Biologie-inspirierte Grenzflächen- und Materialgestaltung

Prof. Dr. Carsten Werner
Tel.: 0351 4658-531
werner@ipfdd.de

Prof. Dr. Brigitte Voit
Tel.: 0351 4658-590
voit@ipfdd.de

Lebende Organismen nutzen rückgekoppelte molekulare Steuerungsmechanismen, um Funktionen und Stoffwechsel-Parameter unter sich ändernden Umgebungsbedingungen anzupassen. Synthetische Materialien verfügen dagegen bisher noch nicht über solche Regulationsmöglichkeiten. Wir konnten nun ein bioresponsives Hydrogel entwickeln, das die Blutgerinnung durch selbständig kontrollierte Freisetzung von Hemmstoffen effektiver unterdrücken kann als alle anderen Materialien, die derzeit klinisch verwendet werden (Nature Communications, dx.doi.org/10.1038/ncomms3168). Das Konzept soll künftig vor allem zur Beschichtung von Medizinprodukten genutzt werden, die im Blutkontakt zur Anwendung kommen. Hierzu bietet das 2013 zur Förderung im Programm Zwanzig20 ausgewählte BMBF Projekt RESPONSE – Partnerschaft für innovative Implantattechnologien (Koordination Universität Rostock) einen idealen Rahmen. Stammzellen sind in der Natur in eine extrazelluläre Matrix aus dreidimensional miteinander verknüpften Biomakromolekülen eingebettet. Um derartige Matrixstrukturen nachvollziehen zu können, wurde in Zusammenarbeit mit Kollegen der Technischen Universität Dresden (Medizinische Fakultät und Zentrum für Regenerative Therapien) im Rahmen des 2013 verlängerten Sonderforschungsbereichs 655 der DFG (From Cells to Tissues) eine Methode entwickelt, mit der die von Zellkulturen produzierte extrazelluläre Matrix auf Oberflächen verankert werden kann. Dieses Prinzip wurde für die Kultur von Blutstammzellen aus dem Knochenmark des Menschen angewandt, die so etwa dreimal schneller vermehrt werden konnten als mit bisher dafür angewandten Kulturbedingungen. Die neue Methodik bietet vielfältige Möglichkeiten zur Entschlüsselung von Signalen, die Stammzellen aus verschiedenen Geweben durch ihre Mikroumgebung steuern lassen und könnte künftig auch für zellbasierte Therapien genutzt werden (Nature Methods, dx.doi.org/10.1038/NMETH2523).

Regenerative Therapien setzen für die Neubildung von Gewebe und Organstrukturen mehr und mehr auf endogene Mechanismen. Zu deren Aktivierung werden Polymer-Matrixsysteme benötigt, die sich erst im Zielgewebe aus löslichen Prekursoren bilden. Ein neues, sehr vielseitiges Prinzip zur Erzeugung multifunktioneller Glycosaminoglycan-Polyethylenglykol-Hybridnetzwerke über Michael-Type-Additionsreaktionen konnte durch regioselektive Peptidfunktionalisierung beider Polymerkomponenten entwickelt werden (Advanced Materials, dx.doi.org/10.1002/adma.201300691). Derartige in situ Matrices stehen auch im Fokus der Forschungen zu immunmodulatorischen Systemen für die dermale Wundheilung, die seit Juni 2013 im Sonderforschungsbereich TR67 der DFG Biomaterialien zur Steuerung von Heilungsprozessen realisiert werden können.

Unter den 2013 abgeschlossenen Graduierungsarbeiten war die mit dem Prädikat „summa cum laude“ ausgezeichnete Dissertation „Tissue engineering of the human corneal endothelium“ von Juliane Teichmann. Zwei erfolgreiche Nachwuchswissenschaftler aus dem Ausland haben sich 2013 entschieden, ihre Arbeit am IPF fortzusetzen: Laura Bray von der Queensland University of Technology, Brisbane, ausgezeichnet mit dem Prime Minister's Queen Elizabeth II Diamond Jubilee Award, und Benjamin Newland von der National University of Ireland, Galway, gefördert durch ein Stipendium des Wellcome Trust, vertiefen für jeweils zwei Jahre die Forschungen des Instituts zu 3D-Kulturmodellen für Tumorzellen sowie zu neuen Behandlungsmethoden für neurodegenerative Erkrankungen.

Gemeinsam mit B CUBE, dem Zentrum für Molekulare Bioingenieurswissenschaft der Technischen Universität Dresden, konnte im September 2013 die Konferenz Engineering Life mit Beiträgen international herausragender Wissenschaftler der Gebiete Biomaterialien, Biophysik und Synthetische Biologie organisiert werden.

Biologie-inspirierte Grenzflächen- und Materialgestaltung

Separation und Wechselwirkungs-untersuchungen von dendritischen Biopolymeren

Susanne Boye, Dietmar Appelhans, Albena Lederer

Die Charakterisierung von dendritischen Biopolymeren und deren Wechselwirkungen untereinander oder zu anderen Molekülen stellt aufgrund der komplexen Zusammensetzung der Probensysteme immer wieder eine Herausforderung dar. Besonders die Standardmethode zur Molmassenbestimmung, die Größenausschlusschromatographie (SEC), gelangt dabei sehr häufig an ihre Grenzen. Gründe dafür sind u.a. die Multifunktionalität dendritischer Polymere und deren Neigung zu adsorptiven Wechselwirkungen mit der stationären Phase. Weiterhin herrschen innerhalb der Trennsäule sehr hohe Scherkräfte, die bei der Untersuchung von dendritischen Biopolymeren und deren Aggregaten oder Biohybridstrukturen zu Degradation der nichtkovalenten Bindungen führen. Aus diesem Grund haben wir für die Auftrennung die Asymmetrische Fluss-Feldflussfraktionierung (AF4) in Kombination mit Lichtstreudetektion (LS) gewählt. Hierbei findet die Auftrennung im Inneren eines schmalen Kanals ohne stationäre Phase statt, indem durch verschieden wirkende Flüsse die Polymere hinsichtlich ihrer Diffusionseigenschaften separiert werden.
Durch ihre Vielseitigkeit und den sehr hohen Trennbereich bietet die AF4-LS die Möglichkeit, sowohl Einzelmoleküle als auch Aggregate oder Hybridstrukturen voneinander zu separieren und zu analysieren. An dieser Stelle möchten wir drei verschiedene Herangehensweisen bei Wechselwirkungsuntersuchungen von dendritischen Glykopolymeren (verzweigte Polymere mit Maltosehülle) erläutern:
1. Auftrennung und Charakterisierung von Einzelmolekülen und Aggregaten:
Gegenstand dieser AF4-LS Untersuchungen sind hochmolekulare Polymere mit Lysin-Dendronen und einer dichten Maltoseschale, die aufgrund der hohen Anzahl an funktionellen Gruppen sehr stark zur Bildung von intermolekularen Wechselwirkungen, wie z.B. H-Brücken neigen. In systematischen Studien wurde der Einfluss der Dendronen-Generationszahl, des pH-Wertes und der Konzentration auf die Molmassen, Radien und Konformationseigenschaften mittels AF4 und gekoppelter Detektion näher betrachtet. Da es mit der AF4-LS möglich ist, sowohl Einzelmoleküle als auch Aggregate zu detektieren, konnte nachgewiesen werden, dass der pH-Wert die Struktur der einzelnen Polymermoleküle beeinflusst und damit das Aggregationsverhalten steuert (Abb. 1) [1].

Keywords
biopolymers
bioconjugates
guest-host-systems
field-flow-fraktionation
light scattering
conformation

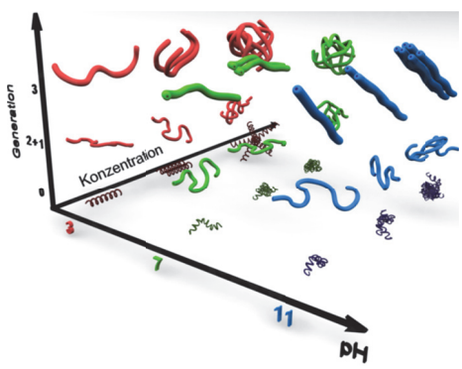

Abb. 1:
Übersicht über die schematischen Molekülstrukturen der Polylysin-dendronisierten Glykopolymere als Einzelmoleküle und Aggregate bei verschiedenen pH-Werten und verschiedenen Konzentrationen

2. Auftrennung und Charakterisierung der Komponenten eines Biokonjugates:
Bei der Untersuchung von Biohybridstrukturen, die auf nichtkovalenten Bindungen zwischen biotinylierten Glykodendrimeren (GD, Polypropylenimin) und Avidin basieren, stellte sich heraus, dass die Herstellung von monodispersen Systemen nicht möglich ist. In jedem Fall herrscht eine Mischung von verschiedenen Komponenten vor. Diese komplexen Systeme wurden erstmals mittels AF4-LS separiert und die einzelnen Bestandteile den jeweiligen Verbindungen zugeordnet und quantifiziert. Dabei wurde der Einfluss u.a. der Biotinligandenanzahl, des Abstandes zwischen GD und Biotin, oder des molekularen Verhältnisses (Avidin: GD) betrachtet. Ein Beispiel einer Biohybridstruktur, gebildet aus Avidin und bivalentem GD (Verhältnis 1:3), ist in der Abb. 2 dargestellt. Es wurde nachgewiesen, dass in dem System sowohl ungebundene Bestandteile als auch definierte Architekturen und komplexe Nanostrukturen mit sehr hohen Molmassen vorhanden sind [2]. In Kombination mit statischer Lichtstreuung und Informationen über den Diffusionskoeffizienten wurde auch eine Aufklärung der Konformationsverteilung möglich.

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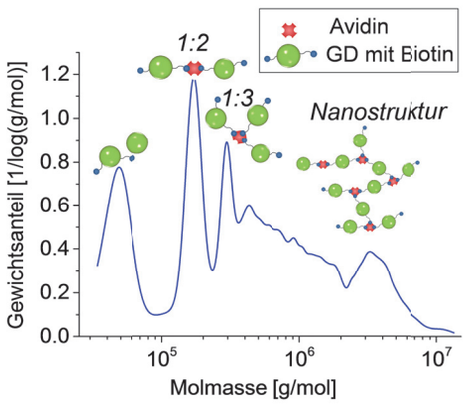

Abb. 2:
Zusammensetzung der Biohybridstrukturen gebildet aus Avidin und Glykodendrimeren (GD, mit zwei Biotinliganden) im Verhältnis 1:3 bestimmt mittels AF4-LS

3. Quantifizierung von Komplexierungskapazität bei Gast-Wirt-Komplexen:
Im Gegensatz zur Bildung von Aggregaten oder Hybridstrukturen sind die Moleküldimensionen bei Aufnahme- oder Freisetzungsstudien von Gastmolekülen in dendritischen Glykopolymeren häufig geringer. Hinzu kommt, dass häufig eine Mischung aus freien und vom Polymer aufgenommenen Gastmolekülen vorliegt. Um die komplexierten Gastmoleküle zu quantifizieren, haben wir zwei verschiedene Herangehensweisen mittels AF4-LS entwickelt: (i) über den Molmassenzuwachs des Gast-Wirt-Komplexes im Vergleich zum reinen Glykopolymer die Zahl der komplexierten Gastmoleküle berechnet. Ist der Anteil an aufgenommen Molekülen zu gering, um mittels LS detektiert zu werden, gibt es die Möglichkeit der (ii) direkten Quantifizierung der Gastmoleküle. Unser Weg beruht auf der Abtrennung der freien Gastmoleküle mittels simultaner Filtration. Dazu wird der Ultrafiltrationseffekt der AF4 genutzt, bei dem der senkrechte Lösungsmittelfluss aus dem Trennkanal durch eine Membran heraus transportiert wird. Durch die Poren der Membran können die kleinen, freien Gastmoleküle abgetrennt und quantitative detektiert bzw. quantifiziert werden. So lässt sich dann bei bekannter Gastmolekülgesamtmenge der komplexierte Anteil berechnen. Bei ersten Testmessungen mit einem hoch verzweigten Glykopolymer und einem Farbstoff korrelierten die Ergebnisse der beiden Methoden sehr gut miteinander [3].
Aktuelle Untersuchungen an Polymer-Wirkstoffsysteme sollen zeigen, wie effektiv sich die simultane Komplexgrößenbestimmung und -quantifizierung auf diese Systeme übertragen lässt.

Kooperation:
Prof. L. Nilsson University Lund

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Anticoagulant hydrogels with responsiveness to different coagulation enzymes

Manfred Maitz, Jan Zitzmann,
Claudia Renneberg, Uwe Freudenberg,
Mikhail Tsurkan, Carsten Werner

Activation of the blood coagulation by blood-contacting devices such as vascular stents or venous catheters is a persisting problem in medicine. Various surface modification strategies including surface passivation and immobilization of anticoagulant molecules have been explored to address the resulting challenges, however, with limited success. Exploring an entirely new concept, we have recently developed a bioresponsive coating, which releases the coagulation inhibitor heparin in response to the activation of the coagulation process when exposed to blood (Fig. 1)[1,2]. Anticoagulant feedback control is achieved by a gel system consisting of heparin crosslinked with a four-armed poly(ethylene glycol) (starPEG) by linker peptides specifically cleaved by the coagulation enzyme thrombin. Cleavage of the linker releases heparin to the liquid phase, where it exerts a massively enhanced anticoagulant activity. The thrombin-cleavable hydrogel outcompetes clinically applied heparin coatings and may offer exciting options for reducing the drug load to the patient and, thus, minimizing side effects in the clinical application of gel-coated products.

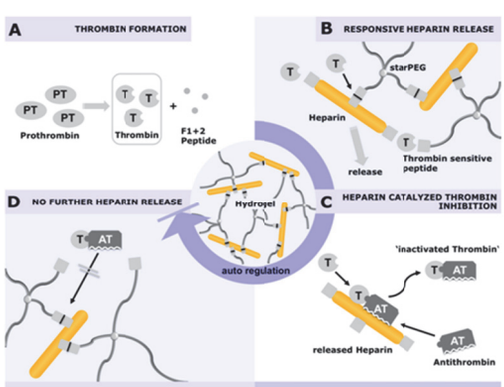

Fig. 1:
Feedback control loop obtained with a thrombin-responsive heparin-releasing hydrogel

Thrombin, the trigger enzyme for the cleavage of this hydrogel, is a 'late' protease in the coagulation cascade reaction. As a consequence, heparin is released from this gel only, when the coagulation reaction is fully activated. Numerical simulations of the coagulation process were performed confirming the assumption that an earlier release of coagulation inhibitor may improve the anticoagulant effect of the gel system. For the experimental verification, hydrogels with comparable network properties were prepared from heparin, starPEG and peptides, which can be either cleaved by the earlier activated coagulation factors kallikrein (Kal-resp), by factor Xa (FXa-resp) or by thrombin at lower or higher rate compared to the previously established, Thrombin-cleavable peptide (Thr-fast, Thr-slow, Thr-std). Coagulation enzyme-mediated gel degradation was verified in buffered solutions of the respective coagulation factors at a concentration of 45 nmol/l each (Fig. 2). The peptides were designed considering the plasma concentration levels of the respective enzymes, i.e. peptides cleaved by the less abundant 'earlier' enzymes were adjusted to exhibit higher turnover rates to ensure the release of pharmacologically active amounts of heparin. There was only minimal cross-reactivity, i.e. the peptides were found to be specifically cleaved by one coagulation factor each.

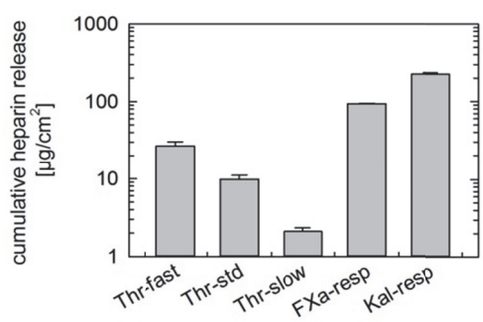

Fig. 2:
Cumulative heparin release from a set of coagulation factor-responsive starPEG-heparin hydrogels crosslinked by different peptides. Gels were exposed for three hours to a solution of the respective coagulation factors.

For an application-oriented evaluation, the hydrogels were exposed to freshly drawn, low dose anticoagulated human whole blood at 37°C. To compare the anticoagulant capacity, the hydrogels were co-incubated with highly pro-coagulant surfaces (binary self-assembled monolayers of 80 % carboxyl-terminated and 20 % methyl terminated alkanethioles on gold) [3]. After two hours incubation, the pro-

Keywords
hydrogel
drug release
feedback control
anticoagulation
hemocompatibility

thrombin F1+2 fragment, a quantitative marker of the plasmatic coagulation and the released heparin concentrations were determined (Fig. 3 A). Hydrogels with accelerated degradation by thrombin (Thr-fast) and kallikrein-responsive hydrogel (Kal-resp) suppressed the coagulation better than the standard thrombin-responsive hydrogel ($p \leftarrow 0.05$), whereas the slow degrading hydrogel exhibited less anticoagulant activity. The hydrogel, which responds to the more upstream coagulation factor FXa had the same anticoagulant capacity as the reference thrombin responsive hydrogel. Quantification of the released heparin (Fig. 3 B) indicates that the kallikrein-responsive hydrogel obtained its good thromboprotective characteristics mainly by the high overall heparin release, which can be attributed to the high sensitivity of the hydrogel to the protease and a low regulating effect of heparin on kallikrein. In contrast, the FXa responsive hydrogel showed only 60 % of the heparin release of the reference hydrogel but produced a similar anticoagulant effect. Apparently, heparin release from bio-responsive hydrogels triggered by earlier activated coagulation factor (FXa) affords anticoagulant effects at lower heparin levels. As a valuable advantage for application, this may allow further minimization of the patient's heparin exposure. At the same time, this hydrogel shows slower consumption and, thus, longer lasting activity. In sum, choosing peptide-crosslinkers cleaved by different coagulation enzymes allows for customizing heparin-releasing hydrogels to respond to different stages of the coagulation cascade for maximizing the anticoagulant effect and for minimizing the required amount of released heparin. These findings further extend the potentialities of the introduced class of coagulation-activated anticoagulant materials.

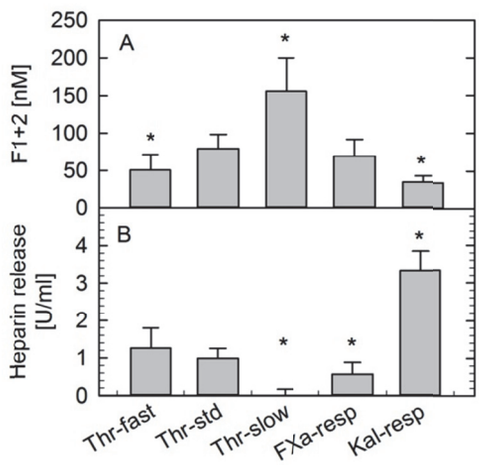

Fig. 3:
(A) Blood coagulation and (B) heparin release observed at incubation of various coagulation responsive hydrogels with whole blood.

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Biologie-inspirierte Grenzflächen- und Materialgestaltung

Biomolecular interactions in hydrogels detected by electrokinetic measurements and surface plasmon resonance

Ralf Zimmermann, Susanne Bartsch, Carsten Werner

Materials mimicking the exogenous signals of extracellular matrices to control cell fate decisions are instrumental for tissue engineering [1,2]. Biohybrid hydrogels containing glycosaminoglycans (GAGs) are particularly promising for that purpose due to the effective binding, protection and sustained release of numerous growth factors [3]. Growth factor binding to GAGs can be attributed to the sulfation pattern of these molecules and is, thus, largely determined by electrostatic interactions between ionized sulfate groups along the GAGs and oppositely charged protein domains. Accordingly, variations of the system net charge resulting from the binding of growth factors might be reflected in the electrokinetic fingerprint of hydrogels, allowing for the electrokinetic detection of growth factor binding.
Aiming at novel electrokinetic sensors for the detection of biomolecular interactions within GAG-based hydrogel materials we employed electrokinectic (streaming potential/current) and surface plasmon resonance (SPR) measurements [4] to analyze the binding of the model protein lysozyme within biohybrid hydrogels consisting of covalently linked star-shaped poly(ethylene glycol) (starPEG) and heparin [5]. Lysozyme was selected as it exhibits a size similar to relevant growth factors and a positive excess charge at physiological pH values. Thin films of the gel material were immobilized on transducer surfaces. The films were shown to be negatively charged due to the ionization of the sulfate and carboxylic acid groups of heparin. The excess conductivity of the hydrogel films (as determined by the electrokinetic measurements) was analyzed applying a recently developed mean-field-approach [6]. The heparin concentration and the crosslinking degree of the hydrogel films were found to be similar to the values for comparable bulk hydrogel materials [5]. The binding of lysozyme to heparin in the hydrogel was analyzed by measurement of the excess conductivity after incubating the films with protein solutions of concentrations between 0.1 and 100 mg/mL taking into account the binding kinetics studied by SPR. The excess conductivity of the hydrogel films significantly decreased with increasing protein concentration within the studied concentration range (Fig. 1). The latter effect can be attributed to the decrease of the number of mobile counter ions in the gel by the electrostatic binding of lysozyme to heparin. It was further shown that lysozyme can be released from the heparin by incubating the hydrogel films with highly concentrated electrolyte solutions. The reversibility of the binding process allows for reuse of the hydrogel films in sensoric applications.
Ongoing studies aim at exploring the binding and release of growth factors of different affinity and size to starPEG-heparin hydrogel films prepared at different molar ratios and using partially desulfated heparin. Furthermore, the miniaturization of the measuring principle and its integration into a lab-on-a-chip system will be investigated.

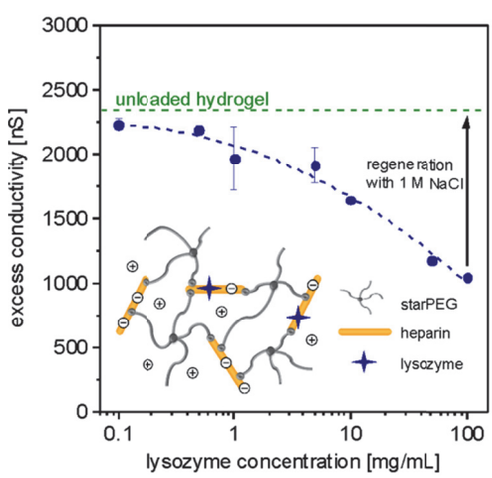

Fig. 1:
Excess conductivity of a starPEG-heparin hydrogel film (ratio starPEG/heparin = 3) in 1 mM KCl solution before and after incubation with different amounts of lysozyme. After incubation with 100 mg/mL lysozyme the gel film was regenerated using a 1 M NaCl solution. The inset illustrates the molecular structure of the gel. For details on gel formation and properties see [5].

Keywords
biohybrid hydrogel
growth factor
electrokinetics
excess conductivity
biosensor
SPR

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Keywords
extracellular matrix
mesenchymal stem cells
haematopoietic stem cells

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Cooperation:
Dr. Juliane Posseckardt,
Prof. Dr. Michael Mertig,
Kurt-Schwabe-Institut für Mess- und Sensortechnik e.V. Meinsberg

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Culture of human hematopoietic stem cells on decellularized cell-derived matrices

Marina Prewitz, F. Philipp Seib,
Malte von Bonin, Jens Friedrichs, Aline Stißel,
Martin Bornhäuser, Carsten Werner

The therapeutic potential of human bone marrow mesenchymal and hematopoietic stem and progenitor cells (MSC and HSPC, respectively) offers great promise for their use in regenerative medicine. A key regulator of these cells is their extracellular matrix microenvironment. However, a major obstacle in defining the exact role of extracellular matrices (ECM) is the lack of suitable methods that recapitulate complex ECM microenvironments in vitro. Our current work therefore concerned a methodology for the reliable anchorage of decellularized extracellular matrices generated from cultured bone marrow MSC to mimic the bone marrow stem cell microenvironment. Cell-secreted ECM was immobilized to culture carriers via maleic anhydride copolymer layers used for covalent binding of fibronectin to the culture carrier surface. Fibronectin was selected for its properties to interact with cell-secreted extracellular matrix proteins, so that culture and decellularization of a cell monolayer on top yields in a superior anchorage of cell-secreted ECM with the opportunity to study the decellularized extracellular phenotype of various cell-derived matrices. The ECM preparations were thoroughly characterized to identify their molecular composition, supra-structural features and nano-mechanical properties.
The obtained MSC-derived ECM substrates served as in vitro culture environments for human HSPC, resulting in a significant increase in proliferation of these cells over a culture period of 7 days, with up to 3-fold expansion of CD34+ cells on MSC-derived matrices without exhaustion of CD34/CD133 double positive cells, and with the potential for long-term (20 weeks) engraftment into NSG (NOD/SCID/IL2 receptor {gamma} chain (null)) mice.
Taken together, we demonstrated the unique ability of cell-derived ECM scaffolds to support expansion and differentiation of bone marrow stem cells in vitro.

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Beyond that, the established methodology enables deciphering and modulating native-like multicomponent ECMs from various cell sources offering exciting options for the in-depth analysis of stem cell regulation by exogenous cues.

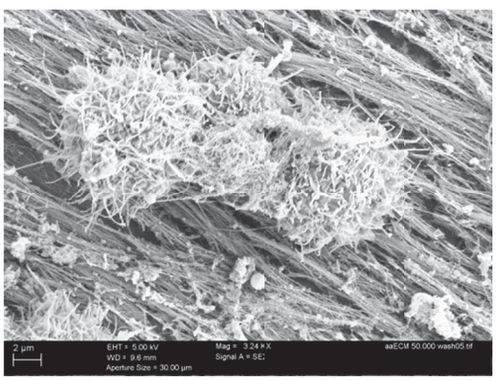

Fig. 1:
Human haematopoietic stem cells in contact with the extracellular matrix that was deposited by human bone marrow mesenchymal stem cells in vitro.

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Cooperation:
Prof. Dr. M. Bornhaeuser,
Universitätsklinikum Dresden

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Multifaceted, in situ forming polymer matrices to induce cell morphogenesis

Mikhail V. Tsurkan, Karolina Chwalek ,
Silvana Prokoph, Andrea Zieris,
Kandice R. Levental, Uwe Freudenberg,
and Carsten Werner

Keywords
hydrogel
biomaterials
growth factors
cell adhesive peptides

Polymer matrices allowing for the precise and independent control over multiple biochemical (biodegradation, presentation of adhesion ligands and soluble effectors) and physical network parameters (mesh size, stiffness) are of particular interest in current bioengineering [1]. Fast self-assembly of such matrices in presence of cells or upon injection into a target tissue is crucial for the three-dimensional stimulation of cells by well-defined material-properties in vitro or in vivo. Several design principles have been explored to create in situ forming materials from synthetic polymers such as poly(ethylene glycol), peptides and glycosaminoglycans (GAGs) [2]. However, previously reported approaches were limited with respect to the control over the polymer network characteristics and did not allow for incorporating hydrophobic signaling molecules. To overcome these limitations, an orthogonal synthesis strategy was developed to produce and characterize a set of PEG-peptide- and glycosaminoglycan-peptide conjugates (Fig. 1) [3]. Stepwise functionalization of starPEG with several peptides resulted in PEG-peptide conjugates displaying multiple functionalities, including different cell adhesion ligands (RGD, SIKVAV) and enzymatically (MMP) cleavable cross-linking units, allowing for the implementation of hydrophobic signaling peptides such as SIKVAV. The GAG building block (heparin) was converted to contain different quantities of maleimide groups to allow for subsequent click reaction to form polymer networks with the above-mentioned PEG-peptide conjugates. A multistep purification technique resulted in ultra-high purity →99.5 % heparin-maleimide conjugates with preserved biological functionality as demonstrated by the reaction with RGD peptides and by the non-covalent conjugation of vascular endothelial growth factor (VEGF). Reacting PEG-peptide and heparin-maleimide-(peptide) conjugates well defined 3D-networks were obtained with

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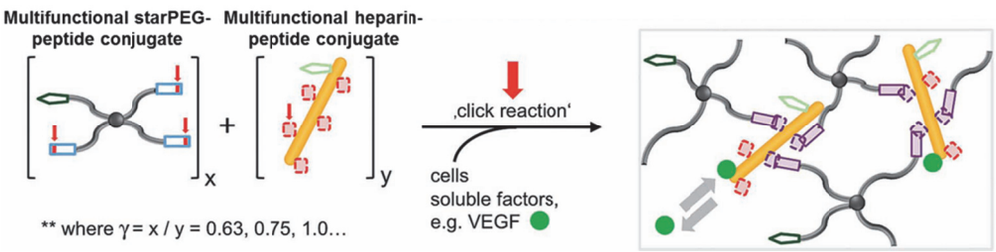

Fig. 1:
Synthetic scheme for the formation of multifunctional in situ forming starPEG-heparin hydrogels (□ = molar ratio of StarPEG/heparin)

independently tunable cell adhesiveness, enzymatic degradability and elasticity. Multi-domain peptide conjugates combining different biological functions and their assembly into cell-instructive hydrogel materials were demonstrated to be suitable for triggering primary human vascular endothelial cells and chicken dorsal root ganglia in 3D cultures (Fig. 2).
The established biohybrid matrix system is concluded to be highly promising for deciphering the response of cells to complex exogenous signals in vitro and in vivo.

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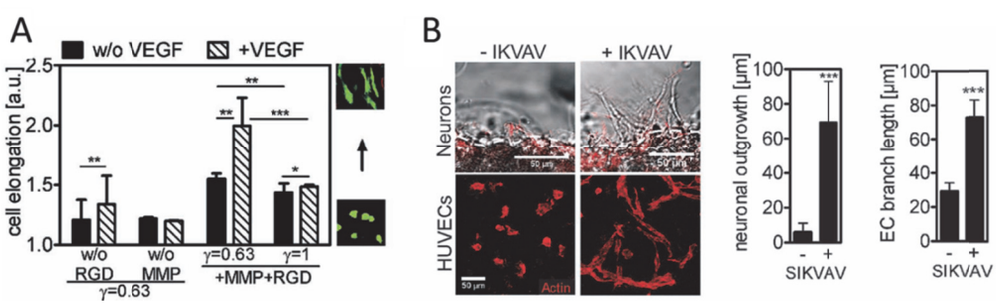


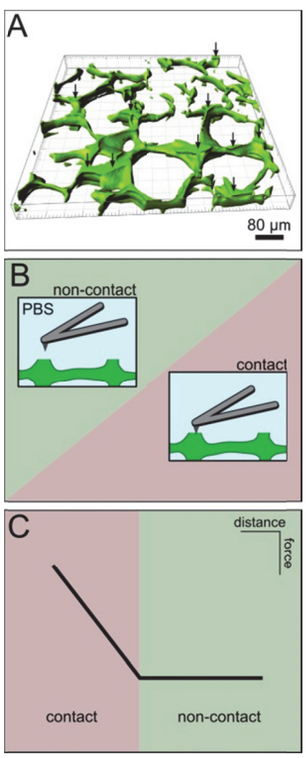
Fig. 2:
(A) Cell elongation (aspect ratio, 1 corresponds to round cell, → 1 corresponds to an elongated morphology) in hydrogels is regulated by cross-linking density γ (molar ratio of StarPEG/heparin = 0.63 vs. γ = 1) and the biomolecular modification of the hydrogels (± RGD, ± MMP-peptide, ± VEGF), (B) SIKVAV peptide promotes EC morphogenesis and neurite outgrowth from the chicken dorsal root ganglia, ± SD.

Biologie-inspirierte Grenzflächen- und Materialgestaltung

Cryogel micromechanics unraveled by atomic force microscopy-based nanoindentation

Jens Friedrichs, Petra B. Welzel, Milauscha Grimmer, Steffen Vogler, Uwe Freudenberg, Carsten Werner

Polymeric macroporous cryogel scaffolds are being developed as three-dimensional analogues of the extracellular matrix for tissue engineering applications. Cryogels are obtained by cooling an aqueous reaction mixture of polymeric gel precursors to sub-zero temperatures. Thereby, two phases are formed: ice crystals and a non-frozen liquid microphase. The gel precursors are concentrated in the liquid microphase. After cryogel formation, the ice crystals can be removed leading to sponge-like structures with interconnected macropores (Fig. 1 A). Compared to bulk hydrogels without macropores, the increased precursor-concentration in the non-frozen liquid microphase of cryogels results in denser polymer networks surrounding the macropores, which is designated as cryoconcentration effect. Due to the cryoconcentration effect, the struts of the sponge-like cryogels can be assumed to exhibit a higher local stiffness than the corresponding bulk hydrogel materials obtained from the same reaction mixture at room temperature. However, this was not demonstrated experimentally, yet. Therefore, we developed and applied an atomic force microscopy-based nanoindentation method to quantify the local mechanical properties of biohybrid glycosaminoglycan-poly(ethylene glycol) cryogels [1,2]. The analytical approach uses very thin sections of the swollen cryogels and thus allows for visualizing the cryogel microarchitecture during nanoindentation by light microscopy (Fig. 1 A). This enables the exact positioning of the AFM tip directly over the struts of open pores (Fig. 1 B). Comparison of mechanical properties of cryogel struts with those of reference bulk hydrogels obtained from the same reaction mixtures at room temperature directly proves, for the first time, that cryoconcentration effects result in higher stiffness of the cryogel pore walls. Combining AFM-based nanoindentation with uniaxial stress-strain compression experiments furthermore provides valuable insights about the effect of cryogelation parameters on integral and local scaffold mechanics allowing for a far-going customization of the cryogels to match a particular tissue elasticity and to induce a desired cellular response.



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Keywords
cryogel
AFM
Nanoindentation

Fig. 1:
(A) Confocal microscopy image of the sponge like microstructure of the PBS swollen cryogel scaffold. Green: cryogel struts. White: PBS filled interconnected pores. Arrows indicate where AFM-based nanoindentation was performed.
(B) Schematic representation of the AFM-nanoindentation measurement principle. The tip of an AFM cantilever is brought into contact with a cryogel strut covered with PBS to probe their local mechanical properties in the swollen state. The applied stress induces a deformation of the tested material proportional to the applied force and the stiffness of the tested region. During the nanoindentation experiments the force exerted on the cantilever as well as cantilever movement are recorded and can be plotted as a force-distance curve (C). Shading represents regimes before and after contact. From the force-distance curve the mechanical properties of the tested material can be extracted.

Biologie-inspirierte Grenzflächen- und Materialgestaltung

Keywords
electrophoresis
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confinement

Electrophoretic transport of charged polymers in cylindrical geometries

Sorin Nedelcu, Jens-Uwe Sommer

Taking as an example the model polyelectrolyte DNA, it is important to know what the sequence of the base pairs in a DNA strand is. To read the full sequence it is typically split into smaller fragments which are sequenced multiple times to find overlapping regions, and later reassembled together. In an electric field, in free solution long charged polymers move with the same velocity irrespective of their size (except for very short fragments in the oligomer range), which makes it difficult to separate them in laboratory setups.

In a first set of simulations, the focus of our work has been on electrophoresis of polyelectrolyte chains in slit geometries [1]. In these simulations, we used longitudinal (parallel to the walls) and transverse applied electric fields (Fig. 1, top).

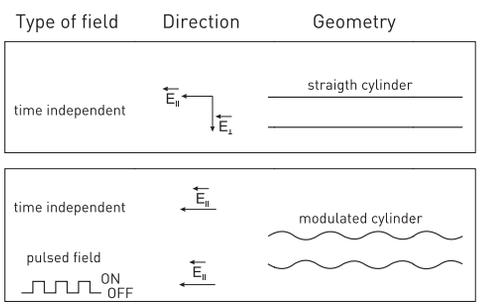

Fig. 1:
Schematic despiction of the type, direction of applied external electric field, and corresponding geometry (in cross-section).

The transverse field led to an asymmetric ion atmosphere around the polyion and modified the net charge of the chain by the number of condensed counterions. Longer polyions were observed to move faster than shorter ones resulting in selective electrophoretic separation. A second set of simulations considered straight cylinder geometries with tapered constrictions [2] (Fig. 1, bottom). In a time-constant applied field in the axial direction, the chain escape time from one cavity into the next could be written as follows

$$\langle \tau \rangle \sim \frac{A}{B^2} \exp\left[A^2 l/(4B)\right] \qquad \text{(Eq. 1)}$$

where the constants A and B depend on the applied electric field, polyelectrolyte length, and characteristic dimensions of the compression-expansion chambers [2].

Two conclusions of our work are important. First, if the longitudinal applied field is kept constant (no change in direction or value), then the array of compression-expansion chambers are more effective in separating charged polymers than straight uniform diameter cylinders. The second conclusion is that the separation efficiency increases even further when the constant field is switched on and off periodically (or otherwise in pulsed applied fields). These findings are summarized in Fig. 2.

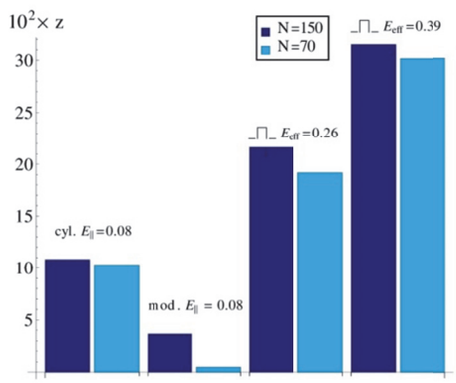

Fig. 2:
In a set time interval, charged chains of different length may move a certain distance z. If the elution order is always the same, one can identify the chains by observing their positions. The bar diagram above compares the distances z for two chains of length N=150 and N=70 in constant (noted cyl.) and variable diameter (noted mod.) cylinders under constant applied fields $E_{||}$, and similar in modulated cylinders (noted mod.) under applied pulse fields of relative strength Eeff (= $E_{||}$ /3). The quantities are reported in Lennard-Jonnes units.

It indicates that the best separation resolution (measured as the difference between the z-values) is obtained in tapered cylinders in constant applied fields, while the fastest separation takes place in tapered constrictions under pulsed electric fields.

Lastly, we studied were straight cylinders with wavy surfaces [3]. Here, one could explicitly measure the fluid flow inside and outside the polyelectrolyte coils, and electrolyte friction.

The ultimate goal of such complex computer experiments is to find a way of individually controlling the dynamics of charged chains, by smart choices of the type of environment, properties of the buffer solution, and applied electric fields.

Sponsor:
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