



Data Article

Data for microbe resistant engineered recombinant spider silk protein based 2D and 3D materials



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ABSTRACT

Data presented in this article describe bacterial and fungal repellent properties of 2D-films and 3D-hydrogels made of different recombinantly produced spider silk proteins based on consensus sequences of Araneus diadematus dragline silk proteins (fibroin 3 and 4). Here, the attachment, growth, and microbial colonization of *Streptococcus mutans* (*S. mutans*) as well as *Candida albicans* (*C. albicans*) on plane and micro-patterned films were visualized by scanning electron microscopy (SEM). Also, microbial

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 Engineered spider silk proteins

viability data are provided of *Escherichia coli* (*E. coli*) and *Pichia pastoris* (*P. pastoris*) on hydrogels made of eADF4(C16) and eADF4(C16)-RGD, quantified using the Alamar blue assay. Experimental results, design of a post-operative contamination model of microbes with mammalian cells, and methods in the data article refer to the research paper "Engineered spider silk-based 2D and 3D materials prevent microbial infestation" published recently [1].

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Specifications Table

Subject	Biotechnology, Biomaterials
Specific subject area	Microbe repellent recombinant spider silk proteins
Type of data	Table, Microscopy images, Figures
How data were acquired	Adhesion of microbial cells on films was analysed using scanning electron microscopy (SEM). Spider silk and polycaprolactone (PCL) films were incubated in microbial solutions (<i>S. mutans</i> and <i>C. albicans</i>) for 12 h and at 37°C. The films were washed with phosphate buffered saline (PBS) and placed in 2.5% v/v glutaraldehyde for 1 h to fix the microbial cells, followed by gradual dehydration using ethanol solutions from 30% (in Milli-Q water) to 100 %. All dried samples were sputter coated with 2 nm platinum and imaged at an accelerating voltage of 2.5 kV using a Zeiss Sigma 660 VP 300, Germany. Adhered microbial cells (<i>E. coli</i> and <i>P. pastoris</i>) on hydrogels were analysed using the CellTiter-Blue assay. Hydrogels incubated with bacterial and yeast cells were washed with PBS and then incubated with 10% CellTiter Blue in PBS for 3 h at 37°C. Transformation of the blue fluorescent dye resazurin into red fluorescent resorufin ($\lambda_{ex} = 530 \text{ nm}$; $\lambda_{em} = 590 \text{ nm}$) was measured using a plate reader (Mithras LB 940, Berthold, Bad Wildbad) with a counting time of 0.5 s.
Data format	Raw data were analysed and processed using Origin and Adobe illustrator.
Parameters for data collection	Experiments were performed by incubating 2D films (eADF4(C16) and PCL) with <i>S. mutans</i> and <i>C. albicans</i> , and 3D hydrogels (eADF4(C16) and eADF4(C16)-RGD) with <i>E. coli</i> and <i>P. pastoris</i> for 12 h and 24 h, respectively, at 37°C.
Description of data collection	Films were dried and imaged using SEM. Hydrogels were incubated with 10 % CellTiter-Blue in PBS for 3 h at 37°C, and transformation of the blue fluorescent dye resazurin into red fluorescent resorufin ($\lambda_{ex} = 530 \text{ nm}$; $\lambda_{em} = 590 \text{ nm}$) was analysed using a plate reader.
Data source location	Institution: University of Bayreuth City/Town/Region: Bayreuth Country: Germany
Data accessibility	The data are supplied with this article.
Related research article	Authors' names: Sushma Kumari, Gregor Lang, Elise DeSimone, Christian Spengler, Vanessa T. Trossmann, Susanne Lückner, Martina Hudel, Karin Jacobs, Norbert Krämer and Thomas Scheibel Title: Engineered spider silk-based 2D and 3D materials prevent microbial infestation Journal: Materials Today DOI: https://doi.org/10.1016/j.mattod.2020.06.009

Value of the Data

- The dataset provides insights into the bacterial and fungal repellence properties of spider silk based 2D-films and 3D-hydrogels as shown in reference (1).
- The data clearly show that both, smooth and patterned eADF films and hydrogels substantially restrict the attachment, growth and microbial colonization of pathogenic bacteria and fungi.

Table 1
Properties of recombinant spider silk proteins

Recombinant spider silk protein	M _w (kDa)	No. of charged amino acid residues at neutral pH (positive/negative)	pI
eADF4(C16)	47.7	0/16	3.5
eADF4(C32NR4)	104.1	2/34	3.5
eADF4(C16)-RGD	48.5	1/17	3.6
eADF4(Ω16)	47.7	0/0	7.7
eADF3(AQ) ₁₂	48.0	0/0	5.5

Charged amino acid residues refer to silk sequences only; the T7 tag, present in all constructs, comprises an additional arginine residue. Values for eADF4(C16), eADF4(C32NR4), and eADF3((AQ)₁₂) were taken from Ref. 2, eADF4(C16)-RGD from Ref. 3, and unpublished data of eADF4(Ω16).

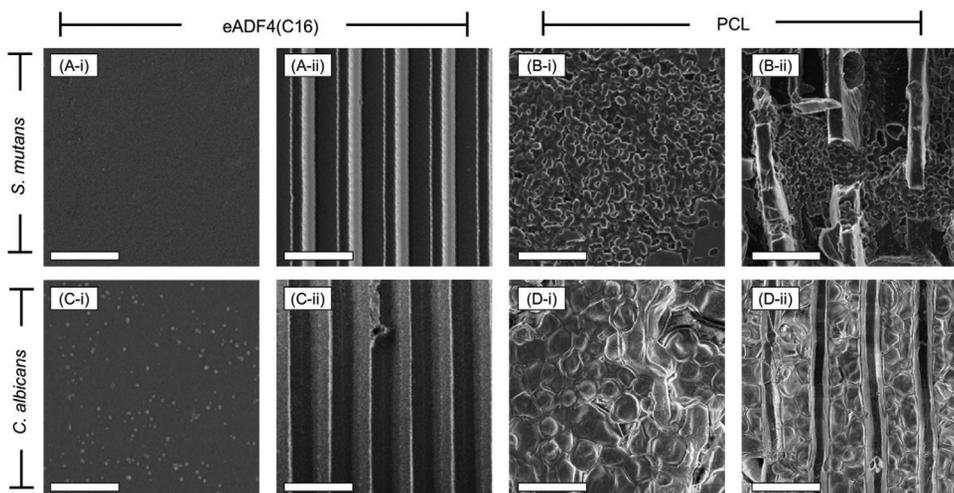


Fig. 1. Bacterial and fungal repellent properties of films made of eADF4(C16). SEM images showing (i) plane and (ii) micro-patterned surfaces of (A & C) eADF4(C16) and (B & D) PCL after 12 h of incubation with (A & B) *S. mutans* and (C & D) *C. albicans* at 37°C. Scale bars = 5 μm.

- The data are useful to design advanced biopolymers that can selectively repel pathogenic microorganisms without actively killing microbes, and, at the same time, promote mammalian cell growth.
- The data is valuable in understanding and examining the microbe-repellent effects of natural spider silk.
- The data is providing information for researchers in the field of biomedical engineering to develop microbe repellent surfaces for biomedical and technical applications.

1. Data Description

Data presented in this article demonstrate that 2D-films and 3D-hydrogels of recombinantly produced proteins show bacterial and fungal repellent properties without killing microbes upon contact. Table 1 summarizes the properties of two engineered *Araneus diadematus* fibroins eADF3 and eADF4 and variants thereof with respect to their molecular weight, net charge and the presence of a terminal assembly domain. SEM images in Fig. 1 clearly show that both, smooth and patterned eADF4(C16) films significantly restrict the attachment and microbial colonization of *S. mutans* as well as *C. albicans*, as compared to polycaprolactone (PCL) films. The viability of *E. coli* and *P. pastoris* on hydrogels of eADF4(C16) and eADF4(C16)-RGD was quantified over 10 days

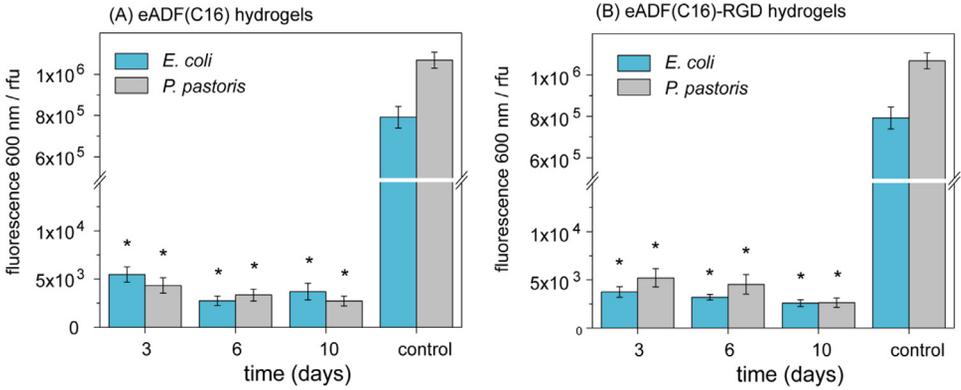


Fig. 2. Bacterial and fungal repellent properties of hydrogels made of two different spider silk variants. Viability of *E. coli* and *P. pastoris* cells on hydrogels of (A) eADF4(C16) and (B) eADF4(C16)-RGD was quantified over 10 days using the CellTiter-Blue assay and analyzing the transformation of the blue fluorescent dye resazurin into red fluorescent resorufin using 530 nm excitation and 600 nm emission filters in a microplate reader. Cell culture treated plates without coating were used as control. Each result is an average of three experiments, and the error bars designate the standard deviations. Student's t-test was performed for statistical analysis, **p* < 0.05.

Table 2
CellTiter-Blue assay data of eADF4(C16) hydrogels in Fig. 2.

Sample name	Days	Fluorescence measurement, Ex 530 nm/Em 600 nm			Average ± stdev	Student's t. test value
		S1	S2	S3		
eADF4(C16)_ <i>E. coli</i>	3	4597	6141	5697	5478 ± 795	1.32 × 10 ⁻⁵
eADF4(C16)_ <i>P. pastoris</i>	3	5220	3625	4184	4343 ± 809	1.14 × 10 ⁻⁶
eADF4(C16)_ <i>E. coli</i>	6	3225	2807	2230	2754 ± 500	1.31 × 10 ⁻⁵
eADF4(C16)_ <i>P. pastoris</i>	6	2662	3536	3843	3347 ± 612	1.13 × 10 ⁻⁶
eADF4(C16)_ <i>E. coli</i>	10	2829	3721	4559	3703 ± 865	1.31 × 10 ⁻⁵
eADF4(C16)_ <i>P. pastoris</i>	10	2502	3316	2368	2729 ± 513	1.13 × 10 ⁻⁶
Control_ <i>E.coli</i>	1	741307	846368	788259	791978 ± 52629	
Control_ <i>P. pastoris</i>	1	1053496	1112205	1039713	1068471 ± 38496	

using the CellTiter-Blue assay and is shown in Fig. 2 and raw data is provided in Table 2 and 3. The experimental design of a post-operative contamination model of bacteria and fungi with mammalian cells in/on hydrogel scaffolds made of eADF4(C16) and RGD-modified eADF4(C16) is shown in Fig. 3. This data specifically demonstrates the aptness of recombinant spider silk in the field of tissue engineering to selectively promote mammalian cell binding and provide microbe repellence at the same time. These data are comprehensively discussed in the reference article [1].

2. Additional information concerning the recombinant spider silk protein sequences

Engineered *Araneus diadematus* fibroins eADF3 and eADF4 and variants thereof are based on the repetitive consensus sequences (modules C and Ω (ADF4), modules A and Q (ADF3)) of the core domains of the naturally occurring fibroins ADF3 and ADF4 of the dragline silk of the European garden spider *A. diadematus*. The number after the modules depict the amount of repeats (e.g. C16 stands for 16 times the C module).

- Amino acid sequence of module C: GSS AAAAAAAAA S GPGGY GPENQGGS GPGGY GPGGP
- Amino acid sequence of module Ω: GSS AAAAAAAAA S GPGGY GPQNQGGS GPGGYGPGGP
- Amino acid sequence of module A: GPYGP GASAAAAAAAA GGYGP GSGQQ
- Amino acid sequence of module Q: GPGQQ GPGQQ GPGQQ GPGQQ

Table 3

CellTiter-Blue assay data of eADF4(C16)-RGD hydrogels in Fig. 2.

Sample name	Days	Fluorescence measurement, Ex 530 nm/Em 600 nm			Average \pm stdev	Student's t. test value
		S1	S2	S3		
eADF4(C16)-RGD_ <i>E. coli</i>	3	3516	4360	3345	3740 \pm 543	1.32 $\times 10^{-5}$
eADF4(C16)-RGD_ <i>P. pastoris</i>	3	4272	6189	5143	5201 \pm 960	1.14 $\times 10^{-6}$
eADF4(C16)-RGD_ <i>E. coli</i>	6	3074	3528	2986	3196 \pm 291	1.31 $\times 10^{-5}$
eADF4(C16)-RGD_ <i>P. pastoris</i>	6	4494	3532	5559	4528 \pm 1013	1.13 $\times 10^{-6}$
eADF4(C16)-RGD_ <i>E. coli</i>	10	2185	2847	2559	2707 \pm 136	1.32 $\times 10^{-5}$
eADF4(C16)-RGD_ <i>P. pastoris</i>	10	2120	2681	3075	2625 \pm 480	1.13 $\times 10^{-6}$

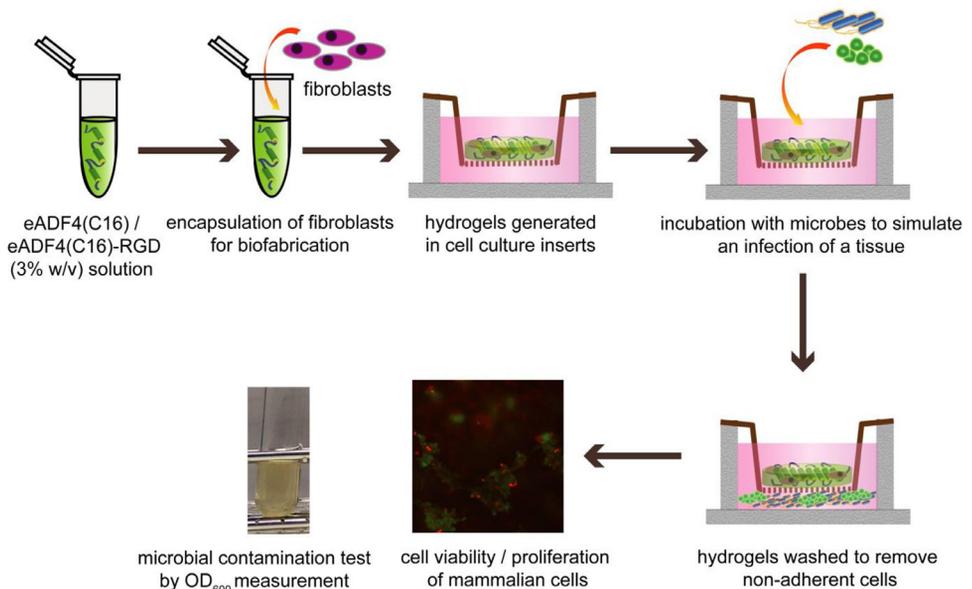


Fig. 3. Schematic illustration demonstrating a post-operative contamination model of microbes (*E. coli* and *P. pastoris*) and mammalian cells (BALB/3T3) in/on hydrogel scaffolds made of eADF4(C16) and eADF4(C16)-RGD. The cell viability of mouse fibroblasts (BALB/3T3) in culture with microbes was evaluated by cell staining with calcein A/M (live cells: green) and ethidium homodimer 1 (dead cells: red), and microbial growth of *E. coli* and *P. pastoris* in fresh media was measured using the optical density at 600 nm (OD₆₀₀).

Abbreviations: RGD, Arginylglycylaspartic acid; NR4, non-repetitive region of ADF4.

Design and production of these recombinant proteins have been described in Detail in references [1] and [2].

3. Experimental design, materials and methods

3.1. Bacteria and yeast culture on films

Streptococcus mutans (DSMZ 20523, Braunschweig) and *Candida albicans* (patient isolate), stored at -80°C , were thawed at room temperature (RT), fractionally spread on Columbia blood

agar (PB 5039A, oxoid, Wesel) and incubated for 48 h at 5 % CO₂ and 37 °C. Afterwards, an overnight culture was prepared in BBL™ Schaedler Broth medium (Becton Dickinson, Sparks MD, USA) and diluted (1:10) with Schaedler Broth medium.

Escherichia coli BL21(DE3)-gold (Novagen, Merck, Darmstadt, Germany), stored at -80 °C, was thawed at RT and inoculated in Luria–Bertani medium (LB) with constant shaking at 150 rpm, 37 °C, until an optical density (OD₆₀₀) between 0.8 and 1 was reached (corresponding to a viable count of approx. 10⁷–10⁸ CFU mL⁻¹). The *E. coli* culture was diluted (1:10) with LB medium.

Pichia pastoris X33 (wild type, Invitrogen, Germany) was inoculated in YPD-media and allowed to grow with constant shaking at 150 rpm for 24 h at 30 °C. The *P. pastoris* culture was diluted (1:10) with YPD medium.

Spider silk and PCL films were taken out of 70 % ethanol, subsequently washed with PBS (8.18 g NaCl, 0.2 g KCl, 0.24 g anhydrous KH₂PO₄, 1.78 g Na₂HPO₄ × 2H₂O, 1 L distilled water, pH 7.4, Sigma Aldrich, St. Louis, Missouri, USA), and incubated in 5 mL of diluted microbial solution (as described above) in petri dishes (Ø 5 cm) for 12 h (5 % CO₂, 37 °C). Then, the films were removed and carefully washed with PBS to remove any non-adherent bacteria and yeast cells, fixed with 2.5 % v/v glutaraldehyde for 1 h, gradually dehydrated with ethanol solutions from 30% (in Milli-Q water) to 100 %, and finally dried at room temperature for subsequent SEM imaging.

Bacterial and yeast cell viability: Adhered *E. coli* and *P. pastoris* on hydrogels were analysed using the CellTiter-Blue assay after culturing for 24 h at 37 °C. Samples incubated with bacterial and yeast cells were washed with phosphate buffered saline (PBS; Sigma-Aldrich) three times and then incubated with 10 % CellTiter-Blue (Promega) in PBS for 3 h at 37 °C. Transformation of the blue fluorescent dye resazurin into red fluorescent resorufin ($\lambda_{\text{ex}} = 530 \text{ nm}$; $\lambda_{\text{em}} = 590 \text{ nm}$) was measured using a plate reader (Mithras LB 940, Berthold, Bad Wildbad) with a counting time of 0.5 s.

Post-operative contamination model: The experimental setup was similar to the previously described ones mimicking post-operative infection in which microbial cells were allowed to adhere on tissue engineering scaffolds for 2–6 h.^[4,5,6] eADF4(C16) and eADF4(C16)-RGD hydrogels with encapsulated BALB/3T3 mouse fibroblasts (i.e. bioinks used for biofabrication) were prepared in hanging cell culture inserts using 24-well plates (Merck Millipore) and then exposed to diluted (1:10, corresponding to OD 0.25) bacterial and yeast cells prepared in DMEM for 6 h, 80 % relative humidity and at 37 °C. Hydrogels were washed three times to remove non-adherent microbes and incubated with fresh DMEM media and cultivated for 10 days under identical conditions. Cell culture medium was changed every 24 h. The cell viability of BALB/3T3 mouse fibroblasts was analyzed using the Live/Dead assay after 3, 6 and 10 days.

Scanning electron microscopy (SEM): To analyze the morphological structure using SEM, dried films and lyophilized hydrogels were fixed to SEM stubs using conductive carbon cement solution (Leit-C, PLANO GmbH). Samples were sputter-coated with 2 nm platinum (Sputter Coater 208 HR with 268 MTM 20, Cressington, Watford, UK.) and then imaged at an accelerating voltage of 2.5 kV using a 270 Zeiss Sigma VP 300 (Zeiss, Oberkochen, Germany) and Field Emission Gun (FEG; Apreo VS, ThermoFisher Scientific/FEI, Germany).

Declaration of Competing Interest

The authors declare the following competing financial interest (s): T.S. is co-founder and share-holder of the AMSilk GmbH.

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