



**QUEEN'S
UNIVERSITY
BELFAST**

Glycopolymer Functionalization of Engineered Spider Silk Protein-based Materials for Improved Cell Adhesion

Hardy, J. G., Pfaff, A., Leal-Egana, A., Mueller, A. H. E., & Scheibel, T. R. (2014). Glycopolymer Functionalization of Engineered Spider Silk Protein-based Materials for Improved Cell Adhesion. *Macromolecular bioscience*, 14(7), 936-942. <https://doi.org/10.1002/mabi.201400020>

Published in:
Macromolecular bioscience

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
[Link to publication record in Queen's University Belfast Research Portal](#)

Publisher rights

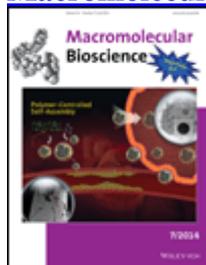
This is the accepted version of the following article: Hardy, J. G., Pfaff, A., Leal-Egaña, A., Müller, A. H. E. and Scheibel, T. R. (2014), Glycopolymer Functionalization of Engineered Spider Silk Protein-based Materials for Improved Cell Adhesion. *Macromol. Biosci.*, 14: 936–942. doi: 10.1002/mabi.201400020, which has been published in final form at <http://onlinelibrary.wiley.com/doi/10.1002/mabi.201400020/abstract;jsessionid=C0ADDD8E20B9165890A0AA37A98A68E2.f02t02>.

General rights

Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.



1. Previous article in issue: Fluorescent Amphiphilic PEG-Peptide-PEG Triblock Conjugate Micelles for Cell Imaging
2. Next article in issue: Magnetic Nanoclusters Engineered by Polymer-Controlled Self-Assembly for the Accurate Diagnosis of Atherosclerotic Plaques via Magnetic Resonance Imaging

[Volume 14, Issue 7](#)

July 2014

Pages 936–942

Communication

Glycopolymer Functionalization of Engineered Spider Silk Protein-based Materials for Improved Cell Adhesion[†]

Authors

- [John G. Hardy](#),
 - 1.
- [André Pfaff](#),
 - 1.
- [Aldo Leal-Egaña](#),
 - 1.
- [Axel H. E. Müller](#),
 -
 - 2.
 - 3.
 -
- [Thomas R. Scheibel](#)
 -
 - 1.
 -

- First published: 2 April 2014 [Full publication history](#)
- DOI: 10.1002/mabi.201400020
- [Citing literature](#)

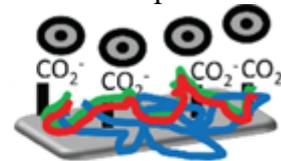
-  score 2
- [Funding Information](#)

- †**Supporting Information** is available at Wiley Online Library or from the author.

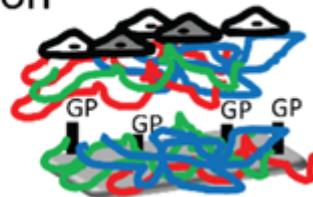
Abstract

Silk protein-based materials are promising biomaterials for application as tissue scaffolds, due to their processability, biocompatibility, and biodegradability. The preparation of films composed of an engineered spider silk protein (eADF4(C16)) and their functionalization with glycopolymers are described. The glycopolymers bind proteins found in the extracellular matrix, providing a biomimetic coating on the films that improves cell adhesion to the surfaces of engineered spider silk films. Such silk-based materials have potential as coatings

A) Poor cell adhesion



B) Glycopolymer functionalization improves cell adhesion



for degradable implantable devices.

[Enhanced Article Feedback](#)

1 Introduction

Implantable biodegradable biomaterials are attractive for the manufacture of biomedical devices such as tissue scaffolds or drug delivery devices.[1] Silk protein-based materials and their composites are attractive for such applications due to their biocompatibility and highly tunable morphologies.[2-4] Consequently a range of such materials have been investigated, typically composed of silkworm fibroins (most commonly that of domesticated *Bombyx mori* silkworms,[2-4] albeit not exclusively)[5] although in recent years silk proteins of other species including bees,[6] caddisfly larvae,[7] lacewings[8] and spiders (produced naturally[9] or recombinantly)[10-14] have also been studied.

Web-weaving spiders (such as *Araneus diadematus* or *Nephila clavipes*) produce a variety of task-specific silk fibers that have been used for centuries by humans in certain parts of the world for applications as diverse as fishing nets and wound dressings. Although naturally spun spider silk fibers show promise for peripheral nerve tissue engineering,[9] harvesting even relatively small quantities of natural silks is an incredibly time consuming and expensive process, and the production of spider silk-like proteins on a scale large enough for industrial applications to be realizable is currently only possible using recombinant DNA technology.[10-14]

Herein we utilize a recombinantly produced engineered silk protein, inspired by the repetitive sequence of one of the major ampullate silk proteins of *A. diadematus* spiders, *A. diadematus* fibroin 4 (ADF4) that has been optimized for high density fermentation in *Escherichia coli* bacteria, namely, eADF4(C16).[\[15\]](#) It can be processed into a number of different materials morphologies, including fibers, films, hydrogels, capsules and particles that are of interest for tissue engineering and drug delivery applications.[\[2-4\]](#) It is possible to utilize various solvents (including water, formic acid and hexafluoroisopropanol (HFIP)) to prepare films composed of eADF4(C16) with tunable chemical, mechanical and topographical properties.[\[16-19\]](#)

During the course of our investigations we have observed that the adhesion of cells to the surfaces of materials composed solely of eADF4(C16) is relatively weak, and have sought to address this by modifying the protein to display cell-adhesive motifs (e.g., the RGD peptide) either chemically or recombinantly,[\[16\]](#) or by altering the surface topography.[\[19\]](#) While this can be effective at improving cell adhesion to the silk-based substrates, the number of cell-adhesive motifs displayed per unit surface area affects not only cell adhesion, but also cell migration and proliferation,[\[20, 21\]](#) and it is therefore challenging to develop materials that would be broadly applicable in an off-the-shelf manner. An attractive alternative method is to develop materials that bind macromolecules from the extracellular matrix (ECM) because the ECM displays cell adhesive moieties at levels that are optimized for the specific tissue in which it is implanted through millions of years of evolution. Moreover, such biomimetic materials are patient-specific personalized medical devices by virtue of the fact that the ECM composition is known to change with age.[\[22, 23\]](#)

The ECM is a complex mixture of proteins, glycoproteins and polysaccharides that interact via a variety of non-covalent supramolecular interactions, and biomimetic composite biomaterials incorporating polysaccharides are interesting because they tend to display low immunogenicity when implanted in vivo.[\[24, 25\]](#)

Synthetic glycopolymers have great potential for biomedical applications because their syntheses are relatively straightforward, and it is therefore possible to develop non-toxic glycopolymer-based materials with novel properties.[\[26-36\]](#) Glycopolymer-functionalized surfaces can bind proteins; glucosamine-based glycopolymers bind wheat germ agglutinin with an affinity that can be enhanced by the glyco-cluster effect,[\[37\]](#) and galactose-based glycopolymers bind lectin RCA₁₂₀.[\[38\]](#) Furthermore, functionalization of chitosan-based materials with wheat germ agglutinin improved the adhesion and viability of fibroblasts on their surfaces,[\[39\]](#) and functionalization of *Bombyx mori* silkworm fibroin-based materials with mannose-based glycopolymers bound the lectin Concanavalin A, and improved the adhesion of myoblasts (L6 skeletal muscle myoblasts) to their surfaces.[\[40\]](#)

Here we describe the modification of the surfaces of eADF4(C16) with glycopolymers that bind proteins found in the ECM, thereby facilitating the formation of a biomimetic coating on the surface of the silk-based material which results in improved adhesion of cells to their surfaces. The syntheses of the glycopolymers are straightforward, scalable and potentially useful to improve cell adhesion to a wide variety of biomaterial substrates.

2 Experimental Section

2.1 Materials

The recombinantly produced protein eADF4(C16) is based on the consensus motif of the repetitive core domain of one of the major ampullate silk fibroins of the garden cross spider (*Araneus diadematus* fibroin 4). The recombinant protein is composed of sixteen repeats of the polypeptide module C (amino acid sequence: GSSAAAAAAAASGPGGYGPENQGPSGPGGYGPGGP), and is referred to hereafter as eADF4(C16). Production and purification of eADF4(C16) was carried out as described previously[15] as was the production and purification of azidopropylamine.[41] Unless otherwise stated, all other chemicals were obtained from Sigma-Aldrich Chemie GmbH and used as supplied.

2.2 Glycopolymer Synthesis

Syntheses of alkyne-capped-poly(-6-*O*-methacryloyl-D-galactopyranose) (PMAGal) derivatives were all carried out in round-bottom flasks sealed with a septum. A representative example, for glycopolymer **1** is as follows: a mixture of the glycomonomer 6-*O*-methacryloyl-1,2:3,4-di-*O*-(isopropylidene)galactopyranose[42] (MAIGal, 1 g, 3.04×10^{-3} mol), the initiator prop-2-ynyl 2-bromo-2-methylpropanoate (30.83 mg, 0.15×10^{-3} mol), and CuCl (15.07 mg, 0.15×10^{-3} mol), in chlorobenzene (3 mL) were degassed for several minutes. After addition of *N,N,N',N'',N'''*-pentamethyldiethylenetriamine (PMDETA, 32 μ L, 0.15×10^{-3} mol) the color of the solution became green, indicating the dissolution of CuCl. The mixture was stirred for 24 h at room temperature, passed through a silica column, and the polymer precipitated from THF into methanol. The protected glycopolymer was analyzed by Gel Permeation Chromatography (GPC) and Matrix-assisted Laser Desorption Ionization Time-of-Flight (MALDI-ToF) Mass Spectrometry as described in the supplementary experimental section. For PMAIGal_DP64 and PMAIGal_DP97 the monomer to initiator to catalyst ratio was [MAIGal]₀/[Initiator]₀/[CuCl]₀/[PMDETA]₀ = 50:1:1:1 and 80:1:1:1, respectively. To deprotect the sugar moieties, PMAIGal was dissolved in 2 mL of trifluoroacetic acid / water (5:1) and stirred for 1 h. The solution was neutralized by the addition of sodium bisulfate, dialyzed against water and finally freeze-dried.

2.3 Film Preparation

Optically clear solutions of the protein eADF4(C16) dissolved in HFIP were cast in 24 well Nunclon Δ surface tissue culture plates. The solvent was allowed to evaporate over a period of 24 h in a fume hood, and the films were then immersed in anhydrous methanol for 1 h prior to drying for a further 48 h under high vacuum. The thickness of the films was determined with high precision digital calipers (Bochem, Germany).

2.4 Film Modification

Films of eADF4(C16) were incubated in an aqueous solution (1 mL) containing 1-ethyl-3-(dimethylaminopropyl)carbodiimide (EDC, 2 mg mL⁻¹), *N*-hydroxysuccinimide (NHS, 3 mg mL⁻¹), and azidopropylamine (2 mg mL⁻¹). After 18 h the solution was removed and the films were washed with water (3 \times 1 mL, 20 min incubation time) and then with ethanol/water (70/30, v/v) and allowed to dry in a fume hood overnight. Films were subsequently incubated in an aqueous solution (1 mL) containing CuSO₄ (at a concentration of 2×10^{-3} M), tris(2-carboxyethyl)phosphine (TCEP, at a concentration of 3×10^{-3} M), and glycopolymer **1**, **2** or **3** at a concentration of 3, 6 or 9 mg mL⁻¹ respectively. After 12 h the solution was removed and the films were washed with ethylenediaminetetraacetic acid (EDTA, 5×10^{-3} M) in phosphate

buffered saline (PBS) until the solution was colorless, after which it was washed three times with PBS, then once with water, and finally with ethanol/water (70/30, v/v) and allowed to dry in a fume hood overnight.

2.5 Fourier Transform (FT) IR Spectroscopic Studies of Films

FT-IR spectra of films were recorded on a liquid nitrogen cooled Bruker Tensor 27 FTIR spectrometer in ATR mode at a resolution of 1 cm^{-1} .

2.6 Water Contact Angle Measurements

Measurements were carried out in line with our previously described methodology.[\[43\]](#) For full experimental details refer to the supplementary information.

2.7 Protein Binding Studies

The adsorption of either rat tail collagen type-1 or laminin-1 mouse protein to the surface of the silk-based films and control substrates (untreated polystyrene and plasma-treated Nunclon Δ surface tissue culture plates) was assessed using a Micro BCA Protein Assay Kit and UV-Vis spectroscopy at 562 nm in accordance with the manufacturer's protocol. For full experimental details refer to the supplementary information.

2.8 In Vitro Cell Adhesion Studies

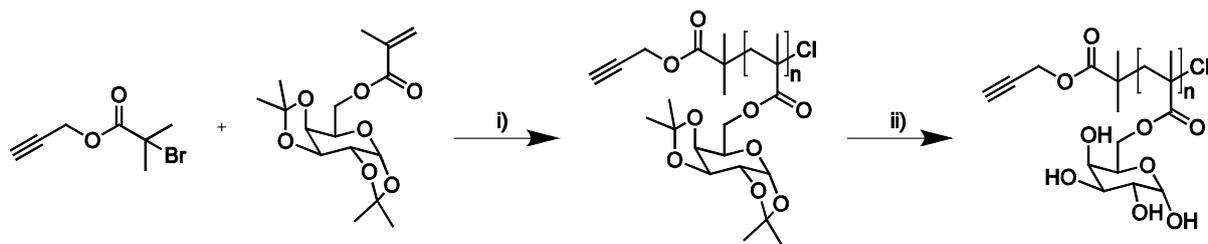
Cell adhesion studies using the fibroblast cell line (M-MSV-BALB/3T3, mouse embryo fibroblasts) were carried out in line with our previously described methodology.[\[26\]](#) For full experimental details refer to the supplementary information.

3 Results and Discussion

3.1 Glycopolymer Synthesis

Three protected glycopolymers were synthesized via reaction of the glycomonomer (MAIGal),[\[42\]](#) the initiator prop-2-ynyl-2-bromo-2-methylpropanoate, CuCl, and PMDETA (at molar ratios of 30: 1: 1: 1, 50: 1: 1: 1 and 80: 1: 1: 1 respectively) in chlorobenzene at room temperature for 24 h (Scheme [1](#)), after which they were purified via passage through a silica column, precipitation from THF solution into methanol, and dialysis. The three protected glycopolymers were analyzed by GPC and MALDI-ToF MS, demonstrating their degrees of polymerization to be 31, 64 and 97 respectively, corresponding to molecular weights of the order of ca. 8 to 30 kDa (see Figure S1 and Table S1, Supporting Information) with polydispersity indices of ≈ 1.1 . The deprotected glycopolymers were isolated via exposure of the polymer to an aqueous solution of trifluoroacetic acid followed by neutralization with sodium bisulfate, dialysis against water and finally freeze-drying.

Scheme 1.



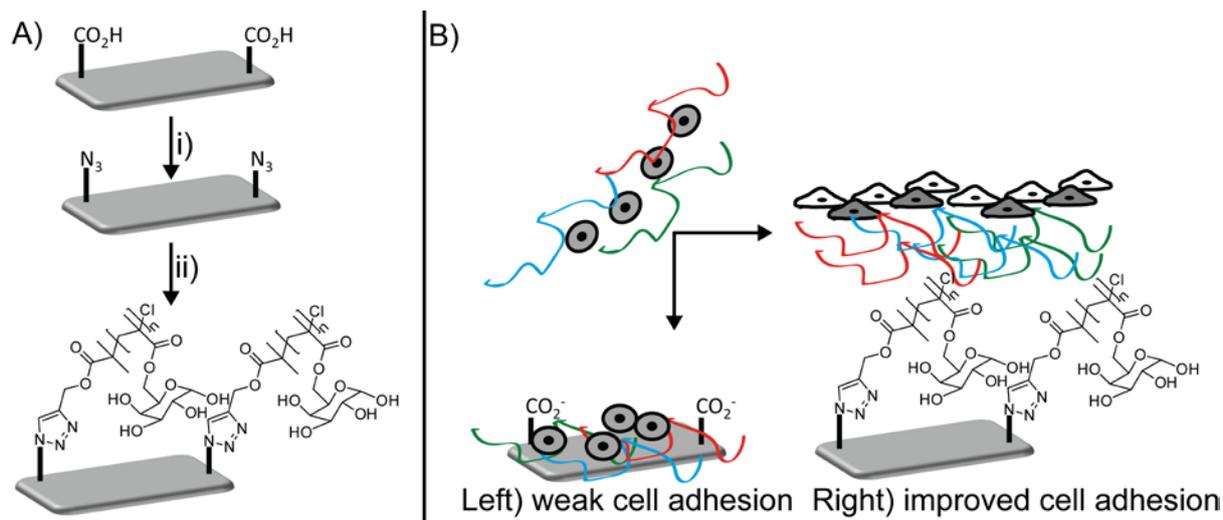
Synthesis of glycopolymers **1–3**. i) CuCl, PMDETA. ii) TFA/H₂O. Glycopolymer **1**, DP = 31; Glycopolymer **2**, DP = 64; Glycopolymer **3**, DP = 97. See also Supporting Information Figure S1 and Table S1.

3.2 Film Preparation and Modification

Films were prepared by casting solutions of eADF4(C16) in HFIP in 24 well tissue culture plates, followed by immersion in methanol to induce β -sheet formation in the silk.^[26] FTIR spectroscopy (Figure S2, Supporting Information) revealed peaks at 1624 cm⁻¹ (amide I), 1520 cm⁻¹ (amide II), and 964 cm⁻¹ (polyalanine), indicative of β -sheet rich spider silk-based materials that are water insoluble. Films had thicknesses of \approx 1 μ m, and would therefore not be expected to be encapsulated by a very thick foreign body capsule if implanted in vivo.^[44]

To modify the surface of the films of engineered spider silk with alkyne-terminated glycopolymers **1–3** (Scheme 2A), azidopropylamine was coupled to the carboxylic acid moieties on the backbone of eADF4(C16) via carbodiimide-mediated amide bond formation with EDC and NHS. The successful coupling of azidopropylamine to the films was straightforward to be confirmed by FT-IR spectroscopy because the characteristic azide absorbance at \approx 2100 cm⁻¹ from azidopropylamine (Figure S3, Supporting Information) was present in the IR spectrum of the modified silk films, eADF4(C16)-N₃ (Figure S4, Supporting Information). Films of eADF4(C16)-N₃ were modified to display glycopolymer **1–3** using azide-alkyne Huisgen cycloaddition chemistry employing CuSO₄ as the copper source and TCEP as the reducing agent to generate the catalytic Cu(I) species. The successful coupling of glycopolymers **1–3** to the films was confirmed by FT-IR spectroscopy using the presence of the characteristic ester absorbance at \approx 1716 cm⁻¹ from the glycopolymers (Figure S5, Supporting Information) and the diminution of the azide (N₃) peak in the films at \approx 2100 cm⁻¹; IR spectra of the glycopolymer modified silk films (eADF4(C16)-**1**, eADF4(C16)-**2**, eADF4(C16)-**3**) are displayed in Supporting Information Figures S6, S7 and S8 respectively. The results of water contact angle measurements (WCA, Table 1) also suggested that the surface functionalization reactions had been successful. Unmodified eADF4(C16) films were relatively hydrophilic (WCA \approx 57°), modification of the hydrophilic carboxylic acid residues to display azides yielding eADF4(C16)-N₃ resulted in more hydrophobic surfaces (WCA \approx 77°), and their subsequent modification with alkyne displaying glycopolymers rendered them somewhat more hydrophilic (WCA \approx 71° for glycopolymers **1–3**) than the azide modified films, yet less hydrophilic than eADF4(C16) films that displayed more carboxylic acid moieties.

Scheme 2.



Surface modification of engineered spider silk films with glycopolymers. A) Chemical modification of engineered spider silk eADF4(C16). i) EDC, NHS, azidopropylamine. ii) CuSO_4 , TCEP and one of glycopolymers 1–3. B) Addition of suspension of cells in media (red, green and blue lines represent proteins) to an engineered spider silk-based film. Left: Unmodified eADF4(C16), upon which cell adhesion is relatively poor. Right: Glycopolymer functionalized eADF4(C16), upon which cell adhesion is improved.

Table 1. Film properties

Substrate	Water contact angle [°]	R_a [μm]	R_q [μm]	R_z DIN [μm]	Collagen-1 binding [$\mu\text{g} \cdot \text{cm}^{-2}$]	Laminin-1 binding [$\mu\text{g} \cdot \text{cm}^{-2}$]	Cell adhesion relative to Nunc Δ [%]	Cell area [μm^2]
Polystyrene cell culture dish	98.3 ± 3.7	N/A	N/A	N/A	1.40 ± 0.54	0.98 ± 0.39	65 ± 9.2	243.2 ± 39.5
Nunclon Δ	81.6 ± 2.9	N/A	N/A	N/A	9.57 ± 3.46	1.19 ± 0.54	100 ± 3.4	562.5 ± 109.3
eADF4(C16)	57.4 ± 3.3	0.4 ± 0.2	0.2 ± 0.1	3.9 ± 1.5	2.14 ± 1.10	0.67 ± 0.08	69 ± 12.8	208.2 ± 43.2
eADF4(C16)- N_3	76.7 ± 2.9	N/A	N/A	N/A	N/A	N/A	N/A	N/A
eADF4(C16)-1	69.3 ± 1.2	0.3 ± 0.1	0.4 ± 0.1	3.8 ± 1.7	2.53 ± 0.90	0.78 ± 0.39	83 ± 11.5	310.9 ± 62.3
eADF4(C16)-2	72.3 ± 4.0	0.3 ± 0.1	0.4 ± 0.1	3.3 ± 0.5	4.40 ± 1.87	0.83 ± 0.38	79 ± 0.6	392.8 ± 80.7
eADF4(C16)-3	71.3 ± 4.1	0.4 ± 0.3	0.5 ± 0.3	6.0 ± 4.0	2.78 ± 0.99	0.63 ± 0.29	83 ± 10.8	329.7 ± 87.2

Since the distribution of glycopolymers on the films could influence the adsorption of proteins on their surfaces and thereby the adhesion of cells, consequently, this was assessed via X-ray photoelectron spectroscopy (XPS) and optical microscopy of films stained with a

carbohydrate-specific fluorescent dye that is commonly used in histochemistry. The peak shapes for the C 1s and O 1s peaks (Supporting Information Figures S9 and S10 respectively) showed no significant differences for each of the different glycopolymer-functionalized films, suggesting that the glycopolymers were distributed homogeneously across the surface of the films. Microscopy of fluorescent periodate Schiff-base stained (Pro-Q Emerald 488) samples revealed that the glycopolymers were distributed across the entire surface of the films (Figure S11, Supporting Information). Although there were some areas that were moderately brighter than others this is likely to be due to the inherent roughness of the film surfaces which was observed by optical profilometry (Supporting Information Figure S12 and Table 1) which correlates to the surface area of the film (and therefore azide moieties) available for chemical modification. There were no statistically significant differences in the surface topographies of the films before or after chemical modification with the glycopolymers. The films typically had average surface roughnesses (R_a) and root-mean-square roughnesses (R_q) of the order of several hundred nanometers (the surface roughness of solution cast films is typically higher than films prepared by spin coating), whereas the average height between the five highest peaks and lowest valleys (R_{zDIN}) revealed micrometer-scale features that had similar dimensions as the areas that were slightly more brightly stained with Pro-Q Emerald 488 (Supporting Information Figures S11 and S12, and Table 1).

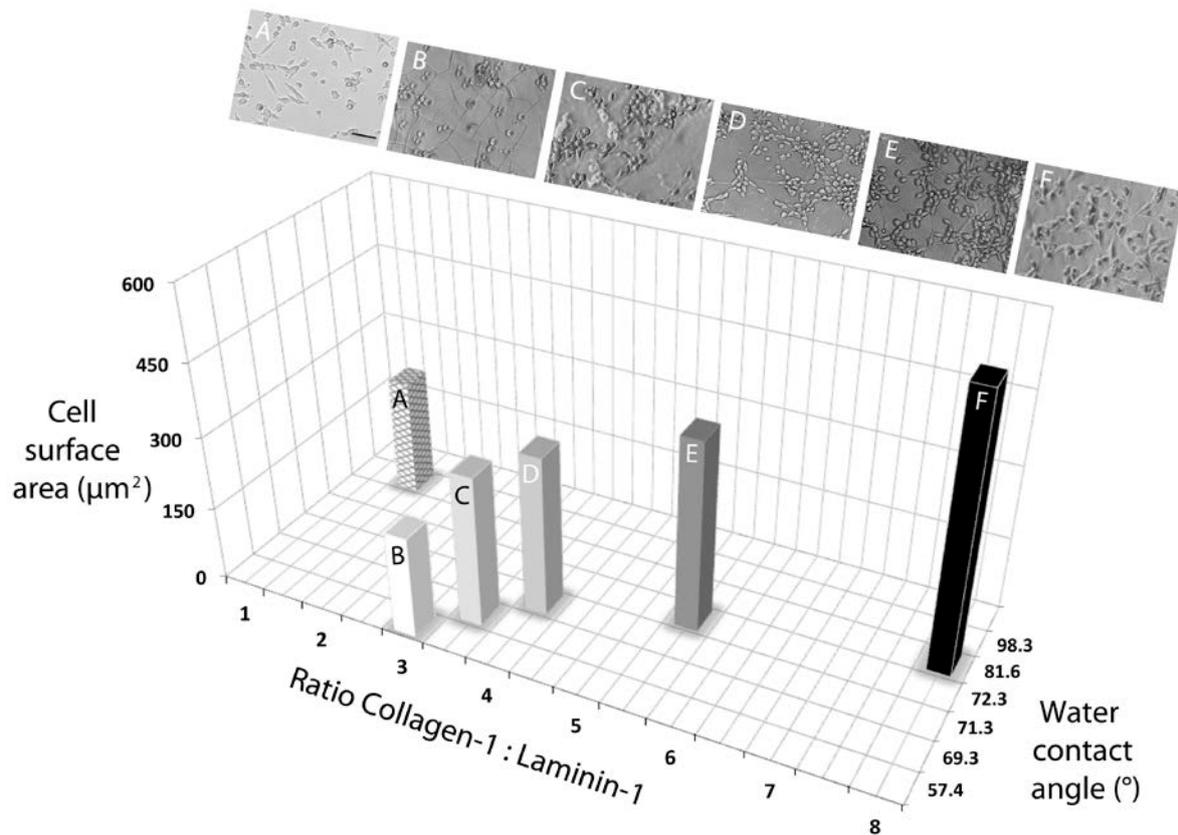
3.3 Protein Binding Studies

Substrates implanted *in vivo* are immediately coated with proteins from blood and interstitial fluids, and these affect the responses of cells adsorbed on their surfaces. Collagens, elastin, fibronectin, laminin and vitronectin are vitally important components of the ECM that play important roles in the adhesion of cells to the ECM. We investigated the adsorption of two ECM proteins (namely rat tail collagen-1 and mouse laminin-1) onto the surfaces of two control substrates (untreated polystyrene tissue culture plates and plasma-treated polystyrene tissue culture plates, that is Nunclon Δ surface plates typically having surface roughnesses of the order of a few nanometers)^[45] and the silk-based films (without/with glycopolymer functionalization) using the BCA Protein Assay (Table 1). Adsorption of collagen-1 and laminin-1 onto untreated polystyrene tissue culture plates (≈ 1.4 or $0.98 \mu\text{g cm}^{-2}$ respectively) is driven purely through hydrophobic interactions, whereas collagen-1 and laminin-1 adsorption onto the more hydrophilic plasma-treated Nunclon Δ surface plates (≈ 9.6 or $1.2 \mu\text{g cm}^{-2}$ respectively) is improved by non-specific hydrogen-bonding interactions. Adsorption of collagen-1 (pI 7.0) onto eADF4(C16) substrates ($\approx 2.1 \mu\text{g cm}^{-2}$) was higher than onto the polystyrene substrates due to non-specific hydrogen bonding interactions (and potentially the higher surface roughness, i.e., higher surface area), yet that of laminin-1 onto eADF4(C16) substrates ($\approx 0.67 \mu\text{g cm}^{-2}$) was lower than onto polystyrene substrates due to charge repulsion between the negatively charged eADF4(C16) substrates (pI 3.5) and laminin-1 (pI 5.4). Functionalization of eADF4(C16) substrates with glycopolymers **1–3** increased the amounts of collagen-1 and laminin-1 adsorbed onto their surfaces (to ≈ 2.8 or $0.75 \mu\text{g cm}^{-2}$ respectively), demonstrating the ability of glycopolymers **1–3** to bind ECM proteins. Interestingly, there was a subtle but noticeable preference for protein adsorption onto surfaces modified with glycopolymer **2** (DP = 64), that adsorbed $\approx 4.4 \mu\text{g cm}^{-2}$ of collagen-1 or $0.83 \mu\text{g cm}^{-2}$ of laminin-1 respectively. The homogeneity of the glycopolymer coatings and similarity in the surface roughnesses of the films suggests that this preference may be related to the freedom of the respective glycopolymers to adapt their conformation upon supramolecular assembly with the proteins, which may be molecular weight dependent.^[46]

3.4 In Vitro Cell Adhesion Studies

With a view to the application of such glycopolymer-modified films as biomaterials, we investigated the adhesion of BALB/3T3 mouse fibroblasts in vitro to their surfaces, and compared the results to the unmodified eADF4(C16) and aforementioned tissue culture plate control substrates (Figure 1 and Table 1). Nunclon Δ surface tissue culture plates which displayed the highest levels of collagen-1 and laminin-1 binding, were also found to display the highest levels of cell adhesion and assigned an arbitrary value of 100%. The number of cells adhered to untreated polystyrene tissue culture plates was much lower ($\approx 65\%$), and moderately improved on eADF4(C16) ($\approx 69\%$), and markedly improved on the glycopolymer-modified substrates ($\approx 80\%$). Furthermore, the cell morphologies were found to differ on the various substrates. The majority of the fibroblasts were spread out on the Nunclon Δ surface tissue culture plates (with surface areas of $\approx 560 \mu\text{m}^2$), whereas they were more rounded (with surface areas of $\approx 243 \mu\text{m}^2$) on the surface of the untreated polystyrene tissue culture plates which is indicative of poorer cell adhesion. Fibroblasts on eADF4(C16) were still more rounded (with surface areas of $\approx 210 \mu\text{m}^2$), which is indicative of even poorer cell adhesion because ECM protein adsorption from the media is low and interactions between the negatively charged surface of the films and the negatively charged cells are weak. Functionalization of the silk films with glycopolymers **1–3** markedly improved the spreading of the fibroblasts on the substrates (fibroblasts covered surface areas of $\approx 310\text{--}390 \mu\text{m}^2$), and those substrates displaying glycopolymer **2** appeared to be the most favorable substrate for the fibroblasts to adhere to (fibroblasts covered surface areas of $\approx 393 \mu\text{m}^2$) which is likely to be due to increased levels of protein adsorption from the cell culture medium.

Figure 1.



The adhesion of mouse embryo fibroblasts (M-MSV-BALB/3T3) on various surfaces is shown. The 3D graph depicts the relationship between the surface chemistry of the films and the surface area of the cells adhered to them. Above: bright field microscope images of the fibroblasts cultured for 6 h in vitro on each of the surfaces. A) Untreated polystyrene tissue culture plates, B) eADF4(C16), C) eADF4(C16)-1, D) eADF4(C16)-3, E) eADF4(C16)-2, F) Nunclon Δ surface tissue culture plates. The scale bar represents 100 μm .

4 Conclusion

Silk protein-based materials are promising materials for tissue engineering and the delivery of drugs and other active ingredients. The modification of the surface of such silk-based materials with ECM protein binding glycopolymers yields a biomimetic surface with markedly improved cell adhesion relative to the unmodified silk. The glycopolymers employed had molecular weights below 40 kDa, which is well below the renal filtration threshold of 70 kDa, facilitating their clearance from the body upon degradation of the underlying silk scaffold (i.e., the scaffolds would be bioerodible in vivo).^[47, 48] The glycopolymer-modified engineered spider silk hybrids have therefore potential for application as biocompatible and bioerodible coatings for bioresorbable implants.

Acknowledgements

The authors thank the Alexander von Humboldt Foundation for a postdoctoral fellowship for J.G.H., and the German Research Foundation (Deutsche Forschungsgemeinschaft, SFB 840 TP A8) for financial support for T.R.S. The authors thank the European Science Foundation for financial support of A.P. and A.H.E. M. via the SONS 2 program (BioSONS project). At the University of Bayreuth we thank Marietta Böhm for GPC measurements, Susanne Edinger for MALDI-ToF MS measurements, and Markus Hecht and Elke Fuchs for assistance with contact angle measurements.

Ancillary

Supporting Information

- 1 O. Z. Fisher, A. Khademhosseini, R. Langer, N. A. Peppas, *Acc. Chem. Res.* 2010, 43, 419.
- 2 C. Vepari, D. L. Kaplan, *Prog. Polym. Sci.* 2007, 32, 991.
- 3 J. G. Hardy, T. R. Scheibel, *Prog. Polym. Sci.* 2010, 35, 1093.
- 4 J. G. Hardy, L. M. Römer, T. R. Scheibel, *Polymer* 2008, 49, 4309.
- 5 S. C. Kundu, B. Kundu, S. Talukdar, S. Bano, S. Nayak, J. Kundu, B. B. Mandal, N. Bhardwaj, M. Botlagunta, B. C. Dash, C. Acharya, A. K. Ghosh, *Biopolymers* 2012, 97, 455.
- 6 T. D. Sutherland, J. S. Church, X. A. Hu, M. G. Huson, D. L. Kaplan, S. Weisman, *PLOS One* 2011, 6, e16489.
- 7 J. B. Addison, N. N. Ashton, W. S. Weber, R. J. Stewart, G. P. Holland, J. L. Yarger, *Biomacromolecules* 2013, 14, 1140.
- 8 F. Bauer, T. Scheibel, *Angew. Chem. Int. Ed.* 2012, 51, 6521.
- 9 C. Radtke, C. Allmeling, K. H. Waldmann, K. Reimers, K. Thies, H. C. Schenk, A. Hillmer, M. Guggenheim, G. Brandes, P. M. Vogt, *PLOS One* 2011, 6, e16990.
- 10 M. Widhe, J. Johansson, M. Hedhammar, A. Rising, *Biopolymers* 2012, 97, 468.
- 11 A. Heidebrecht, T. Scheibel, *Adv. Appl. Microbiol.* 2013, 82, 115.
- 12 J. G. Hardy, T. R. Scheibel, *J. Polym. Sci., Part A: Polym. Chem.* 2009, 47, 3957.
- 13 J. G. Hardy, T. R. Scheibel, *Biochem. Soc. Trans.* 2009, 37, 677.
- 14 A. Rising, M. Widhe, J. Johansson, M. Hedhammar, *Cell. Mol. Life Sci.* 2011, 68, 169.
- 15 D. Huemmerich, C. W. Helsen, S. Quedzuweit, J. Oschmann, R. Rudolph, T. Scheibel, *Biochemistry* 2004, 43, 13604.
- 16 S. Wohlrab, S. Muller, A. Schmidt, S. Neubauer, H. Kessler, A. Leal-Egana, T. Scheibel, *Biomaterials* 2012, 33, 6650.
- 17 S. Wohlrab, K. Spiess, T. Scheibel, *J. Mater. Chem.* 2012, 22, 22050.
- 18 K. Spiess, R. Ene, C. D. Keenan, J. Senker, F. Kremer, T. Scheibel, *J. Mater. Chem.* 2011, 21, 13594.
- 19 F. Bauer, S. Wohlrab, T. Scheibel, *Biomater. Sci.* 2013, 1, 1244.
- 20 G. Maheshwari, G. Brown, D. A. Lauffenburger, A. Wells, L. G. Griffith, *J. Cell. Sci.* 2000, 113, 1677.
- 21 E. Ruoslahti, M. D. Pierschbacher, *Science* 1987, 238, 491.
- 22 I. Gadjanski, K. Spiller, G. Vunjak-Novakovic, *Stem Cell Rev. Rep.* 2012, 8, 863.
- 23 C. Frantz, K. M. Stewart, V. M. Weaver, *J. Cell Sci.* 2010, 123, 4195.
- 24 B. Voit, D. Appelhans, *Macromol. Chem. Phys.* 2010, 211, 727.
- 25 C. Schatz, S. Lecommandoux, *Macromol. Rapid Comm.* 2010, 31, 1664.
- 26 D. Cunliffe, S. Pennadam, C. Alexander, *Eur. Polym. J.* 2004, 40, 5.

- 27 G. Yilmaz, C. R. Becer, *Eur. Polym. J.* 2013, 49, 30461.
- 28 S. L. Flitsch, *Curr. Opin. Chem. Biol.* 2000, 4, 619.
- 29 J. L. J. Blanco, C. O. Mellet, J. M. G. Fernandez, *Chem. Soc. Rev.* 2013, 42, 4518.
- 30 V. Ladmiral, E. Melia, D. M. Haddleton, *Eur. Polym. J.* 2004, 40, 431.
- 31 S. Slavin, J. Burns, D. M. Haddleton, C. R. Becer, *Eur. Polym. J.* 2011, 47, 435.
- 32 V. Vazquez-Dorbatt, J. Lee, E. W. Lin, H. D. Maynard, *Chem. Bio. Chem.* 2012, 13, 2478.
- 33 M. Ahmed, P. Wattanaarsakit, R. Narain, *Eur. Polym. J.* 2013, 49, 3010.
- 34 L. You, H. Schlaad, *J. Am. Chem. Soc.* 2006, 128, 13336.
- 35 K. Babiuch, C. R. Becer, M. Gottschaldt, J. T. Delaney, J. Weisser, B. Beer, R. Wyrwa, M. Schnabelrauch, U. S. Schubert, *Macromol. Biosci.* 2011, 11, 535.
- 36 Q. Yang, M. Strathmann, A. Rumpf, G. Schaule, M. Ulbricht, *ACS Appl. Mater. Int.* 2010, 2, 3555.
- 37 A. Pfaff, A. H. E. Muller, *Macromolecules* 2011, 44, 1266.
- 38 A. Pfaff, L. Barner, A. H. E. Muller, A. M. Granville, *Eur. Polym. J.* 2011, 47, 805.
- 39 Y. C. Wang, S. H. Kao, H. J. Hsieh, *Biomacromolecules* 2003, 4, 224.
- 40 S. Das, D. Pati, N. Tiwari, A. Nisal, S. Sen Gupta, *Biomacromolecules* 2012, 13, 3695.
- 41 N. S. Hatzakis, H. Engelkamp, K. Velonia, J. Hofkens, P. C. M. Christianen, A. Svendsen, S. A. Patkar, J. Vind, J. C. Maan, A. E. Rowan, R. J. M. Nolte, *Chem. Commun.* 2006, 2012.
- 42 T. P. Bird, W. A. P. Black, J. A. Colquhoun, E. T. Dewar, D. Rutherford, *J. Chem. Soc. C* 1966, 1913.
- 43 J. G. Hardy, A. Leal-Egana, T. R. Scheibel, *Macromol. Biosci.* 2013, 13, 1431.
- 44 P. H. Zeplin, N. C. Maksimovikj, M. C. Jordan, J. Nickel, G. Lang, A. H. Leimer, L. Römer, T. Scheibel, *Adv. Funct. Mater.* 2014, doi: [10.1002/adfm.201302813](https://doi.org/10.1002/adfm.201302813).
- 45 A. S. Zeiger, B. Hinton, K. J. Van Vliet, *Acta Biomater.* 2013, 9, 7354.
- 46 F. Klappenberger, M. E. Canas-Ventura, S. Clair, S. Pons, U. Schlickum, Z. R. Qu, H. Brune, K. Kern, T. Strunskus, C. Woll, A. Comisso, A. De Vita, M. Ruben, J. V. Barth, *ChemPhysChem* 2007, 8, 1782.
- 47 M. J. Knauf, D. P. Bell, P. Hirtzer, Z. P. Luo, J. D. Young, N. V. Katre, *J. Biol. Chem.* 1988, 263, 15064.
- 48 B. Chen, K. Jerger, J. M. J. Frechet, F. C. Szoka, *J. Controlled Release* 2009, 140, 203.