# Biologie-inspirierte Grenzflächen- und Materialgestaltung

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Eine zentrale Herausforderung des ST2 ist die Entwicklung von Polymermatrices, die die Kombination definierter physikalischer und biomolekularer Signale der extrazellulären Matrix von Zellen realisieren lassen und damit therapeutische und diagnostische Technologien ermöglichen.
Hierzu konnte im Berichtszeitraum eine auf Glycosaminoglykanen, Poly(ethylenglykolen) und funktionellen Peptiden beruhende Materialplattform um selektiv desulfatierte Heparinderivate erweitert werden, was die Steuerbarkeit der Bereitstellung von Wachstumsfaktoren aus den resultierenden Biohybrid-Gele wesentlich erleichtert. Die Netzwerkeigenschaften der Materialien wurden durch Kraftfeld-Methoden theoretisch erkundet, sodass eine unabhängige Modulation der zell-instruktiven Matrix-Parameter erreicht werden konnte (Biomacromolecules 15 (2014) 4439-4446). Für die lokalisierte Stimulation von Zellen in multifunktionellen Hydrogelen konnten eine neuartige Fotostrukturierungsmethodik (Advanced Healthcare Materials 2014, DOI: 10.1002/adhm.201400395) und ein Verfahren zur Herstellung makroporöser Cryogele mit definiert abgestuften mechanischen Eigenschaften entwickelt werden (Advanced Healthcare Materials 3 (2014) 1849-1853).
Diese Arbeiten unterstützen Forschungsprojekte, die auf die Regeneration von Geweben und Organen in vivo abzielen, ermöglichen aber gleichzeitig auch die Entwicklung von komplexeren, dreidimensionalen Modellen für die Untersuchung von physiologischen Prozessen und Erkrankungen in vitro. So konnten Hydrogel-basierte Ko-Kulturen von humanen mesenchymalen Stammzellen, Gefäß-Endothelzellen und verschiedenen Krebszellen etabliert werden, die es erlauben, die Interaktion von kapillaren Gefäßstrukturen mit Krebszellen zu untersuchen und in dieser Weise Wirkstoffe zu testen (Scientific Reports 4 (2014) Article number: 4414; Advanced Drug Delivery Reviews 79-80 (2014) 30-39). Die Nutzung solcher Mikrogewebe als realistische in vitro Modelle ist vielversprechend für künftige Anwendungen in der biologischen Grundlagenforschung ebenso wie in pharmazeutischen Untersuchungen und soll daher im Rahmen neuer Projekte weiterverfolgt werden.

Zum verbesserten Targeting von Folsäure-exprimierten Rezeptoren an Krebszellen wurden pH-schaltbare Polymersomen entwickelt (Small 2014, DOI: 10.1002/smll.201402581).
Eine weitere wesentliche Aufgabe des ST2 ist die Erkundung von technologisch relevanten Funktionen in der belebten Natur und die Übertragung der zugrundeliegenden Prinzipien in synthetische Materialien. Mit Hilfe einer Reverse-Imprinting-Lithographie-Methode konnte René Hensel Strukturprinzipien der antiadhäsiven Haut von Collembolen in synthetischen Polymermaterialien nachvollziehen (Advanced Materials 26 (2014) 2029-2033). Seine Dissertation zu diesem Thema wurde 2014 abgeschlossen und mit dem Prädikat „summa cum laude“ ausgezeichnet.
Andererseits konnten Schichtstruktur und chemische Konstitution der Collembolenhaut aufgeklärt werden, was weiterführende Möglichkeiten für die Entwicklung Biologie-analoger antiadhäsiver Beschichtungen, speziell für die Minimierung der mikrobiellen Besiedlung von Oberflächen eröffnet (Journal of the Royal Society Interface 11 (2014) 20140619).
Die Forschungen zu diesem Thema wurden durch den Innovationspreis des Fördervereins des IPF sowie durch den International Bionic Award 2014 der Schauenburg-Stiftung geehrt. Ein Kurzfilm des Teams wurde mit dem Fast Forward Science Award von Wissenschaft im Dialog prämiert.
In Beiträgen zu 2014 erschienenen Büchern konnten profilbestimmende Arbeitsrichtungen des ST2 zusammenfassend dargestellt werden (Stem Cells: From Basic Research to Therapy, CRC Press; Biointerfaces: Where Material Meets Biology, RSC Press; Bio-inspired materials for biomedical engineering, Wiley).
Philipp Seib, der nach längerer Tätigkeit am IPF und an der Tufts University, Boston, USA, eine Professur an der University of Strathclyde, Glasgow, UK, innehat, wurde zum IPF Fellow ernannt. Schwerpunkt seiner Forschungen sind in vivo Tumormodelle auf Basis von Polymer-Scaffolds.
Lars Renner konnte im Ergebnis der ersten Open Topic Ausschreibung des IPF als Nachwuchsgruppenleiter gewonnen werden. Ziel seiner Arbeitsgruppe ist es, Interaktionen von Proteinen und Lipiden in Mikroorganismen bei deren Wechselwirkung mit Oberflächen aufzuklären.

# Biologie-inspirierte Grenzflächen- und Materialgestaltung

## Enzymatic biohybrid structures fabricated by avidin-biotin conjugations: Controlling sizes and enzyme activity over sequential conjugations and filtration steps

Franka Ennen, Philipp Fenner, Georgi Stoychev, Susanne Boye, Albena Lederer, Brigitte Voit, Dietmar Appelhans

Biohybrid structures composed of avidin and biotinylated glycodendrimers (bGD) are promising delivery vehicles, on the one hand, because of the high biocompatibility of oligosaccharide modified dendrimers.[1, 2] On the other hand because their sizes can be fine-tuned by the bGD´s degree of biotinylation and the ligand-receptor stoichiometry.[3] In addition, the formation of supramolecular associates through the conjugation of avidin with bGD´s further facilitate the potential for targeted delivery through the EPR effect due to their high molecular weights. Along with the latter, it has been found that avidin has the ability to bind to certain lectins on cancer cell surface.[4-8] Moreover, the additional attachment of an enzyme makes them suitable vehicles in directed enzyme prodrug cancer therapies (DEPT). One enzyme-prodrug system of interest is horseradish peroxidase/indole 3 acetic acid. Since HRP transforms IAA into cytoxins, this enzyme prodrug combination has been proposed for anticancer therapy.[9] Importantly, it was found that neither the horseradish peroxidase (HRP) nor the indole 3 acetic acid (IAA) harm cells [10] and their apoptotic effects on various cancer cell types have been studied in vitro.[11-16]
Thus we conjugated a bivalent biotinylated enzyme (bHRP) and a tetravalent biotinylated glycodendrimer (PPI-DS-C6B4) to avidin (Figure 1a). The successful fabrication of avidin-glycodendrimer-enzyme biohybrid structures (BHS) was proven by dynamic light scattering (DLS) (Figure 1b), where a non-quantitative conversion of the biotinylated compounds with avidin was found (Table 1).

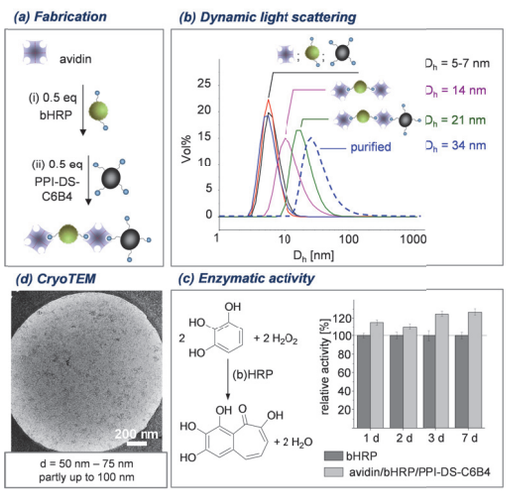


**Fig. 1:**
**(a) Fabrication of enzymatic biohybrid structures (BHS): addition of 0.5 eq bHRP to 1 eq avidin, (ii) addition of 0.5 eq PPI-DS-C6B4; (b) DLS volume plot of the single components, preconjugates and final enzymatic BHS; (c) Enhanced enzymatic activity of avidin-enzyme-glycodendrimer BHS; (d) CryoTEM image of avidin-glycodendrimer-enzyme BHS. Note: the fabrication images only illustrate the fabrication approach and do not aim to image the actual composition.**

An important key step was the purification of BHS by hollow fiber filtration (HFF). This allowed us the removal of non-bound particles and smaller conjugation adducts to isolate defined BHS with a $D_h$ of about 34 nm (Figure 1b). Besides the fact that the conjugation of such biotinylated compounds crucially depends on their properties (e.g. degree of biotinylation or charge of the participating components) applying the right conjugation sequence (avidin/bHRP/PPI-DS-C6B4 vs. avidin/PPI-DS-C6B4/bHRP) resulted in significantly enhanced enzymatic activity of avidin/bHRP/PPI-DS-C6B4 nanostructures. This is a pivotal prerequisite for a successful application of those BHS in DEPT. More importantly, we were able to apply asymmetric flow field flow fractionation (AF4) coupled to static and dynamic light scattering in order to get deeper insights in their molar masses and dispersity. With this technique the determination of shape factor (~ 2.7) was possible indicating an anisotropic geometry (Table 2).

**Keywords**
**avidin-biotin conjugation**
**glycodendrimers**
**enzyme**
**directed enzyme prodrug**
**cancer therapies**

**Table 1:**
**Amount of converted biotinylated compound in avidin/bHRP//PPI-DS-C6B4 BHS**

| amount of bound biotinylated compounds | |
|---|---|
| applied molar ratio | determined molar ratio |
| 1/0.50/0.50 | 1/0.20/0.15 |

**Table 2:**
**Number and mass average of molar masses, dispersity and shape factor of avidin/bHRP/PPI-DS-C6B4 BHS**

| $M_n$ | $M_w$ | Đ | $R_g/R_h$ |
|---|---|---|---|
| [g/mol] | [g/mol] | $M_w/M_n$ | |
| 586 000 | 1 259 000 | 2.15 | 2.67 |

In summary, we demonstrated the elucidation of adequate fabrication sequence in order to fabricate defined BHS. This also led to significantly enhanced enzymatic activities of the enzyme horseradish peroxidase embedded in avidin-glycodendrimer associates. The analytical method AF4 can be used in order to determine important physical properties of the BHS (Table 2). Consequently, the next step will be to evaluate their potential in targeted drug delivery.


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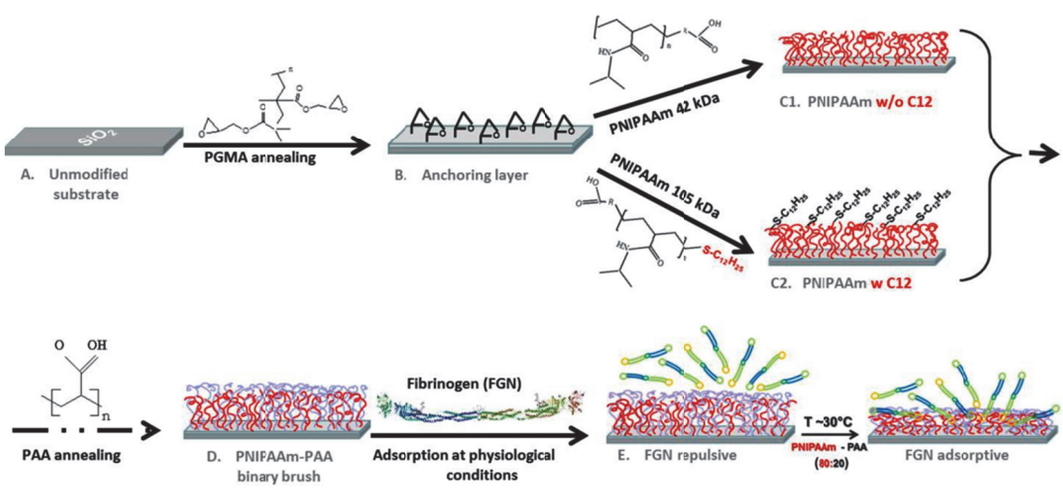
# Biologie-inspirierte Grenzflächen- und Materialgestaltung

## Nanostructured biointerfaces: thermo-responsive polymer brushes for the control of protein interaction

Evmorfia Psarra, Ulla König, Andreas Janke, Cornelia Bellmann, Eva Bittrich, Klaus-Jochen Eichhorn, Manfred Stamm, Petra Uhlmann

Controlling the reversibility, quantity and extent of biomolecule interaction at interfaces has a significant relevance for bioscience applications. Therefore there is the outmost need to adapt the interfaces of solid materials, when considering the longevity and successful function of materials in biological environments. The advancement of functionality and performance is the major issue of surface engineering aiming the production of responsive materials that specifically control biological responses. Currently, nanostructured materials have been investigated with respect to an enhanced manipulation of their physical, biochemical and biological cues which have an immense potential for technological achievements. Nano-scaled polymer brushes are composed of end-tethered linear polymers, which are unidirectional stretched away from the surface due to excluded volume interactions owing a sufficient grafting density. They are very promising materials for the modulation of interactions with biological systems because of their distinct collaborative response to environmental changes and their potential to serve as a toolbox for bottom-up approaches. In this context, the interfacial characteristics of poly(N-isopropylacrylamide) PNIPAAm polymer brushes grafted to model silica surfaces were investigated in regard to their interactions with dissolved proteins. Because of a phase transition at the lower critical solution temperature (LCST) PNIPAAm brushes are able to alter interfacial properties in response to temperature stimuli. Two different PNIPAAm species were used to prepare brushes by the *grafting-to*-method. One polymer type possessed an additional hydrophobic terminal group (dodecyl trithiocarbonate, C12). Because it is known, that hydrophobic moieties can influence protein interaction considerably, the aim of our study was to investigate the impact of the polymer brush nanoarchitectonics on model protein adsorption. In order to design nanostructured biointerfaces, the temperature triggered fibrinogen (FGN) adsorption was studied both on homo polymer PNIPAAm brushes with and without the hydrophobic terminal functionalization, and further on binary brushes made of the polyelectrolyte poly (acrylic acid) (PAA) and one of the prior described two PNIPAAm species:

**Keywords**
**stimuli-response**
**polymer brushes**
**protein adsorption**
**PNIPAAm**




**Scheme 1:**
**research subject: A-D stepwise explanation of binary brush preparation PNIPAAm-PAA with different PNIPAAm species; E example of subsequent stimuli responsive FGN adsorption onto binary brush**

# Biologie-inspirierte Grenzflächen- und Materialgestaltung

Due to the introduction of the hydrophobic C12 terminus in the brush nanostructure the interfacial properties of both the homo PNIPAAm brushes and their resultant binary brushes showed pronounced differences. In summary, the hydrophobic C12 terminus has an influence on the wettability, the phase transition temperature, the electrokinetic properties, and the morphology of the PNIPAAm polymer brushes. Figure 1, illustrates the effect on FGN adsorption, measured *in situ* by spectroscopic ellipsometry.

As expected, the adsorbed amount of FGN was generally increasing by PNIPAAm w C12, i.e. the modified PNIPAAm brush with hydrophobic terminus was able to adsorb FGN at 37 °C, significantly. Accordingly, homo PNIPAAm brushes lost their proven protein resistant character above the LCST. By using binary PNIPAAm-PAA brushes with different composition; the stimuli dependent FGN adsorption was even more distinct.

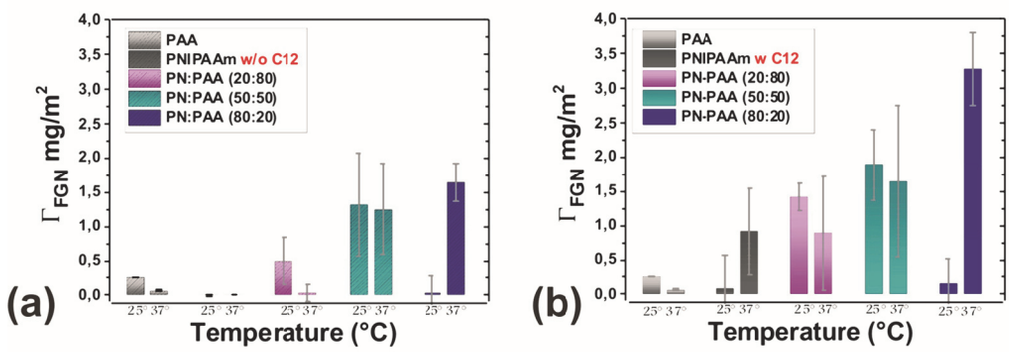



**Fig. 1:**
**Adsorbed amount of FGN on homo- and binary polymer brushes, without C12 (a) and with C12 (b). The experiments were performed in PBS at pH 7.4.**

From Fig. 1, it can also be derived that the ratio of the binary brush components is crucial for protein adsorption. Clearly, the FGN adsorption effect is considerable amplified by introducing a hydrophobic terminus. The interplay of both binary brush components offers the chance to create new material properties. This example shows that bottom-up techniques with nano-scaled polymer brushes have a large potential to act as smart building blocks with multiple functions. They are able to generate well-defined biomimetic and tunable nano-biointerfaces, capable of imitating dynamic properties of natural biological systems.

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Prof. S. Minko, University of Georgia, USA

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# Biologie-inspirierte Grenzflächen- und Materialgestaltung

## Drug delivery and cell interaction of polyelectrolyte complex particle coatings for bone healing

Bernhard Torger, David Vehlow, Birgit Urban, Christin Striegler, Dietmar Appelhans, Martin Müller

**Keywords**
**osteoporosis**
**bone substituting material**
**cytocompatibility**
**drug delivery**
**polyelectrolyte complex**
**bisphosphonate**

The therapy of fractures and defects in the systemically diseased hard tissue is one of the great challenges in medicine. Artificial allogenic bone substituting materials (BSM) are used for bone remodelling and tissue regeneration, but have problems or fail in the case of systemic bone diseases osteoporosis (OP) and multiple myeloma (MM).
This work aims at the development of an adhesive nanoscaled carrier system for bone therapeutic drugs usable for the functionalization and improvement of BSM. We have chosen biocompatible polyelectrolyte (PEL) complex (PEC) nanoparticles (NP) [1] fabricated by mixing polycation and polyanion solutions and loaded by relevant therapeutics such as bisphosphonates.
In the framework of a Collaborative Research Center of DFG clinicians, cell biologists and material scientists have raised multiple requirements concerning properties of such a nanoparticular carrier systems. In the following we give results on three of those requirements obtained by our related part project.

Adhesiveness in contact to water
Fig. 1 shows FTIR spectra on thin films of pure zoledronate (ZOL), ZOL/poly(ethyleneimine) (PEI), ZOL/cellulosesulfate (CS) and ZOL loaded PEC NP (ZOL/PEI/CS) before (black) and after contact to phosphate buffer (blue) [2].

Significantly, only the ternary ZOL/PEI/CS NP system has sufficient adhesiveness. Dried PEC NP films are claimed to bind at surfaces in certain analogy to latex systems by ad- and cohesive forces upon water removal (deswelling) [3].

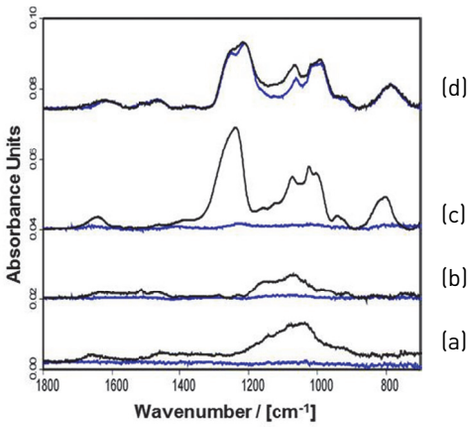


**Fig. 1:**
**FTIR spectra of casted ZOL containing films at Ge model substrates. (a) pure ZOL, (b) mixture ZOL/PEI -1:4, (c) mixture ZOL/CS -1:4 and (d) mixture ZOL/[PEI/CS-0.9]-1:4 before contact to water and after contact to water (from bottom to top).**

Cytocompatibility
Fig. 2 shows concentration profiles of metabolic activity of human mesenchymal stromal cells (hMSC) cultured above casted PEC NP films of two compositions.

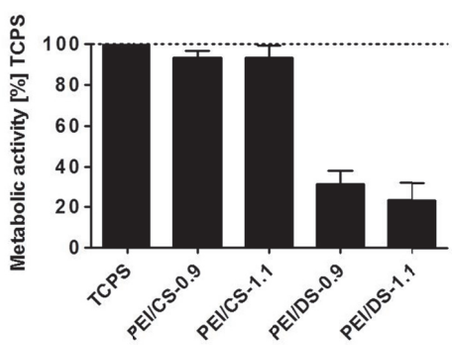


**Fig. 2:**
**Metabolic activity of hMSC cultured onto immobilized NP of PEI/CS and PEI/DS (PEC-0.9, PEC-1.1) in comparison to tissue culture polystyrene (TCPS, control). Detailed conditions and parameters can be found therein [4].**

While PEI/dextansulfate (DS) results in a strong dependence of metabolic activity on concentration, PEI/CS results in no such dependence suggesting better cyto-compatibility. We suggest that toxicologically suspected branched PEI is better masked by linear CS than by branched DS due to steric reasons [4]. Moreover, net charge (PEC-0.9 vs. PEC-1.1) showed no effect on metabolic activity of hMSC.

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**Keywords**
**springtail**
**biomimetics**
**omniphobicity**
**polymer membrane**
**reverse imprint**
**lithography**

Retarded drug release
Fig. 3 shows drug release curves of risedronate (RIS) from casted films of PEC NP (poly(l-lysine)/CS) in comparison to pure RIS films.

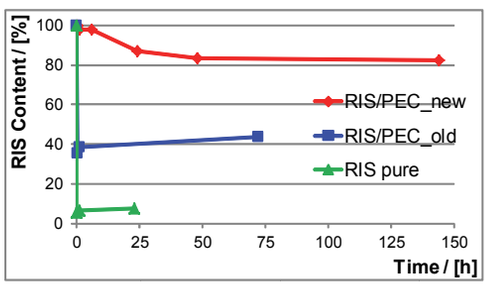


**Fig. 3.**
**Decrease of the relative RIS content from pure drug (green) and drug loaded PLL/CS particle films (blue, red) casted onto Ge substrate in contact to release medium. Blue and red curve denote release profile for old and new PEC/NP preparation.**

Obviously, there is a higher retardation for RIS loaded at PEC NP. Furthermore, the initial burst and residual drug content for PEC NP could be significantly enhanced using a new preparation protocol. We claim electrostatic binding and physical entrapment within the compact PEC phase as main factors of the drug release behavior. Branched polycations like PEI and maltose modified PEI may show a specific affinity to phosphate related compounds like bisphosphonates or others (ATP).

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## Biologically inspired omniphobic surfaces by reverse imprint lithography

Ralf Helbig, René Hensel, Julia Nickerl, Carsten Werner

Springtails (Collembola) are soil dwelling arthropods, living in decaying material and on plants. The skin-breathing animals avoid suffocation by an extremely effective and robust omniphobic cuticula [1]. The anti-adhesive skin consists of nanoscopic granules and interconnecting ridges, which together form hexagonal or rhombic comblike patterns, as exemplarily shown in Figure 1. Recent analyses [2,3] demonstrated the predominant role of the cross-sectional profiles of the submicron-structures irrespective of the surface chemistry. Furthermore, the comblike arrangement of the surface features was found to be mechanically stable, allowing for high shear load dissipation. Mimicking this effectively liquid-repellent and mechanically stable morphology in engineered materials may offer exciting opportunities for demanding applications.

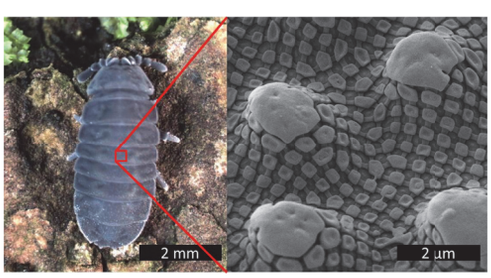


**Fig. 1:**
***Tetrodontophora bielanensis* with comblike skin pattern**

Therefore, a polymer membrane that resembles the springtail cuticle morphology was developed by means of a novel reverse imprinting method (Figure 2). A silicon master structure was produced in a two-tier patterning process to serve as template for feature replication (Figure 2 top). Subsequently, perfluoropolyether dimethacrylate (PFPEdma) templates were cast from the silicon master. The cavities of this mold were filled with poly(ethylene glycol) dimethacrylate (PEGdma) prepolymer which was finally UV-cross-linked. The resultant

membrane is flexible and free-standing and, thus, transferable to various nonflat substrate materials. The omniphobic performance of the polymer membrane was demonstrated by plastron collapse and long term immersion tests [4]. The mechanical durability of the membranes was shown by wear tests using a nanotribometer.

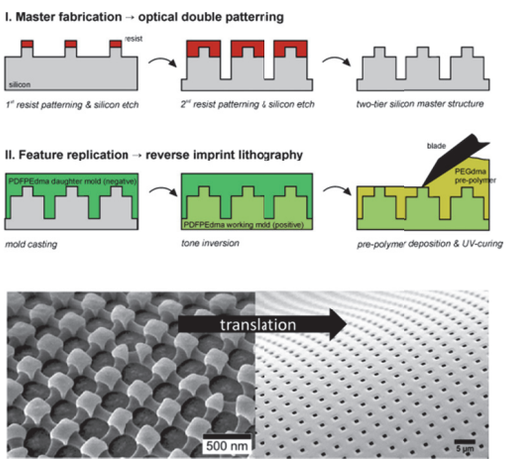


**Fig. 2:**
**(top, down left, down right) process scheme for membrane fabrication, springtail surface, flexible polymer membrane**

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## Neutrophil extracellular traps on hydrophobic surfaces as a cause of pro-coagulant effects

Marion Fischer, Claudia Sperling, Carsten Werner

**Keywords**
**hemocompatibility**
**biomaterials**
**immune responses**

The formation of neutrophil extracelluar traps (NETs) is a facet of innate immunity and results in the release of DNA and granular proteins upon activation of white blood cells. These expelled DNA fibers and adherent enzymes represent a highly efficient defense mechanism of neutrophils against invading bacteria and fungi. The increased levels of DNA in whole blood, however, can be related to adverse host responses. In clinical settings, persistently exposed NETs were found to be correlated to tissue damage [1] and thrombosis [2]. Whilst there is a growing body of evidence for NET-related effects in disease states, biomaterials-induced NET formation has not been addressed yet but may provide valuable insights in inflammatory and thrombotic events.
Using an *in vitro* whole blood incubation model, we demonstrated for the first time the capacity of biomaterials to induce NET formation and identified NETs on surfaces after whole blood incubation on hydrophobic materials (Fig. 1).

NET quantification by detection of extracellular DNA revealed higher levels for highly hydrophobic PTFE, followed by the moderately hydrophobic SAM-$CH_3$ pointing to a correlation of NETosis to materials hydrophobicity. Adherent neutrophils on PTFE and SAM-$CH_3$ underwent activation, exhibited high degrees of spreading and frustrated phagocytosis, possibly triggering NETosis.
We further evaluated prothrombotic events on NET-positive surfaces. Earlier studies showed the role of extracellular chromatin in thrombosis through the recruitment of platelets and red blood cells [3, 4].

# Biologie-inspirierte Grenzflächen- und Materialgestaltung

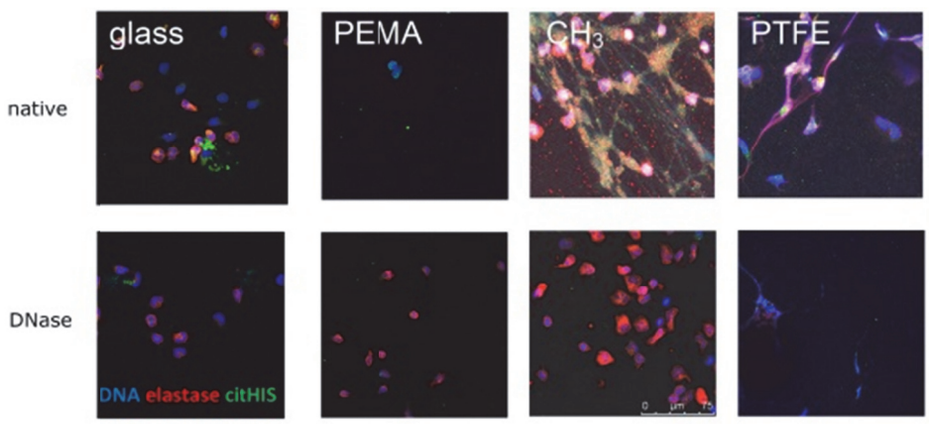


**Fig. 1:**
**Fluorescent micrographs of surfaces after whole blood incubation for 4h. Immunostaining was done for DNA (blue), elastase (red) and citrullinated histone ("citHIS", green).**

We likewise found increased granulocyte-platelet conjugate formation and platelet degranulation upon whole blood incubation of NET-positive PTFE and SAM-$CH_3$ surfaces (Fig. 2).

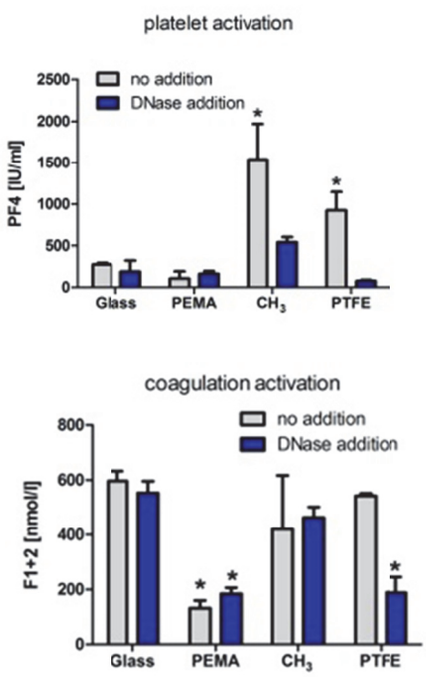


**Fig. 2:**
**top: Platelet factor 4 (PF4) and bottom: fragment F1+2 detected in plasma after surface whole blood incubation. Statistical evaluation: One way ANOVA with post Turkey-test: PF4: '*$CH_3$' and '*PTFE' significantly different to all other samples, F1+2: '* PEMA' significantly different to all other samples except for PTFE DNase, '*PTFE DNase' is significantly different to all other samples except PEMA and PEMA DNase**

In line with the observed platelet activation, NET-forming surfaces exhibited significantly increased coagulation activation. The NET-related prothrombotic events are most probably mediated by binding and activation of FXIIa, in concert with the observed platelet activation processes.
Finally, digestion of NETs with 100 U/ml DNase was performed to prevent surface-mediated NETosis in our *in vitro* setup. NET-degradation by DNase I treatment significantly reduced platelet activation on NET-positive -$CH_3$ and PTFE. This is consistent with previous work of Fuchs et al. using perfusion chambers [5]. NET degradation furthermore significantly reduced plasmatic coagulation (F1+2 fragment) on PTFE.
Taken together, our data suggest that prevention of NET formation deserves consideration in the design of hemocompatible surfaces for blood-contacting biomedical devices.


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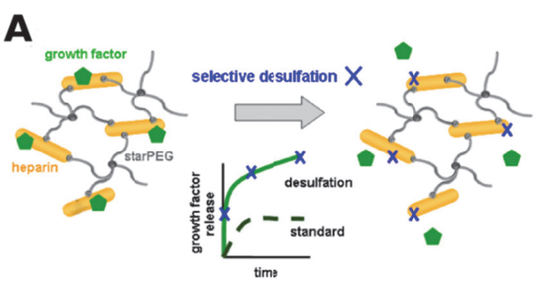
# Biologie-inspirierte Grenzflächen- und Materialgestaltung

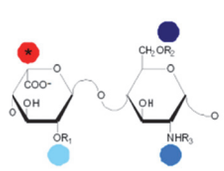
## Biohybrid networks of selectively desulfated glycosaminoglycans for tunable growth factor delivery

Andrea Zieris, Ron Dockhorn, Anika Röhrich, Ralf Zimmermann, Martin Müller, Petra B. Welzel, Mikhail V. Tsurkan, Jens-Uwe Sommer, Uwe Freudenberg, Carsten Werner

The glycosaminoglycan (GAG) heparan sulfate of the mammalian extracellular matrix regulates storage and presentation of various growth factors [1]. Specific sulfation patterns of GAG govern the electrostatic complexation of biomolecules and thus allow for modulating the release profiles of growth factors from GAG-based hydrogels (Fig. 1A).




B



| | $R_1$ | $R_2$ | $R_3$ | theoretical number of sulfate groups* per disaccharide | determined number of sulfate groups* per disaccharide |
|---|---|---|---|---|---|
| unmodified heparin | $SO_3^-$ | $SO_3^-$ | $SO_3^-$ | 3 | |
| N-DSH, NAc | $SO_3^-$ | $SO_3^-$ | Ac | 2 | 2.07 ± 0.24 |
| 6-O-DSH, NAc | $SO_3^-$ | H | Ac | 1 | 1.32 ± 0.09 |
| cDSH, NAc | H | H | Ac | 0 | 0.33 ± 0.09 |

* based on average composition of major heparin sequence

**Fig. 1:**
**Selectively desulfated heparin derivatives used in the formation of biohybrid hydrogels for adjusting growth factor release profiles. (A) Modulated growth factor delivery from hydrogels with desulfated heparin building blocks (schematic view). (B) Functional groups at the 2-O-, 6-O-, and N-positions of heparin and its desulfated derivatives, expected residual sulfate contents, and analytically determined sulfate contents (from both polyelectrolyte titration and ATR-FTIR).**

To explore this options, selectively desulfated heparin derivatives mimicking naturally occurring GAGs such as chondroitin sulfate, heparan sulfate and hyaluronic acid were prepared and thoroughly characterized by means of polyelectrolyte titration, HPLC and ATR-FTIR (Fig. 1B). The desulfated heparin derivatives acting as multivalent cross-linkers were covalently converted with star-shaped poly(ethylene glycol) (StarPEG)into binary polymer networks covering a wide range of viscoelastic properties (1.3 kPa-14.7 kPa). The impact of the GAG sulfation pattern on the reactivity of the polymeric gel building blocks and the resulting network characteristics of the synthesized hydrogels were theoretically evaluated by mean field methods [2, 3] and experimentally analyzed by rheometry and swelling measurements (Fig. 2I). Sulfation-dependent differences of reactivity and miscibility of the heparin derivatives were shown to determine network formation. The sulfation pattern of the StarPEG-GAG hydrogels was confirmed to rule growth factor binding and release [4]. Based on these findings, a quantitative design concept for customizing growth factor affinity and physical characteristics was introduced (Fig. 2II) and validated by adjusting a set of hydrogels with similar mechanical properties (Fig. 2 II) but different sulfation pattern, allowing for a graduated release of fibroblast growth factor 2 (Fig. 2III).

In sum, a set of selectively desulfated heparin derivatives with tailored biomolecular and physicochemical properties was utilized for customizing the growth factor delivery from GAG-based hydrogels with independently adjusted physical properties. The obtained materials offer an unprecedented level of control over cell-instructive matrix characteristics and can therefore be instrumental in applications relying on the microenvironmental regulation of cellular fate decisions.

**Keywords**
**hydrogel**
**biomaterials**
**growth factors**
**mechanical properties**
**mean field**
**drug delivery**

# Biologie-inspirierte Grenzflächen- und Materialgestaltung

**Keywords**
**extracellular matrix**
**microwell array**
**hematopioetic stem and progenitor cells fate decision**
**competitive repopulation assay**

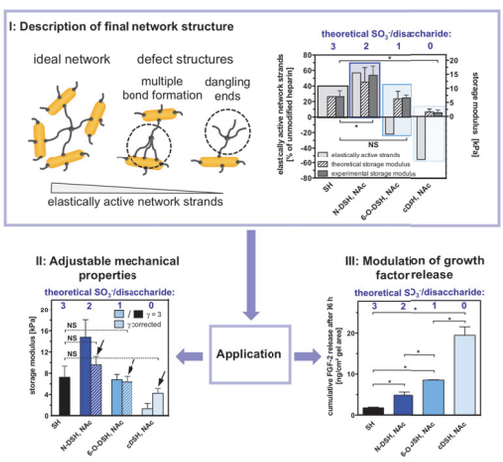



**Fig. 2:**
**Network structure, mechanical properties, and growth factor release in dependence on GAG sulfation pattern of desulfated heparin derivatives used for gel formation. Variations in the elastically active network strands (I, right) as well as the experimentally determined and theoretically calculated storage moduli revealed the presence of defect structures within the network (I, left). On the basis of the results of the mean field model, the molar ratio of the hydrogel building blocks ($\gamma$ = molar ratio of StarPEG/ heparin) was adjusted to obtain networks with similar mechanical properties but different sulfation patterns (SH vs heparin derivates, see arrows, II). Altered sulfation patterns thus enable a far-going modulation of FGF-2 release from the produced hydrogel matrices (III). Statistics: mean ± standard deviation, * indicates $p < 0.05$, and NS indicates nonsignificant differences.**
**(Figure 1 and 2 from reference 4 (copyright?).**

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## Space constraints govern fate of hematopoietic stem and progenitor cells in vitro

Eike Müller, Carsten Werner

Hematopoietic stem and progenitor cells (HSPC) of adult mammalian organisms are known to reseed in a specialized microenvironment within the bone marrow (BM) referred to as *stem cell niche*. This microenvironment is made up of various cell types, biopolymers of the extracellular matrix (ECM) and signaling molecules, tightly regulating life-long hematopoiesis through a balance of HSPC self-renewal, differentiation, dormancy and apoptosis.[1] Although the hematopoietic system is studied already for more than four decades, our knowledge about the control of HSPC function within their niche(s) is still very limited and effective methods to maintain and expand HSPC for applications in regenerative medicine are lacking.[2] Therefore, deciphering exogenous cues that determine stem cell fate decisions is a persisting challenge of cell biology and bioengineering. In an effort to unravel the role of spatial constraints in the cell-instructive characteristics of bone marrow microenvironments, we recently demonstrated that microcavity arrays functionalized with FN result in a more state of human HSPC (hHSPC). Specifically, spatial restriction of individual hHSPC within FN coated microcavities was observed to better maintain a CD34+ and CD133+ phenotype.[3] However, as surface markers are only of limited indicative value, competitive repopulation studies in mice, representing the 'gold standard' in the analysis of HSPC functionality were applied to evaluate the stemness of the cells after in vitro culture in adhesive single-cell and multi-cell microcavities, respectively (Fig.1).

# Biologie-inspirierte Grenzflächen- und Materialgestaltung

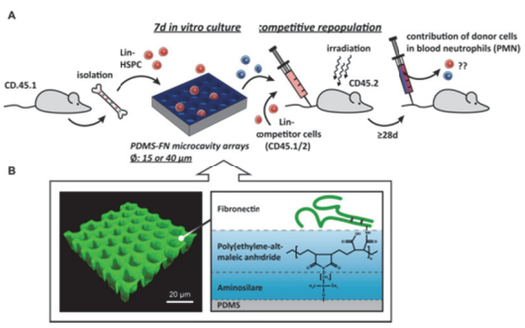
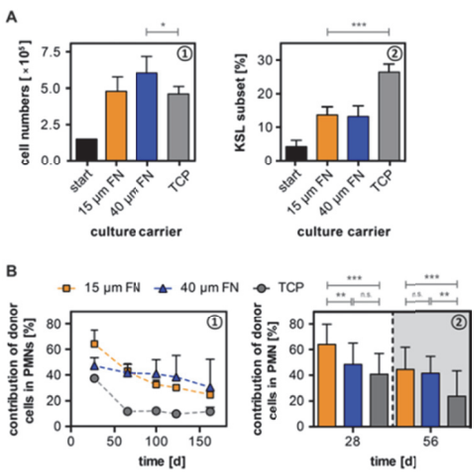



**Fig.1:**
**Setup for HSPC *in vitro* culture and repopulation experiments.**
**(A) Lin- cells were isolated and cultured in serum free medium on PDMS-FN microcavities during 7 days, 1 x $10^6$ cultured cells (CD45.1) were injected into lethally irradiated recipients (CD45.2) with 5 x $10^5$ total bone marrow cells (CD45.1xCD45.2), peripheral blood chimerism was analyzed by flow cytometry.**
**(B) Confocal laser scanning microscopy (CLSM) image of a PDMS micropattern, demonstrating the homogeneous immobilization of FN (green). FN was covalently linked to maleic acid copolymer layers, which in turn were immobilized via imide bonds to aminosilane functionalized PDMS surfaces.**

FN coated culture carriers containing cavities of 10 µm depth and diameters of 15 and 40 µm were prepared to provide adhesive single-cell and multi-cell compartments for the culture of HSPC enriched murine BM cells for 7 days *in vitro* (Fig.1B). Our data show that the expansion of murine immature hematopoietic cells lacking the expression of antigens indicative for a mature cell lineage, designated as lineage negative (Lin-) HSPC, can be controlled by exposure to FN coated microcavities of a particular size as previously observed for CD133+ human HSPC in culture. [3] Analyzing the population of CD117+ (Kit), Sca-1+, and Lin- (KSL) cells, known to contain the main HSPC fraction, at day 7 we observed a drastic, about 3-fold, increase of the KSL fraction of cells grown on FN functionalized microcavity arrays and an up to 6-fold increase on TCP (Fig.2A). No influence of cavity size on the frequency of KSL cells was detected. These findings may imply that although adhesion to FN supports proliferation of Lin- cells, HSPC on FN are more quiescent than on TCP.




Next we analyzed the repopulation efficacy of the *in vitro* cultured HSPC. Adherent cells were detached from the microcavity arrays on day 7 of the cultures and 1 x $10^6$ of the whole harvested population combined with 5 x $10^5$ freshly isolated Lin- competitor cells were transplanted into lethally irradiated wild type recipient mice. The success of the repopulation was monitored over a time period of up to 162 days by analyzing the relative contribution of donor cells to short-lived polymorphonuclear neutrophils (PMN), indicative for the donor cell contribution to the hematopoietic stem cell compartment, using flow cytometry. Repopulation experiments demonstrated the functionality of HSPC cultured on FN coated microcavity arrays in terms of their ability to contribute to the formation of PMN in the recipient (Fig.2B). Although the total KSL number of HSPC was lower after culture on either type of FN coated carrier compared to TCP we found a drastically improved repopulation potential of these cells, confirming the supportive effect of adhesive interactions in the maintenance of HSPC *in vitro*. Furthermore, during the first 28 days after transplantation cells that had been exposed to small cavities in culture showed a significantly higher relative contribution to PMN compared to cells grown in large cavities or on TCP. These findings indicate an influence of both surface coating and cavity size on the short-term engraftment efficiencies. Monitoring the relative PMN contribution over the following weeks we observed an improved donor contribution if the donor cells were grown on FN functionalized microcavities in comparison to TCP. However, cavity size had

**Fig.2:**
***In vitro* expansion and competitive repopulation to assess HSPC functionality. (A) Overall cell numbers of murine BM derived Lin- cells cultivated for 7 d on top of TCP or FN coated PDMS microcavity arrays with various diameters (left). Percentage of cells from the KSL subset in the total cell population(right). (B) Murine Lin- cells (CD45.1) grown on top of FN functionalized 15 µm or 40 µm sized cavities and TCP for 7 days were collected and $10^6$ cells together with 5 x $10^5$ freshly isolated competitor BM cells (CD45.1xCD45.2) transplanted into each lethally irradiated recipient mouse (CD45.2). The relative contribution of donor cells to PMN of the peripheral blood was analyzed via cytometry.**

**Keywords**
**3D culture model**
**breast cancer**
**prostate cancer**
**microenvironment**

no strong impact on long-term HSC (LT-HSC), as no differences between the contribution of cells cultured on FN functionalized arrays of 15 µm and 40 µm diameter microcavities was detected after a period of 56 days (Fig.2B). Taken together, our data suggest that *ex vivo* cultivation of HSPC on small, adhesive microcavities efficiently prevents differentiation into more committed progenitors selectively maintaining ST-HSC and LT-HSC. As demonstrated by this report, the developed set of cell-adhesive microcavity arrays provides a useful tool for studies on the role of spatial constraints and adhesive interactions in stem and progenitor cell fate control *in vitro*.

Co-operation:
Prof. Claudia Waskow, Dr. Tatyana Grinenko, Institute for Immunology, Technische Universität Dresden
Prof. Tilo Pompe, Institute of Biochemistry, Universität Leipzig

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## Tissue-engineered 3D tumour angiogenesis models: potential technologies for anti-cancer drug discovery

Laura J. Bray, Marcus Binner, Uwe Freudenberg, Carsten Werner

Despite advancement in detection methods and treatments, breast and prostate cancer remain major causes of death. Therefore it is imperative that we gain a greater understanding of the progression from localized to advanced cancer using relevant physiological systems. There is also a current need to upgrade the existing practice in culturing tumour cells and modelling tumour angiogenesis and metastasis to bone *in vitro*, in order to create experimental models with greater clinical relevance.
Like many other cancer cells, breast and prostate cancer cells have been extensively studied in two dimensional (2D) cultures, through which a significant understanding of cancer biology has been gained. However, the culture of cells on rigid 2D substrates, such as tissue culture plastic, does not recreate the dynamic and highly complex tissue microenvironment. In the body, cells are embedded in an extracellular matrix (ECM) that provides not only architectural support, but also delivers signals to cells that direct their behaviour. Therefore, emerging approaches in engineering tumour microenvironments have focused on the development of synthetic matrices, such as PEG-based hydrogels, that are able to deliver defined cues through the incorporation of biochemical and biological functionalities [1]. We have previously demonstrated that such hydrogels support the co-cultivation of vascular endothelial cells (EC) with tumours [2, 3]. We have now extended this body of work to study the ability to create an ECM-like model to study breast and prostate cancer cell growth in 3D. Also, we investigated the ability to produce a tri-culture mimicking tumour angiogenesis with cancer spheroids, vascular EC and mesenchymal stem cells (MSCs).
Cultures prepared in matrix metalloproteinase (MMP)-cleavable starPEG-heparin hydrogels displayed spheroid formation in contrast to adherent growth on tissue culture plastic. Cancer cell lines were able to be co-cultivated

with EC and MSC. These engineered constructs can routinely recreate breast and prostate tumour vascularisation. The multiple cell types cultured within this model were less sensitive to chemotherapy when compared with two dimensional (2D) cultures. These results have indicated that our model provides a similar chemotherapeutic response to that found with widely published animal models, allowing for more relevant *in vitro* culture conditions. This model opens the way for future extensive chemotherapeutic combination treatment analyses. Moreover, the described *in vitro* angiogenesis model can be instrumental to identify components of the vascular development process that would be ideal therapeutic targets.

In conclusion, we have formed 3D *in vitro* bioengineered tumour angiogenesis microenvironments within a glycosaminoglycan-based hydrogel culture system. These multiparametric bioengineered tumour angiogenesis models are able to more closely mimic *in vivo* models than 2D cultures. We anticipate these new biomimetic tumour microenvironments to provide a valuable new tool to answer intricate biological questions and improve our limited comprehension of the role of microenvironmental and vascular signals in cancer progression. Insights gained with additional explorations using this model may lead to the identification of more effective treatment options for breast and prostate cancer patients.

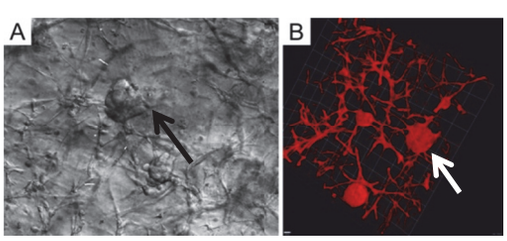


**Fig. 1:**
**3D model of prostate cancer angiogenesis. Images depict light microscope (A) and confocal 3D projection (B) of prostate cancer cells co-cultured with vascular cells and mesenchymal stromal cells for 14 days in starPEG-heparin hydrogels. Arrows demarcate tumour spheroids.**

Sponsor:
Australia Awards, Leibniz Association, Deutsche Forschungsgemeinschaft

Co-operation:
Prof. D.W. Hutmacher, Queensland University of Technology, Australia

[1] M.V. Tsurkan, K. Chwalek, S. Prokoph, A. Zieris, K.R. Levental, U. Freudenberg, C. Werner, Defined polymer-peptide conjugates to form cell-instructive starPEG-heparin matrices in situ, Adv Mater, 25 (2013) 2606-2610.

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# Biologie-inspirierte Grenzflächen- und Materialgestaltung

**Keywords**
**immunomodulation**
**hydrogel**
**wound healing**
**macrophages**

## Immunomodulatory hydrogels to support dermal wound healing

Lucas Schirmer, Passant Atallah, Uwe Freudenberg, Carsten Werner

Impaired wound healing and the resulting chronic wounds pose a serious threat and cause significant morbidity and mortality. Facing the increasing population age, the growing epidemic of metabolic syndrome and the current lack of causal therapies more effective treatments of chronic wounds are urgently needed.
Within the complex interaction of leukocytes, fibroblasts, endothelial and epithelial cells in the wound environment, macrophages play a central role in the regulation of inflammation and the subsequent cell proliferation. [1] Promotion of the pro-regenerative (M2) macrophage phenotype by gel-based delivery of anti-inflammatory cytokines (such as IL4, IL10 and IL13) can therefore be reasonably expected to improve dermal wound healing. For that purpose, we utilize a hydrogel platform based on star-shaped poly(ethylene glycol) (starPEG) and a set of glycosamino-glycans (GAGs) with different sulfation patterns for the tunable release of cytokines. [2], [3]

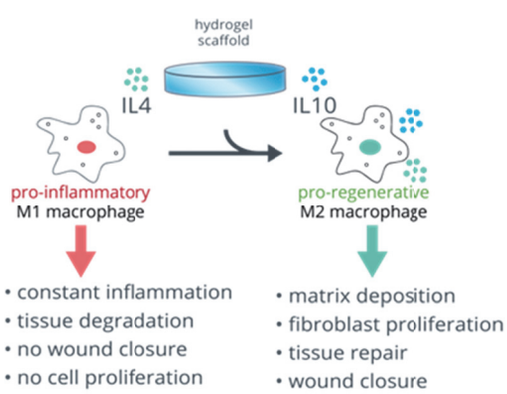


**Fig. 1:**
**Concept of starPEG-heparin hydrogel based immunomodulation of wound macrophages**

To characterize the release of the pro-regenerative cytokines, IL4, IL10 and IL13 were loaded to starPEG-GAG hydrogels at different concentrations and their release at 37°C in IMDM medium + 0.1% BSA was observed over 7 days by ELISA-techniques.

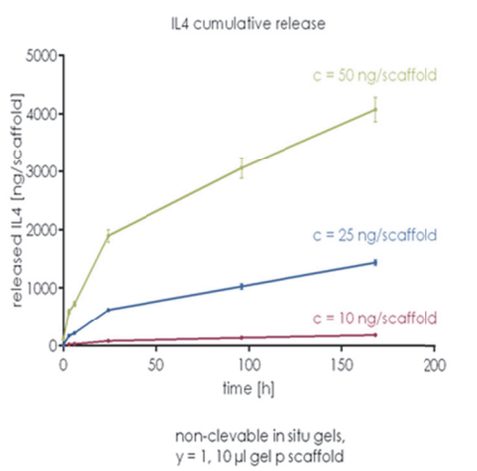


**Fig. 2:**
**IL4 release from starPEG-heparin hydrogels loaded with different concentrations**

IL4 and IL13 showed a similar release with an initial burst within the first 24 hours and a subsequent continuous slow release observed up to 168 hours. After 168 hours, a cumulative amount of about 8% of the loaded cytokine had been released from the hydrogel scaffolds. Since the release is directly correlated to the loading concentration, the initial loading could be used to tune the released amount (Figure 2, data for IL13 not shown). In contrast, preliminary results for IL10 show a much slower release (data not shown) of only 4% after 168 hours.
To test the effect of pro-regenerative cytokines released from the GAG-based hydrogels, bone marrow derived macrophages (BMDM) were isolated from C57BL/6J mice and challenged with 10 ng/ml LPS. Cytokine loaded starPEG-GAG hydrogel discs were added to the BMDM cultures without any direct contact to the cells. The culture was incubated for 72 h. Subsequently the cell culture supernatant was tested for inflammatory markers (Figure 3).

# Biologie-inspirierte Grenzflächen- und Materialgestaltung

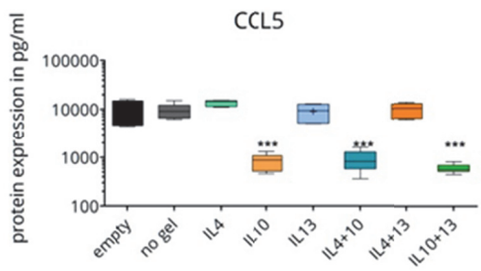


**Fig. 3 :**
**Protein expression of CCL5 after 72h culture with anti-inflammatory cytokine-loaded hydrogel discs**

Our data show that cytokine-loaded starPEG-GAG hydrogels can have a significant effect on the response of the macrophages to inflammatory cues. While hydrogels loaded with IL10 could significantly reduce the expression of several inflammatory markers, hydrogels loaded with either IL4 or IL13 strongly increased the expression of regenerative genes (data not shown). The combined hydrogel-based delivery of IL4 or IL13 together with IL10 caused a reduction of the inflammatory response and in parallel an increase in the regenerative behavior of the macrophages. In sum, the results show that interleukin-functionalized starPEG-GAG hydrogels effectively modulate the inflammatory response of macrophages in culture and may therefore create valuable new options for the treatment of chronic skin wounds.

Sponsor:
Transregio Sonderforschungsbereich (SFB) 67 "Matrixengineering" der Deutschen Forschungsgemeinschaft (DFG)

Co-operation:
Prof. J. Simon, Dr. U. Anderegg, Dr. S. Franz, Medizinische Fakultät der Universität Leipzig, Klinik für Dermatologie, Venerologie und Dermatologie

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# Biologie-inspirierte Grenzflächen- und Materialgestaltung

**Keywords**
**lipid bilayer membrane**
**Monte Carlo simulation**
**polymer adsorption,**
**polymer translocation**
**amphiphilic copolymer**
**balanced hydrophobicity**
**induced permeability**

## Localization and passive transport of copolymers interacting with lipid bilayers

Marco Werner, Hauke Rabbel, Jens-Uwe Sommer

In order to support the systematic development of non-toxic cargo systems into living cells, it is helpful to investigate the modes of self-assembly of macromolecules such as proteins, peptides or polymers in interaction with lipid membranes on a fundamental level. Therefore, we use a generic, dynamic Monte Carlo model to simulate coarse grained phospholipids, polymers, and solvent molecules, where short-range repulsive interactions between components of different hydrophobicity are implemented. The high computational efficiency of the model allows to directly count passive translocation events of the polymers across the membrane (Fig. 1, upper part).

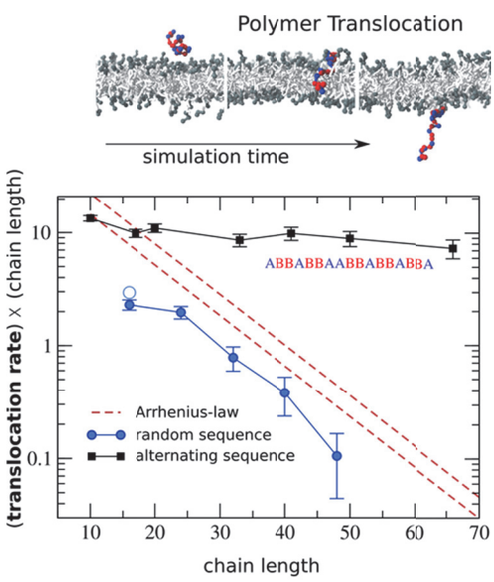



**Fig. 1:**
**Translocation rates of random copolymers and alternating copolymers at the balanced point (containing 60% *B*-type monomers) as functions of chain length [2].**

In previous work, we have analyzed the rate of translocations of *homopolymers*, where all chain monomers had been of the same type, and the hydrophobicity of the polymer had been adjusted by means of the hydrophobic interactions of each monomer. We found that there is a certain intermediate hydrophobicity of the polymer, where we observe a maximum of translocation rates [1].
These results are now compared with those for *random copolymers*, which are composed of hydrophilic (*A*) and hydrophobic (*B*) monomers. The average hydropbobicity of the *random copolymer* has been adjusted by the fractions of *A* and *B* monomers of the chain [2]. Also for these systems we find a maximum of translocation rates at an intermediate fraction of hydrophobic monomers.
Interestingly, both for homopolymers and for random copolymers we were able to demonstrate that the point of maximum translocation rates is controlled by a point of a balanced solubility of the polymer with respect to the solvent phase and the bilayer's core. This corresponds to the scenario, where the bilayer's core neither acts as a diffusion barrier nor as a potential trap for the polymer as a whole, and the polymer is equally soluble/insoluble in the bilayer's core and in solvent.
As compared to homopolymers, amphiphilic copolymers show considerably stronger localization close to the bilayer-solvent interface, where they can reorganize in order to increase the number of *A*- and *B*-type monomers located in their preferred environments. Copolymer adsorption at the bilayer-solvent interfaces reduces the translocation rate as compared to homo-polymers. By choosing particular sequences of *A*- and *B*-monomers consisting in small, homogeneously distributed blocks, however, polymer adsorption can be partly circumvented and translocation rates increase as compared to random *A*/*B*-sequences (see Fig. 1, lower part).

Polymers close to the point of balanced hydrophobicity induce perturbations in the lipid ordering and locally increase the lipid flip-flop rate. Dynamic and static perturbations induced by homopolymers as well as random copolymers lead to an increased permeability of the membrane with respect to solvent, see Fig. 2.

# Biologie-inspirierte Grenzflächen- und Materialgestaltung

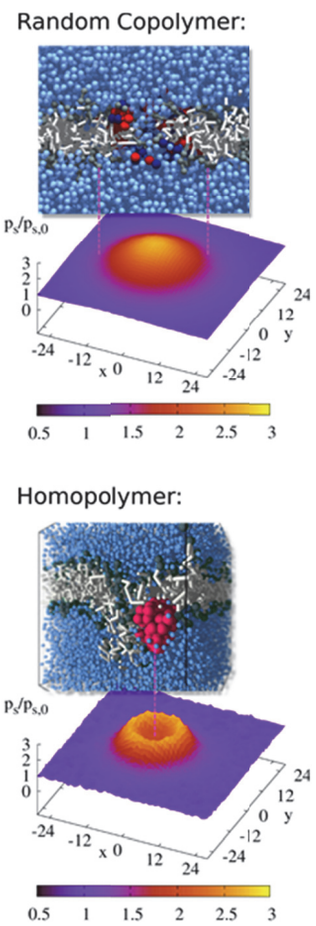
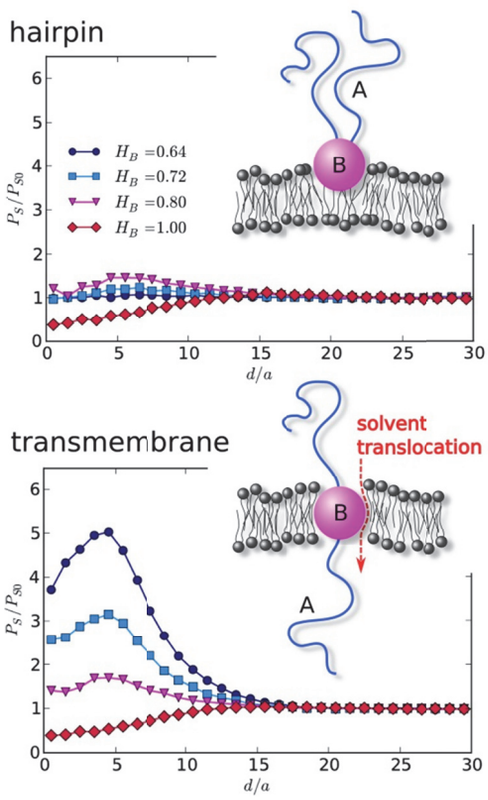



**Fig. 2:**
**Polymer-induced permeabilities, $P_s$, relative to the unperturbed membrane, $P_{s,o}$, for random copolymers and homopolymers of balanced hydropbobicity and as function of the projected distance from the chain along the bilayer midplane [1,2].**

Simulation results suggest that random copolymers induce transient pores of short life-time, whose position is mostly correlated to the center of mass position of the polymer. Homopolymers with a balanced hydrophobicity are neither compatible with solvent nor with the lipid tail phase. They collapse into droplet-like structures (globules), blocking solvent molecules. When located inside the membrane, permeability with respect to solvent is increased at the interface between the polymer globule and the lipid tail phase. This corresponds to a hollow-cylindrical transient pore. By linking two hydrophilic blocks to the polymer globule in a trans-membrane state, see Fig. 3, one can anchor the polymer in the bilayer and stabilize the transient pore for a longer time [3].




To conclude, our results underline the significance of a balanced hydropbobicity for polymer translocation as well as polymer-induced permeability. Furthermore, we reveal some aspects of the rich manifold of membrane-polymer interactions and self-assembly as function of amphiphilicity and the sequence of amphiphilic copolymers based on a generic model, which can be considered as basic functional modes of membrane-active proto-proteines and transient pores.


[1] M. Werner, J.-U. Sommer, V. A. Baulin, Soft Matter 46(8), 11714 (2012)
[2] M. Werner, J.-U. Sommer, Biomacromolecules 16 (1), 125 (2015)
[3] H. Rabbel, M. Werner, J.-U. Sommer, in preparation


**Fig. 3:**
**Polymer induced relative change of solvent permeability for triblock copolymers consisting of hydrophilic *A*-blocks and a *B*-block of adjustable hydrophobicity, $H_B$, as function of the projected distance, *d*, from the center of mass of the B-block. The balanced hydrophobicity of the B-block corresponds to $H_B$=0.68.**
**Induced permeability in the transmembrane state [3] is increased as compared to the hairpin state or the homopolymer [1].**