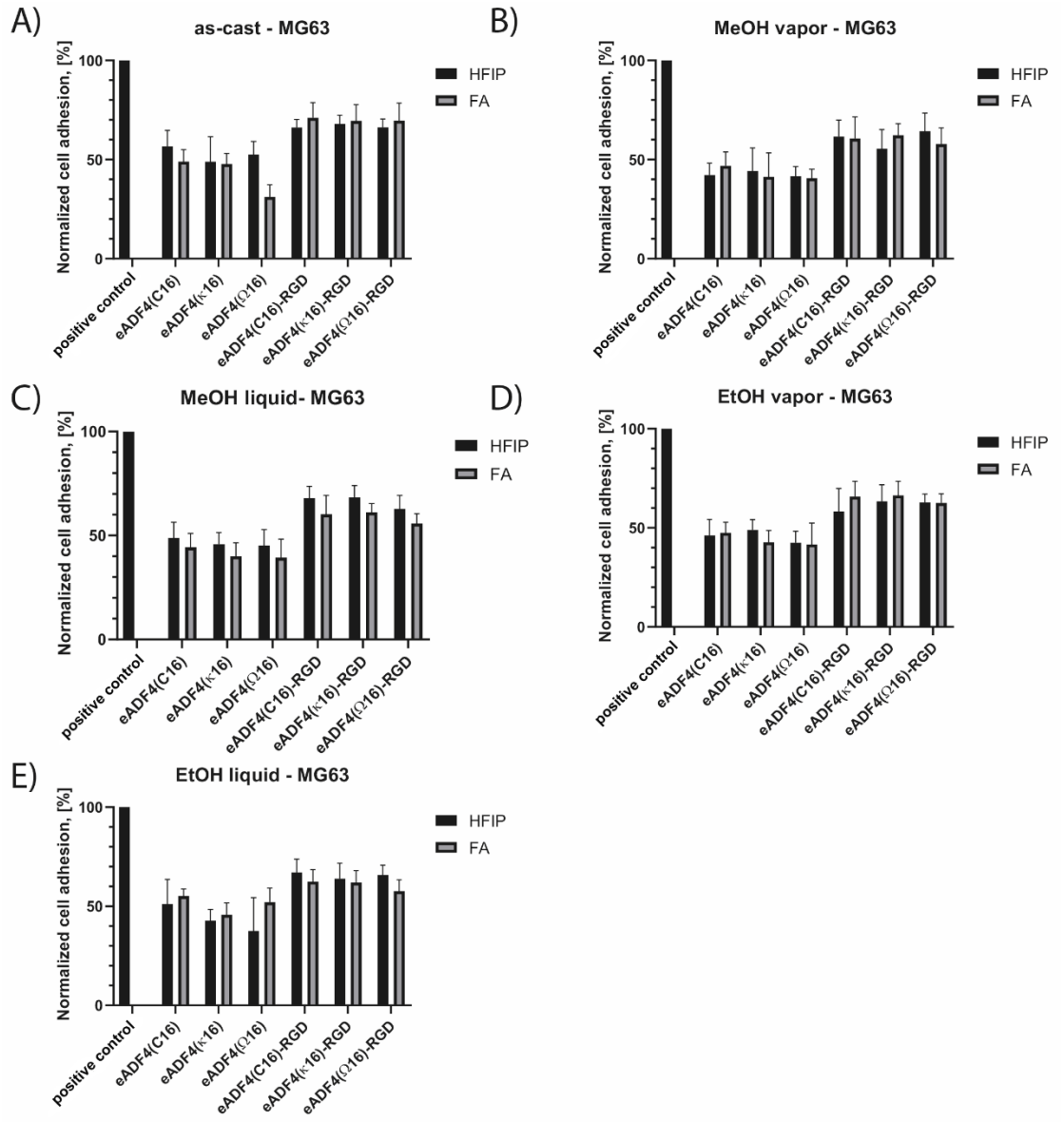

Figure S2. Cell adhesion of MG63 on films made of eADF4 variants (**A**) as cast or post-treated with (**B**) MeOH vapor, (**C**) MeOH liquid, (**D**) EtOH vapor, and (**E**) EtOH liquid.

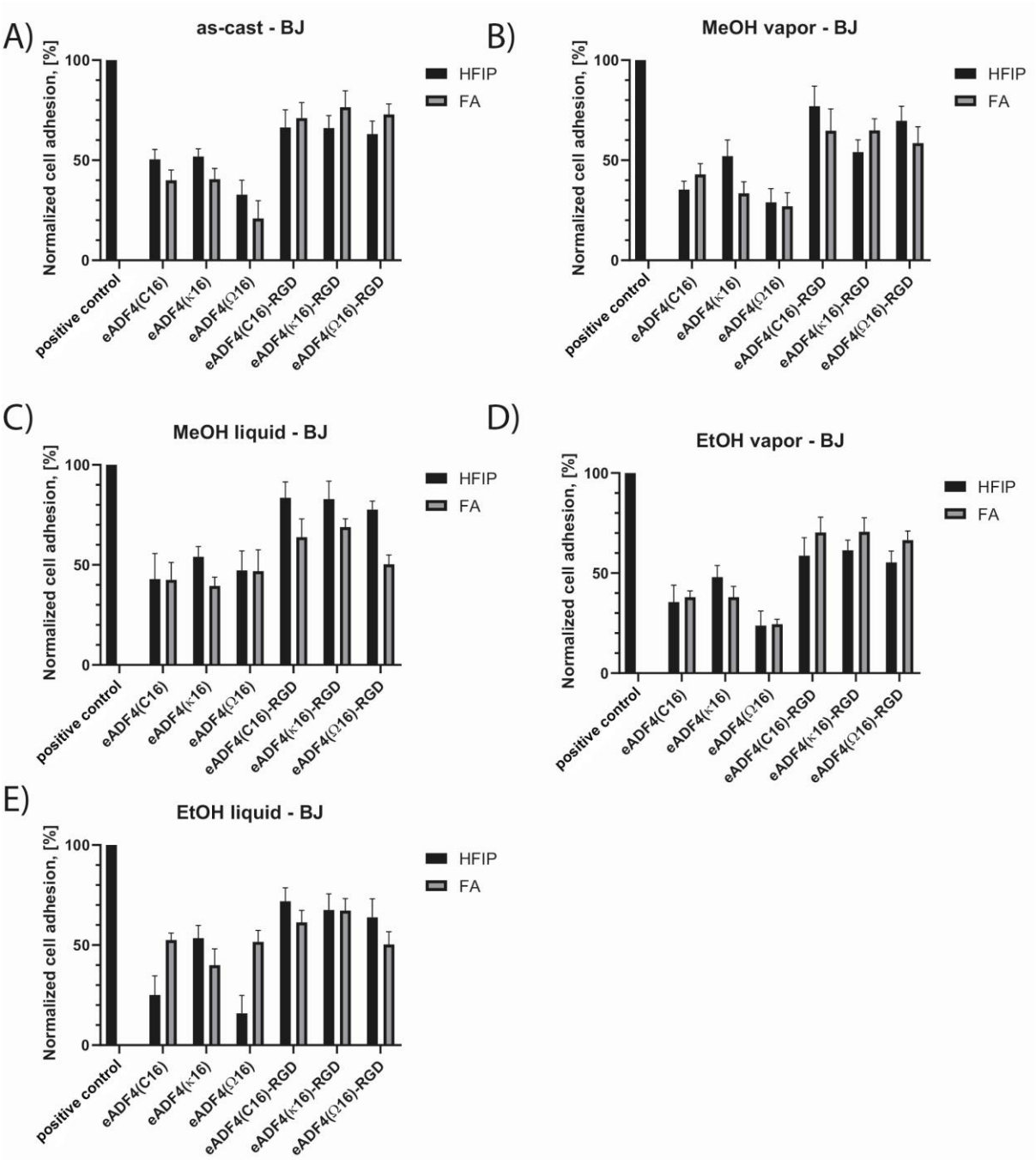

Figure S3. Cell adhesion on films made of BJ of eADF4 variants (**A**) as cast or post-treated with (**B**) MeOH vapor, (**C**) MeOH liquid, (**D**) EtOH vapor, (**E**) EtOH liquid.

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