Biomimetische Grenzflächen und Matrices

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Biomimetische Materialien, Werkstoffe die Strukturen und Funktionen lebender Materie nachvollziehen und über in der Natur vorkommende Systeme hinausgehend kombinieren, stellen eine besondere Herausforderung der Materialforschung dar und spielen in verschiedensten innovativen Technologien eine immer wichtigere Rolle. Polymere sind aufgrund ihrer ausgeprägten Variabilität in chemischen und physikalischen Eigenschaften und der Möglichkeit zur Erzeugung hochspezifisch interagierender Molekülarchitekturen die wichtigste Basis für Biologie-inspirierte Materialien.

Biomaterialforschung am IPF wird im Rahmen des mit dem Institut für Werkstoffwissenschaft der Technischen Universität Dresden etablierten Max-Bergmann-Zentrums für Biomaterialien Dresden realisiert. Der Notwendigkeit zur Einbeziehung von Expertise aus verschiedenen Teilgebieten der Lebenswissenschaften wird durch enge Verzahnung mit dem Dresdener Exzellenzcluster für Regenerative Therapien Dresden (CRTD), dem Zentrum für Innovationskompetenz für Molekulare Bioingenieurswissenschaft (ZIK B CUBE) und der Graduiertenschule Biomedicine & Bioengineering (DIGS BB) Rechnung getragen.

Im Fokus der Forschungsprojekte stehen die Aufklärung und Steuerung von Ladungs- und Strukturbildungsprozessen an Materialgrenzflächen im Kontakt zu wässrigen Medien/Biofluids, das mechanistische Verständnis und die selektive Kontrolle der Fremdkörper-Abwehr des Blutes beim Kontakt mit Materialoberflächen sowie der Aufbau und die Anwendung modularer Polymermatrices zur Steuerung zellulärer Eigenschaften und morphogenetischer Prozesse für Regenerative Therapien.

Besonders hervorzuheben unter den Ergebnissen des Jahres 2010 sind die Fortschritte bei der Gestaltung bioresponsiver Hydrogel-Matrices (S. 57 f. und *Chem. Communications* 46 (2010) 1141-1143) und der definierten Funktionalisierung dieser Gelsysteme mit Wachstumsfaktoren (S. 58 f. *Biomaterials* 31 (2010) 7985-7994). Weitere wichtige Ergebnisse sind die Etablierung einer neuartigen Organkulturmethode zur Untersuchung der Wirkung von exogenen Signalen auf Stammzellen (PloS ONE 5 (2010) e105) und die Aufklärung der Wirkung des Tissue Factors auf die materialinduzierte Aktivierung der Blutgerinnung (*Biomaterials* 31 (2010) 2498-2507).
Für das Gebiet der Elektrokinetischen Phänomene wurde mit der Herausgabe des ersten Themenheftes der Zeitschrift *Current Opinion in Colloid and Interface Science* (Volume 15 (2010), gemeinsam mit Hans Lyklema) eine neue Plattform geschaffen und zur Darstellung des Wissenstandes zu Ionenadsorptionsprozessen an Grenzflächen in wässrigen Medien genutzt wurde (*Current Opinion in Colloid and Interface Science* 15 (2010) 196-202).

Vier Dissertationsarbeiten wurden erfolgreich verteidigt (Dimitar Stamov, Yvonne Müller, Marion Fischer, Babette Lanfer), die Arbeit von Babette Lanfer zur Entwicklung eines mikrofluidischen Verfahrens für die gerichtete Immobilisierung von rekonstituierten Extrazellulärmatrices (*Tissue Engineering Part A* 16 (2010) 1103-1113) wurde mit *summa cum laude* ausgezeichnet. Dr. Tilo Pompe wurde der Ruf auf die W2-Professur für Biophysikalische Chemie an der Universität Leipzig erteilt.

Neue Projekte, zu denen 2010 die Arbeit aufgenommen wurde, sind u. a. DFG-Projekte zur Wirkungsweise von immobilisierten Inhibitoren der Blutgerinnung, zum Einfluss der Verankerung von Zelladhäsionsliganden und zur Steuerung der Freisetzung von Morphogenen aus Hydrogelen, ein Projekt im Rahmen des SFB 655 „From Cells to Tissues“ (Polymermatrices zur Generierung von interaktiven Nischen für Blutstammzellen) und das BMBF-geförderte Systembiologie-Netzwerk „Die Virtuelle Leber“.

Im Rahmen der 3. Conference on Stem Cells and Tissue Regeneration fand im Juli 2010 das Max-Bergmann-Symposium statt (eingeladene Vorträge von M. Lutolf, D. Mooney, P. Shastri, M. Stevens, S. Sakyiama Elbert), im November das auf stimuli-responsive Polymere konzentrierte IPF-Kolloquium (mit Beiträgen von M. Cohen Stuart, W. Huck, R. Netz, R. Ulijn u. a.).

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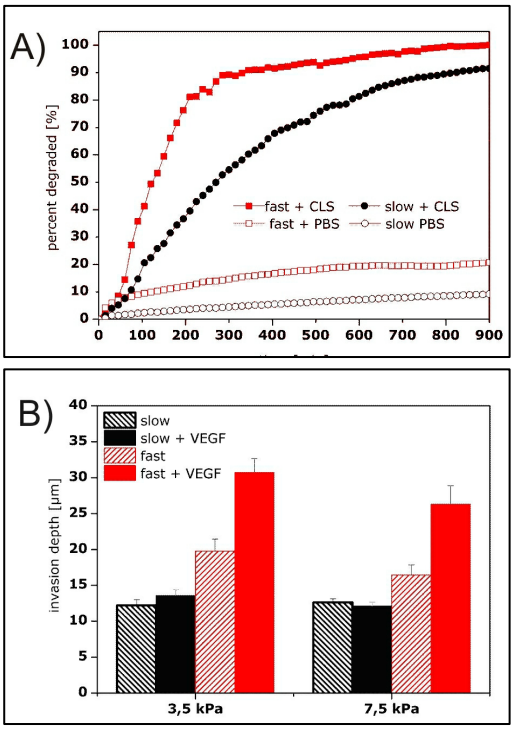
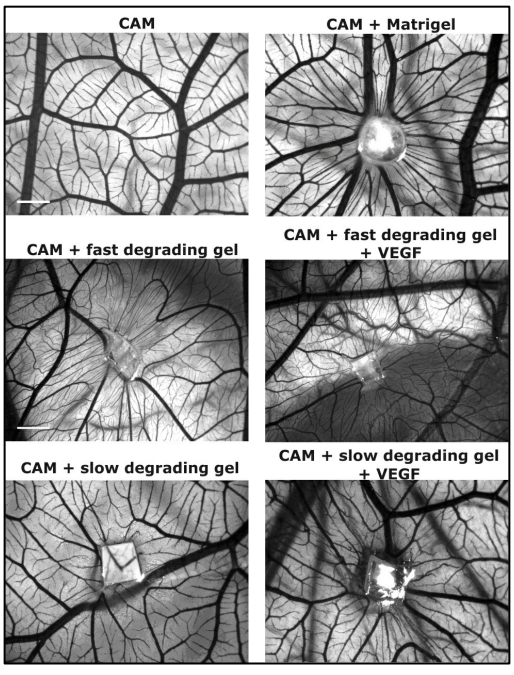
Biohybrid hydrogels based on starPEG and heparin to promote vascularization

Karolina Chwalek, Kandice R. Levental, Mikhail V. Tsurkan, Uwe Freudenberg, Carsten Werner

Natural extracellular matrix (ECM) undergoes dynamic remodeling, which enables cell expansion and migration. Therefore, biodegradable hydrogels with similar cell responsive characteristics receive the greatest attention in material science. The implementation of chemical linkages sensitive to cell-secreted proteases is a common strategy to make hydrogels responsive to cell-mediated degradation [1]. We have developed a new strategy for the controlled degradation of previously characterized starPEG-heparin hydrogels [2] using enzymatically cleavable peptide units to crosslink the hydrogels. For a far-reaching modulation of the degradation speed, we use two different methods to conjugate the enzymatic cleavable peptides to the starPEG building block, using either ester- or maleimide bonds (indicated as "fast" and "slow" degradable, Fig. 1).



The results show (Fig. 1 A), that ester-linked enzymatically cleavable gels undergo unspecific degradation in PBS solution, whereas maleimide linked gels were stable. Using collagenase to mimic enzymatic cleavage, the ester-linked gels undergo significantly faster degradation compared with the maleimide bond gels suggesting an beneficial effect of both specific (protein-mediated) and unspecific (ester bond hydrolysis) degradation (Fig. 1 A, ester-linked gels are indicated as "fast", maleimide linked gels are indicated as "slow"). Cell experiments demonstrate the erosion speed of the biomaterial to have a direct impact on endothelial cell (EC) behavior. It could be shown that the initial physical properties are less important for viability, but that the degradation characteristics massively influence the EC migration rate (Fig. 1 B). Degradation characteristics are primarily influenced via the chemical character of ester or maleimide bond (hydrolytic labile vs. hydrolytic stable) and secondarily influenced (less pronounced) by the crosslinking degree (stiffness of the material). Additionally, a proangiogenic response of the endothelial cells could be evoked through VEGF (a growth factor) release from the scaffolds (Fig. 1 B). The positive effect of VEGF released from the scaffolds was further shown, *in vivo,* in chicken chorioallantoic membrane assays.



Keywords:
angiogenesis
cell morphology
degradation
endothelial cell
CAM assay

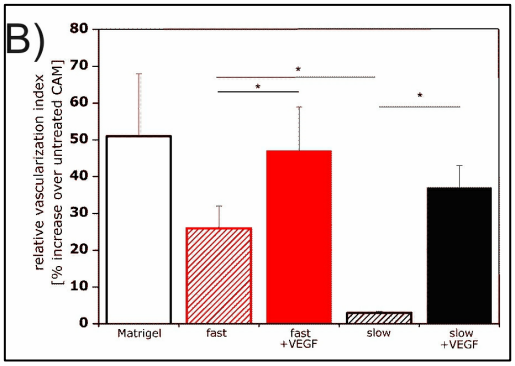
Fig. 1:
(A) Degradation of biodegradable starPEG-heparin hydrogels. Degradation was performed in phosphate buffered saline (PBS) and collagenase solution (CLS).
(B) Quantification of the invasion depth showed that 3D migration speed of cells is directly correlated to the invasion depth.

Fig. 2 A:
Chicken embryo chorioallantoic membrane (CAM) angiogenesis assay: Visualization by light microscopy

Keywords:
biohybrid hydrogel
stromal-derived factor-1
cardiac progenitor cell
migration
growth factor release
system

Fig. 2 B:
Chicken embryo chorio-allantoic membrane (CAM) angiogenesis assay: Relative vascularization index

VEGF-loaded gels of both types promoted angiogenesis comparable with the use of Matrigel, included as a positive control (Fig. 2).



Results obtained from the *in vivo* CAM assay indicate that biodegradable starPEG-heparin hydrogels loaded with growth factor are able to attract new blood vessels within a short period of time and that they are fully biocompatible, thus making them promising candidates for the development of engineered tissues.

Sponsors:
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Biomimetic starPEG-heparin hydrogels are well-defined delivery matrices for SDF-1 to promote cardiac progenitor cell chemotaxis

Silvana Prokoph, Kandice R. Levental, Uwe Freudenberg, Carsten Werner

Promoting effective tissue repair after myocardial infarction is an important research area due to the limited dividing capacity of cardiomyocytes that restricts the regeneration of ischemic tissue. Following a myocardial infarction, a gradient of stromal cell-derived factor-1 (SDF-1) initiates homing of circulating endothelial progenitor cells (EPCs) thought to improve the neovascularization of ischemic tissue. To enhance recruitment of EPCs and further aid neovascularization of the injured myocardium, a novel biomimetic hydrogel system was utilized as an effective delivery vehicle to provide SDF-1 at the site of the injury. This hydrogel system combines the flexible and nonadhesive synthetic poly(ethylene glycol) (starPEG) as the main structural component with the natural occurring glycosaminoglycan heparin as a biofunctional building block. In particular, the high affinity of heparin to reversibly bind different cytokines and chemokines, such as SDF-1, is used to modulate the functionality of the matrices. Moreover, the design concept of the starPEG-heparin hydrogels allows for an independent tuning of physical properties while maintaining a constant level of biofunctionalization. ELISA and radiolabeling studies were performed to determine the uptake and release profiles of the hydrogels with varying cross-linking degrees (e. g., mechanical properties). A constant level of SDF-1 functionalization (e. g., initial immobilized amounts) was found across hydrogels with varying degrees of crosslinking. Furthermore, the amount of SDF-1 released by the scaffolds correlates well with the concentration of initially applied SDF-1, thus enabling the specific adaptation of the released cytokine amount for the desired application (Fig. 1). Additionally, hydrogels crosslinked via matrix metalloprotease-(MMP-) sensitive peptides could be applied to increase the amount of SDF-1 released.

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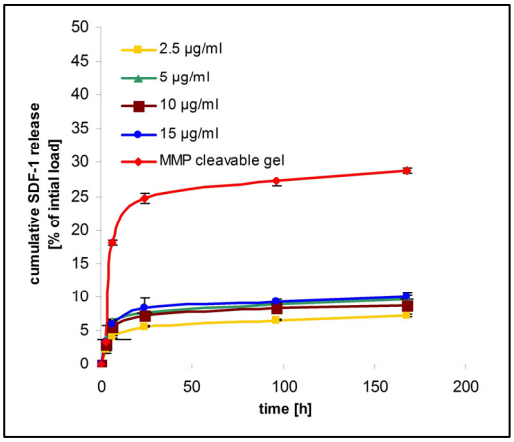

Fig. 1:
Percentage of SDF-1 released by starPEG heparin hydrogels

To examine the attraction of EPCs to SDF-1-loaded starPEG-heparin gels, the chemotactic properties of starPEG-heparin gels were investigated in a transwell migration assay. Studies revealed that EPC migration is enhanced by establishing a gradient of SDF-1 by the hydrogels in a dose dependent manner with a migration maximum at SDF-1 loading concentrations of 10 µg/ml. Equivalent experiments with soluble SDF-1 in the bottom well instead of SDF-1 released by the biohybrid gel system showed a similar but slightly decreased migration of the EPCs, suggesting that the long-term gradient provided by the hydrogels is beneficial in promoting chemotactic migration (Fig. 2).

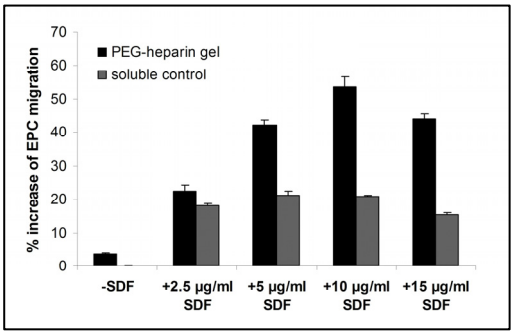

Fig. 2:
Fold-increase of EPC-migration as determined by transwell-assay with SDF-1 loaded MMP-cleavable gels

Moreover, vertical migration of EPCs from collagen gels into MMP-cleavable starPEG-heparin gels loaded with different concentrations of SDF-1 confirmed the chemotactic properties of the hydrogel system. In this context, the MMP-sensitive peptides incorporated as crosslinking moieties allowed for cell-mediated remodeling and invasion into the hydrogels. Increasing amounts of SDF-1 immobilized in the matrix likewise augmented the migration depth of the EPCs into the gels (Fig. 3).

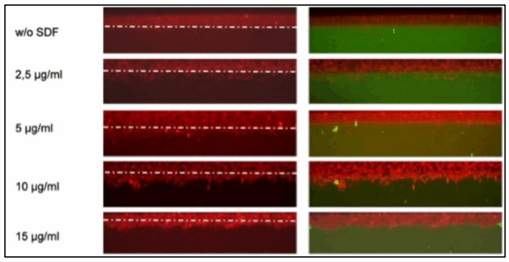

Fig. 3:
Vertical migration of EPCs into SDF-1 loaded starPEG heparin hydrogels (red color: cells migrating into the green colored gel body)

Moreover, initial *in vivo* experiments in nude mice showed that subcutaneously implanted SDF1-loaded hydrogels increased cell infiltration, vessel formation, and matrix remodeling compared with control-hydrogels without SDF-1 (Fig. 4).

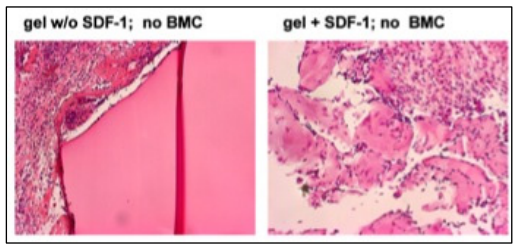

Fig. 4:
SDF-1 loaded hydrogels implanted for 7 days subcutaneous into the back of nude mice show significantly increased cell infiltration, matrix remodeling and vessel formation compared to gels without SDF-1

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Cooperation:
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Institute for Cardiovascular Regeneration

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Keywords:
lipid bilayer
stimuli-responsive polymers
diffusion
interfacial forces

Lipid bilayer membranes on multistimuli-responsive poly(N-isopropylacrylamide) copolymer cushions

Martin Kaufmann, Yunfei Jia, Carsten Werner, Tilo Pompe

Artificial lipid bilayers formed on solid surface supports are used as models of cell membranes to study fundamentals of physical, chemical, as well as biological nature. Polymer cushions used as support provide space for membrane-incorporated proteins to prevent protein denaturation and enable lateral mobility.
Our approach was divided into three subprojects. In a first step stimuli-responsive polymer cushions were produced, which allow a tunability of the cushion state due to the pH- and temperature-dependent swelling state. Secondly, lipid bilayers were formed on the cushions (Fig. 1) and their lateral mobility was examined in response to the swelling state. In a third step membrane proteins like adhesion receptors will be integrated to probe their function in dependence of swelling state of the pH- and temperature-dependent polymer cushion.
The stimuli-reponsive polymer cushions were based on carboxyl-containing poly(N-isopropylacrylamide) (PNIPAAm) copolymers surface-grafted as thin film in a brush-like manner. Their swelling transitions were characterized by quartz crystal microbalance with dissipation monitoring. They were found to be tuneable over a wide range, distinctly defined by the local and global balance of hydrophilic and hydrophobic interactions along the copolymer chains of variable composition [1].

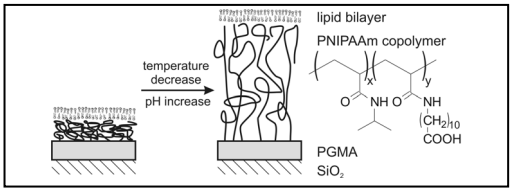

Fig. 1:
Layer composition and chemical structure of the PNIPAAm copolymer cushioned system. The thin PGMA film served as an anchor on SiO_2 for the PNIPAAm copolymer layer.

Drying and rehydration of a cationic lipid mixture was found to successfully produce a homogeneous lipid bilayer on top of the PNIPAAm cushion. Fluorescence recovery after photobleaching (FRAP) was used as a method to determine the diffusion coefficient of lipids within the bilayer (Fig. 2). The results revealed that lipid mobility in cushion supported bilayers was distinctively higher in comparison to solid glass support. In contradiction to the initial expectations, modulation of temperature and pH led to weak variations in lipid mobility that did not correlate with the PNIPAAm cushion swelling state. The results suggested a minimal coupling of the lipid bilayer with PNIPAAm polymer cushions by interfacial forces [2].

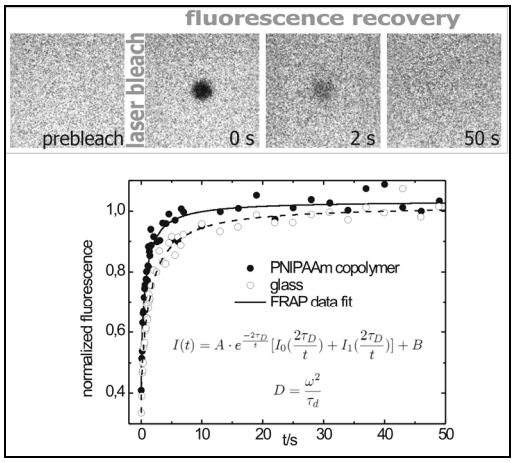

Fig. 2:
Characterization of the lateral lipid mobility by FRAP using a confocal laser scanning microscope. Fluorescent labeled lipids within a disc-shaped spot (Ø = 10 µm) are bleached by an intense laser pulse. Due to the lipid mobility the bleached lipids mix with unbleached ones. The resulting recovery intensity curve was fitted to determine the diffusion coefficient.

In ongoing work the transmembrane adhesion receptor $\alpha_5\beta_1$-integrin is investigated in the cushion supported lipid bilayer membranes as it has a major function in cell adhesion. The focus is set on the impact of the tuneable frictional drag between integrins and polymer cushion, which is expected to lower mobility, influence integrin activation and clustering processes.

Sponsor:
Deutsche Forschungsgemeinschaft

Cooperation:
D. Kuckling, Universität Paderborn
Ü. Coskün, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden

[1] M. Kaufmann, Y. Jia, L. Renner, S. Gupta, D. Kuckling, C. Werner, T. Pompe: Soft Matter 6 (2010), 937–944
[2] M. Kaufmann, Y. Jia, C. Werner, T. Pompe: Langmuir 27 (2011), 513-516

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Aligned collagen fibrils to induce directional cell growth in cellulose hollow fiber membranes

Babette Lanfer, Ralf Zimmermann, Yvonne Müller, Uwe Freudenberg, Carsten Werner

Polymeric microtubes offer valuable options for various regenerative therapies by serving as engineered nerve conduits, capillary blood vessels and tubular units of endocrine or secretory organs. In any of these applications, tubes have to support tissue compartmentalization and cell adhesion. The directionality of adherent cells often plays an important role for the functionality of tube-based constructs; examples include the axonal outgrowth of neural cells and the organization of vascular endothelial cells. To induce cellular orientation by structured extracellular matrix templates we developed and applied a microfluidic method to coat the lumen surface of cellulose hollow fiber membranes (inner diameter 200 µm) with aligned collagen I fibrils. Carboxylic acid groups on the interior sides of the hollow fiber membranes were converted into active esters to enable the subsequent covalent attachment of collagen fibrils. A recently introduced shear-flow technique was adapted to control the directional orientation upon deposition of collagen fibrils on the wall of the hollow fibers [1-3]. Examination by confocal scanning microscopy revealed that the collagen fibrils were well aligned in flow direction.

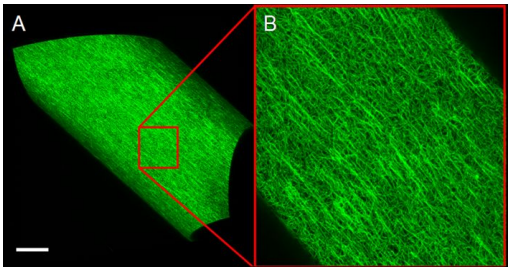
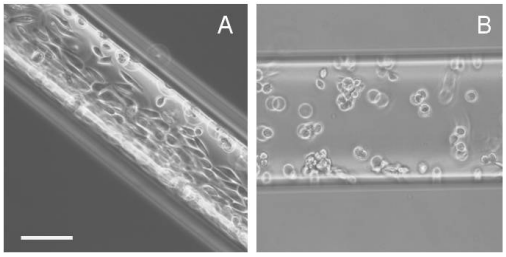


Fig. 1:
Aligned FITC-labeled (green) collagen fibrils reconstituted on the lumen surface of cellulose hollow fibers scale bar 100 µm

In a second step, a heparin solution was streamed across the reconstituted collagen matrix coating. FITC-labeled heparin was found to be co-localized with the fibrillar collagen matrices, allowing for the effective conjugation of heparin-binding morphogens.



Culture experiments with fibroblasts L929 revealed that cells grown within cellulose hollow fibers carrying directionally aligned collagen fibrils conform to the fibril orientation and exhibit an elongated morphology. Ongoing work aims at fabricating multifunctional tubular scaffolds combining aligned collagen I fibrils with biochemical cues according to the requirements of different cell types and applications.

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Keywords:
aligned collagen
extracellular matrix
cellulose
tissue engineering

Fig. 2:
Fibroblasts L929 in cellulose fiber membranes align on oriented collagen fibrils (A) in contrast to low adherence and random morphology on non-aligned collagen fibrils (B). Scale bar 100 µm

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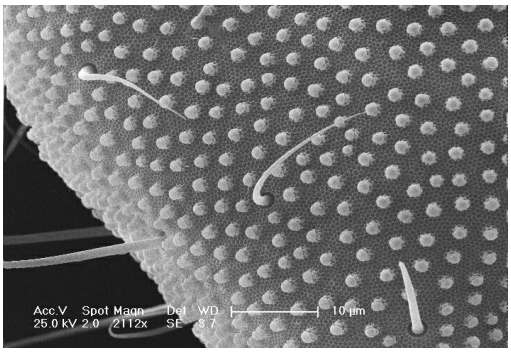
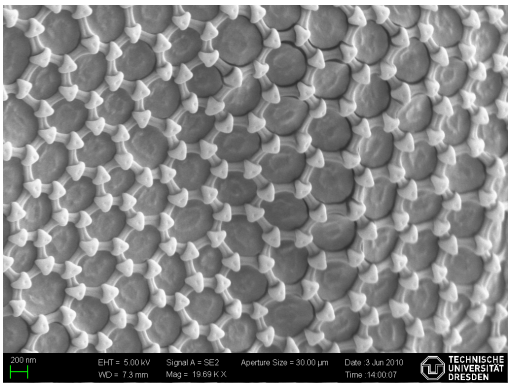
Keywords:
antiadhesive
hierarchical structures
self-cleaning surfaces
Collembola
Staphylococcus epidermidis

Revealing design principles of Collembolan skin

Ralf Helbig, Ana Cordeiro, Carsten Werner

Water-repellent and self-cleaning plant surfaces have recently gained much interest. These features result from hierarchically aligned structural elements, and reflect adaptation to humid environments and to selective pressure from pathogens.





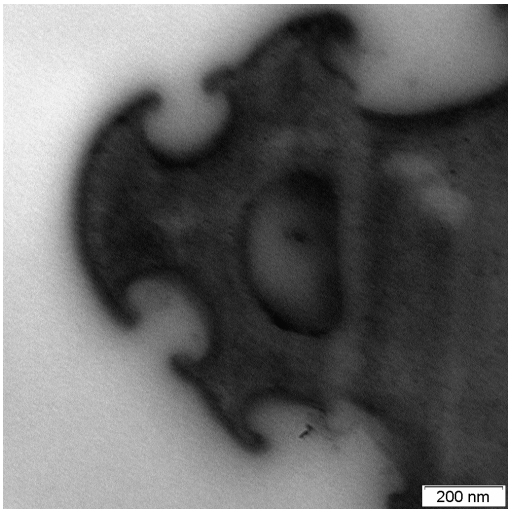


Fig. 1:
Microstructure: Bristles and papillous shapes (top)
Nanostructures: Comb alignment of ridges that connect small granules (centre)
Mushroom cross section: Overhanging shapes of the granules and ridges (bottom)

Springtails (Collembola, Entognatha), arthropods which live in soil, in decaying material, and on plants, have adapted to even more demanding conditions by evolving extremely effective and robust anti-adhesive skin patterns (Fig. 1). However, details of these unique properties and their structural basis are still unknown. Collembolan skin can resist wetting by many organic liquids (ethanol or tridecane) and at elevated pressures (3-4 bar). A combination of bristles and a comb-like hexagonal mesh (comb diameter between 400 and 900 nm) of interconnected nanoscopic granules (sizes around 250 nm) distinguish the skin of springtails from anti-adhesive plant surfaces. Furthermore, the mushroom-shaped cross-sections of ridges and granules were revealed to be a highly effective design principle of Collembolan skin.
In line with the non-wetting properties, collembolan skin was also found to exhibit outstanding repellence to particles and bacterial or fungal contamination. None of the microscopically investigated samples in our study ever showed a trace of any adhering material. Furthermore, it was observed that *Escherichia coli*, *Staphylococcus epidermidis* and *Candida albicans*, which represent Gram-positive bacteria, Gram-negative bacteria, and fungi, respectively, do not attach to the skin after massive exposure for several days. Artificial hole structures, mimicking the comb alignment on Collembola, showed significant advantages of the original periodicity of about 600 nm compared with larger periodicities regarding a decreasing initial attachment of *Staphylococcus epidermidis*, i. e., a globular microbe (Fig. 2).

This tendency was even maintained for both the original hydrophilic SU-8 photoresist and the Teflon AF dip coated hydrophobized surface. If hole periodicity is in line with bacterial size (~ 1000 nm), the microbial attachment is maximized due to a maximized contact area.

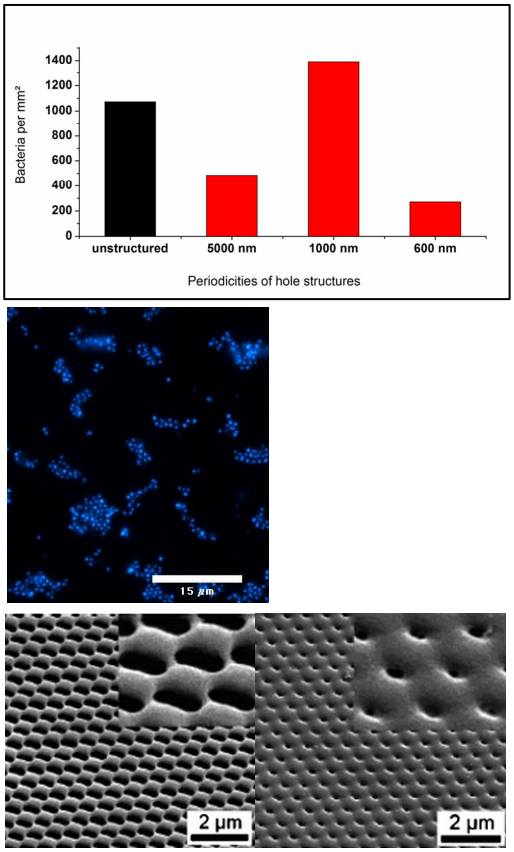


Fig. 2:
Adhesion of Staphylococcus epidermidis on structured SU-8 (after 2 min) (top);
DAPI labeled Staphylo¬coccus epidermidis (centre);
Laser interference patterned photo resist (bottom)

Cooperation:
C. Neinhuis, Technische Universität Dresden, Institute of Botany
E. Jacobs, Technische Universität Dresden, Institute of Medical Microbiology and Hygiene
A. Lasagni, Fraunhofer Institute for Material and Beam Technology, Dresden

Electrokinetics reveals diffuse segment distribution of a poly(N-isopropylacrylamide-*co*-carboxyacrylamide) soft thin films

Ralf Zimmermann, Martin Kaufmann, Carsten Werner

Stimuli-responsive materials, as engineered from thermo-, light- or pH-sensitive polymers, have gained widespread interest in numerous scientific areas and industrial applications, e. g., biosensing, bioadhesion, drug delivery, tissue engineering and microfluidics. A mandatory prerequisite for optimizing the performance of these materials is a complete analysis of the interfacial properties of their constitutive polymer films. This necessarily includes charge formation and screening in aqueous environment and how charge affects the film structure under different environmental conditions.
In order to unravel correlations between charging and structure of poly(N-isopropylacrylamide-*co*-carboxyacrylamide) (PNiPAAM-co-CarboxyAAM) soft thin films, we performed streaming current, surface conductivity and swelling (ellipsometry) measurements over a broad range of pH and salt concentrations (pH 2.5-10, 0.1-10 mM KCl) at constant temperature (22 °C). The films were shown to be negatively charged due to ionization of the carboxylic acid groups in the repeat unit of the copolymer. For a given salt concentration, the absolute value of the streaming current exhibited an unconventional, non-monotonous dependence on pH with the presence of a maximum at pH~6.4. This maximum is most pronounced at low electrolyte concentrations and gradually disappears with increasing salinity (Fig. 1). Complementary ellipsometry data further revealed that the average film thickness increases by a factor ~2.2 (i. e., from 40 to 87 nm as derived from ellipsometry data applying a box model) with increasing pH over the whole range of salt concentrations examined. The greater the solution salt concentration, the lower the pH value where expansion of the hydrogel layer started to take place. The dependence of film thickness on pH and electrolyte concentration remarkably followed that obtained for surface conductivity.

Keywords:
interfacial charge formation
electrohydrodynamics
diffuse soft interfaces
stimuli-responsive polymer films

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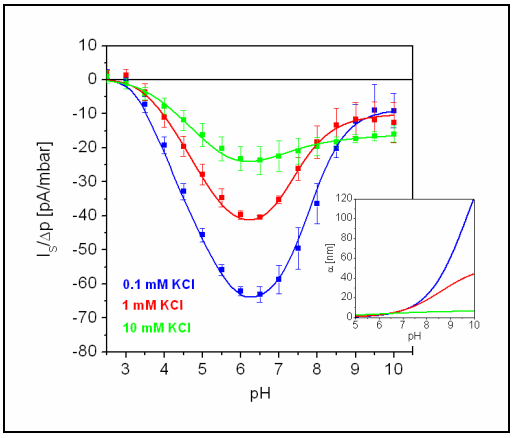

Fig. 1:
Ratio streaming current over applied pressure, $I_S/\Delta p$, as a function of pH for 0.1, 1 and 10 mM KCl solution. Experimental data: symbols. Solid lines represent reconstruction of the data by means of the theory detailed in Refs. [1, 2]. The inset shows the dependence of the softness parameter α on pH and salt concentration necessary for reproducing the experimental electrokinetic data in the pH range 2.5-10.

The streaming current and surface conductivity results could be consistently interpreted on a quantitative basis using the theory we previously derived for electrokinetics of charged diffuse (heterogeneous) soft thin films [1], complemented in this study by the derivation of a general expression for the surface conductivity of such systems [2]. In particular, the maximum in streaming current *versus* pH was unambiguously attributed to the presence of an interphasial gradient in polymer segment density following heterogeneous expansion of the chains within the film upon swelling when increasing pH (see increase of the softness parameter α in Fig. 1). Quantitative inspection of the data further suggested that p*K* values of ionogenic groups in the film, as derived from streaming current and surface conductivity data, differ by ~0.9 pH unit. This difference is attributed to the impact of position-dependent hydrophobicity across the film on the ionization degree of carboxylic sites.
Due to the versatile application of stimuli-responsive hydrogel films (see above), the results will contribute to the better understanding of interactions and transport processes at those surfaces. The introduced methodology of combined electrokinetic and swelling experiments in combination with adequate numerical solution of the governing electrohydrodynamic equations allows a comprehensive and consistent characterization of polymer surfaces in contact with aqueous solution, and thus provides a basis for further progress in polymer surface engineering.

Cooperation:
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Application of reversible inhibitors for hemocompatible release systems

Manfred Maitz

Surface immobilization of coagulation inhibitors has been previously developed as an effective method for the formation of actively hemocompatible surfaces [1]. The blood vessel wall, as the physiologically best hemocompatible surface, not only contributes anti-thrombin properties but additionally activates the anti-coagulant Protein C pathway. The combination of these two effects is desired for more advanced actively hemocompatible coatings. Coagulation factors like thrombin and the anticoagulant enzyme Protein C are structurally related serine proteases. Competitive inhibitor molecules targeting the reactive center of the enzymes show cross specifity. In appropriate equilibrium situations, an inhibitor bound to the reactive center of one molecule may be released to bind preferably to the other enzyme. As indicated in Fig. 1, this can be used to build up an "on demand" Protein C release system using an immobilized reversible inhibitor preloaded with activated protein C.

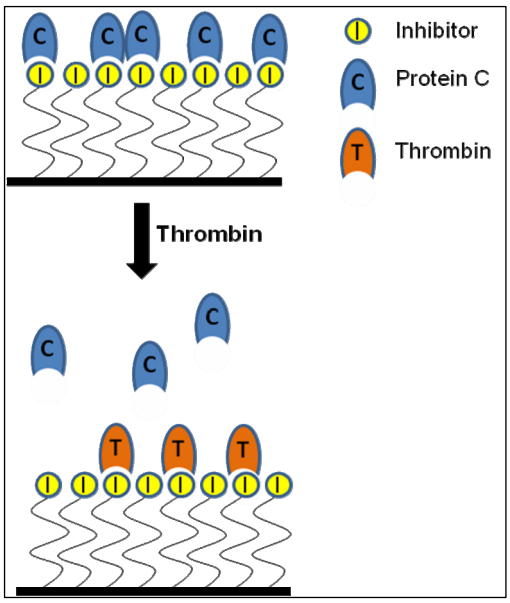

Fig. 1:
Principle of the inhibitor based hemocompatible release system

In the presence of thrombin, Protein C is released from the inhibitor while the coagulation factor thrombin binds and becomes inactivated.
Seven commercial and in-house synthesized thrombin inhibitors (I-1 to I-7) were immobilized and tested for their suitability for this concept by loading with radio-labeled Protein C. The Protein C elution by buffers with thrombin or with the non-competitive albumin (BSA) was probed (Fig. 2).

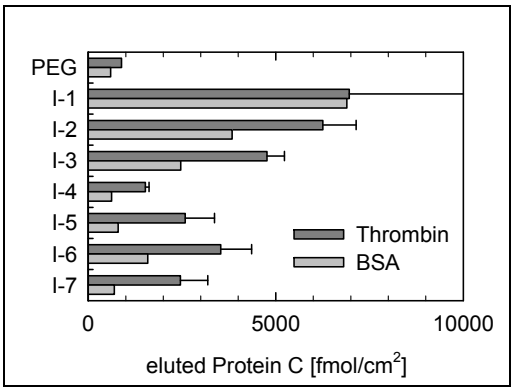

Fig. 2:
Release of pre-loaded Protein C from various surface immobilized inhibitors in presence or absence of the alternative inhibitor ligand thrombin

All surfaces released more Protein C in the presence of thrombin, which competes with Protein C for the inhibitor compared with the pure albumin-containing buffer. The various inhibitors showed different affinities and selectivities for Protein C. I-1 showed fastest release of Protein C without sensitivity for the composition of the elution medium, whereas the release from I-4 to I-7 was much slower but obviously triggered by thrombin.
Thus, a release system has been assembled for the anticoagulant Protein C that is responsive to an enhanced coagulation situation and has direct thrombin inhibiting properties. The system will be further evaluated and optimized in blood plasma, and in whole blood incubation assays.

Sponsor:
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[1] Gouzy, M.-F. et al.: Biomaterials 25 (2004) 3493

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