Biologie-inspirierte Grenzflächen- und Materialgestaltung

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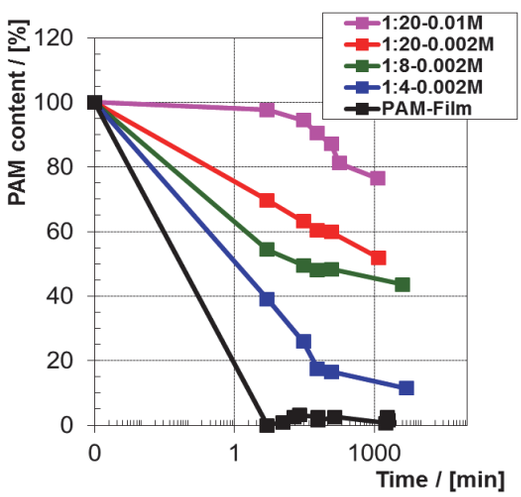
Biologie-inspirierte Polymersysteme machen bisher unerreichte Materialeigenschaften zugänglich, die es ermöglichen neue technologische Herausforderungen zu beantworten. Im Jahr 2012 konnten wir mit Hilfe einer neuen Nanoreplikationsmethode zeigen, wie durch das Zusammenwirken von chemischer Konstitution und skalenübergreifender Strukturierung an der Cutikula von Collembolen Benetzung und mikrobielle Besiedlung effektiv verhindert werden können (NPG Asia Materials 2013) und wie sich das Bauprinzip der anti-adhäsiven Haut mit theoretischen Ansätzen generalisieren lässt (Langmuir 2013). Um die Adhäsion von Mikroorganismen an funktionalisierten Materialoberflächen quantitativ bewerten zu können, wurde eine Rasterkraftmikroskopie-Methode etabliert und zur Untersuchung der anti-adhäsiven Wirkung immobilisierter Enzyme auf marine Bakterien angewandt (Macromolecular Rapid Communications 33 (2012) 1453-1458). Diese und andere Themen werden in enger Zusammenarbeit mit dem Zentrum für Innovationskompetenz B CUBE an der TU Dresden verfolgt.
Die Entwicklung von speziellen analytischen Ansätzen und die Nutzung theoretischer Methoden erwiesen sich auch bei Forschungsarbeiten zu Biohybrid-Polymeren für regenerative Therapien als vorteilhaft. Nachdem bereits mit Kraftfeldmethoden Möglichkeiten zur Entkopplung von mechanischen und biomolekularen Matrixeigenschaften für die gezielte Stimulation von Stamm- und Vorläuferzellen identifiziert werden konnten (Advanced Functional Materials 22 (2012) 1391-1398), wurden weiterführend mit Hilfe eines neuen elektrokinetischen Analyseverfahrens die Ladungs- und Netzwerkeigenschaften der entsprechenden Glycosaminoglycan-Polyethylenglykol-Gele quantitativ erfasst (Analytical Chemistry 84 (2012) 9592-9595). Das so noch besser erschlossene Matrixsystem konnte durch Sekundärfunktionalisierung vielfältig angepasst und so im Rahmen des Zentrums für Regenerative Therapien Dresden (CRTD) und darüber hinaus in translationalen Projekten genutzt werden. Beispielsweise konnte in Kooperationen mit Stefanie Dimmeler, Zentrum für Kardiologie der Universität Frankfurt, und Annette Beck-Sickinger, Institut für Biochemie Universität Leipzig, gezeigt werden, dass durch entsprechende Gele erzeugte Konzentrationsgradienten des chemotaktischen Zytokins SDF1 die migratorische Aktivität von humanen endothelialen Vorläuferzellen effektiv steuern lassen (Biomaterials 33 (2012) 4792-4800, Journal of Controlled Release 162 (2012) 68-75). Mit Hilfe einer speziellen Cryo-Gelierungsmethode konnten weiterhin Biohybridgel-Matrices mit Porenstrukturen im Mikrometerbereich für die dreidimensionale Stimulation von Zellen entwickelt werden (Biomacromolecules 13 (2012) 2349-2358).
Zu den 2012 eingeworbenen Projekten gehören das EU-Projekt „Bioactivated hierarchical hydrogels as zonal implants for articular cartilage regeneration (HYDROZONES)“ und das DFG-Projekt „Mechanisch stabile anti-adhäsive Polymeroberflächen durch biomimetische Strukturierung“ (mit A. Lasagni, Fraunhofer IWS).
Zwei langjährige Mitarbeiter wurden 2012 berufen: Kandice Levental auf eine Assistant Professorship für Integrative Biology and Pharmacology an der University of Texas, Houston, Texas und Philipp Seib - nach einem Postdoc-Aufenthalt an der Tufts University - auf eine Lecturer-Position an der University of Strathclyde, Glasgow, Schottland.
Unter den 2012 abgeschlossenen Graduierungsarbeiten war auch die mit dem Prädikat „summa cum laude“ ausgezeichnete Arbeit „Modulation of growth factor functionality through immobilization in starPEG-heparin networks“ von Andrea Zieris (Stipendiatin der International Graduate School für Biomedicine and Bioengineering - DIGS BB).

Biologie-inspirierte Grenzflächen- und Materialgestaltung

Release of bone therapeutic drugs from polyelectrolyte complex films

Martin Müller, Bernhard Torger,
David Vehlow, Bernd Keßler, Birgit Urban

Functionalization of bone substitution materials by drugs or local drug delivery systems is a highly relevant strategy to improve bone healing [1]. Recently, we reported on polyelectrolyte (PEL) complex (PEC) nanoparticles, that were loaded by the bisphosphonate (BP) pamidronate (PAM) and deposited as adhesive films onto planar Ge model substrates [2]. BPs are known to inhibit osteoclastic activity via the farnesyl pathway favoring osteoblastic bone formation, and are widely used as therapeutics for systemic bone diseases like osteoporosis. A retarded release of PAM under conservation and adhesive stability of the bare PEC particle film was shown by in situ ATR-FTIR spectroscopy, monitoring the depletion of PAM in the cast PEC film matrix. Various factors of PAM release were studied. A PEC system based on poly(ethyleneimine) (PEI) and cellulose sulfate (CS) was used because these compounds are easily available and especially branched/linear PEL combinations might feature high structural densities enabling better drug entrapment. Results on factors influencing PAM release can be found in Fig. 1, where the relative PAM content with respect to the initial dry state is plotted versus time in contact with the release medium. The lowest initial burst behavior and slowest release kinetics were obtained for the highest PEC concentration (0.01M) and lowest PAM/PEC ratio (1:20). Furthermore, kinetic analysis based on the Ritger-Peppas model revealed values of $b << 0.5$ for PAM/PEC samples cast from 0.002M dispersions, suggesting dissolution of dried PAM in the PEC matrix. However, PAM/PEC samples cast from 0.01M dispersions revealed values of b close to 0.5, suggesting hindered dissolution or diffusion due to a more dense PEC matrix and lower drug/surface area ratio. A model describing drug retention in PEC particle films is described therein [2].
Actual and future studies on PECs address screening of further drug/PEL combinations and casting techniques. Recently the release of streptomycin used for tuberculous osteomyelitis therapy, from PEL complex layers fabricated by the layer-by-layer technique was reported [3]. Furthermore, PECs will be applied at relevant BSM and biocompatibility and interaction to bone cell cultures studied [4]. In conclusion, surface adhesive drug-loaded PEC particles are promising drug delivery systems to functionalize bone substitution materials.



Sponsor:
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Cooperation:
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M. Gelinsky, T. Hanke, U. Hempel, Technische Universität Dresden
R. Schwartz-Albiez, Deutsches Krebsforschungszentrum Heidelberg

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Keywords
bone diseases
osteoporosis
bone substituting material
drug delivery
polyelectrolyte complex
bone therapeutic drug
bisphosphonate

Abb. 1:
Decrease of the relative PAM content in drug loaded PEC (PEI/CS) particle films casted onto Ge substrate in contact to the release medium. The pure PAM film (black) is compared to PAM/PEC films for various PAM/PEC ratios (1:4, 1:8, 1:12) and concentrations (0.002 vs. 0.01M).

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Keywords

Quantifying the effect of covalently immobilized enzymes on biofilm formation by atomic force microscopy-based single-cell force spectroscopy

Jens Friedrichs, Andrea Zieris,
Silvana Prokoph, Carsten Werner

Biofouling is the undesired deposition of microorganisms and their extracellular polymeric substrates on man-made surfaces. This phenomenon can occur in an extremely wide range of situations, from the colonization of medical devices to the production of ultra-pure, drinking and process water and the fouling of ship hulls, pipelines and reservoirs. Recently, we have developed and thoroughly characterized a model system to investigate the influence of biofilm-degrading enzymes, covalently immobilized onto maleic anhydride copolymer films, on the attachment of major marine foulers [1, 2].

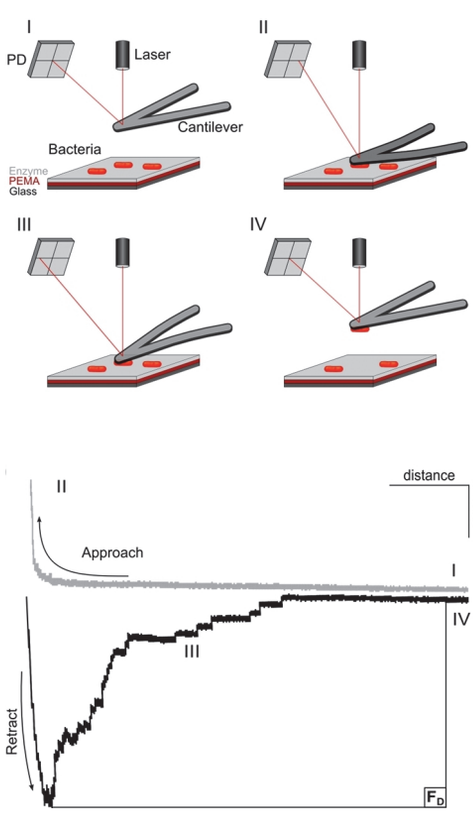

Fig. 1:
Single-cell force spectroscopy setup.
Top: (I) To measure the force acting on the AFM cantilever, the cantilever deflection is determined using a laser beam reflected by the back of the cantilever onto a multi-segment photodiode (PD). Bacteria were preincubated on the covalently immobilized enzymes. (II) A functionalized cantilever was lowered towards an isolated bacteria bound to the enzymes on the polymer-coated supports until a preset force was reached. (III) After a given contact time, the cantilever was retracted thereby completely detaching the bacteria from the support (IV).
Bottom: Force-distance curve showing steps (I), (II), (III), and (IV) corresponding to those outlined above. The maximal detachment force (FD) was extracted from the retraction force-distance curve. The approach and retraction force-distance curves are colored grey and black, respectively.

Here, we have developed a novel atomic force microscopy-based single-cell force spectroscopy assay to quantify the adhesion of bacterial cells to surfaces. In the assay, bacteria were allowed to attach to a surface for a given period of time. Subsequently, an AFM cantilever functionalized with adhesive proteins was used to detach individual bacteria from the surface (Fig. 1).
The assay was applied to quantify the effect of two biofilm-degrading enzymes, the protease Subtilisin A and glycoside hydrolase cellulase, on the adhesion of a biofilm-forming bacterial strain. For Subtilisin A a clear difference in the recorded detachment forces between active and inactive enzyme layers could be detected. In contrast, relatively similar detachment forces were recorded when detaching bacteria from active and inactive glycoside hydrolase cellulase layers (Fig. 2). Since the protease Subtilisin A had a profound effect on bacterial adhesion whereas the glycoside hydrolase cellulase had not, it could be assumed that mainly proteins mediated the initial attachment of the analyzed bacterial strain to these surfaces, whereas polysaccharides are less important in the attachment.

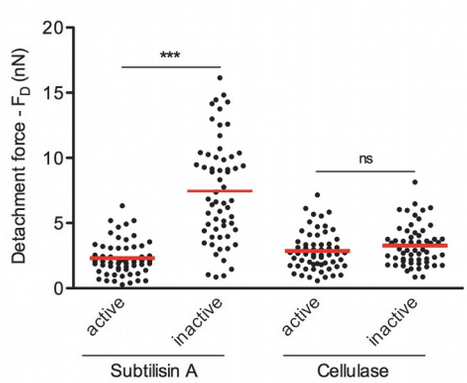

Fig. 2:
Adhesion strength of biofilm-forming bacteria Cobetia marina to active and inactive biofilm-degrading enzymes. Data sets are presented in scatter dot plots, where each dot represents the detachment force of a single bacterium. Black lines mark the mean.

Sponsor:
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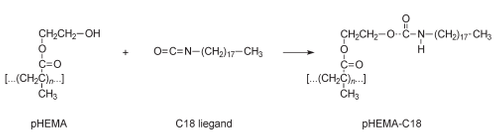
Biologie-inspirierte Grenzflächen- und Materialgestaltung

The effect of octadecyl chain immobilization on the hemocompatibility of poly (2-hydroxyethyl methacrylate)

Marion Fischer, Claudia Sperling, Carsten Werner

Thrombotic complications encountered with blood contacting devices still are of clinical relevance. These complications are related to coagulation events that occur at the blood-material interface being triggered by initial protein adsorption and subsequent cellular activation processes.
Following the idea of self-renewable albumin layers as non-coagulant, shielding interfaces hydrogel materials were decorated with varying amounts of alkyl chains, which can mediate attachment of albumin through its fatty acid binding sites [1].



Scheme 1:
Derivatization of poly (2-hydroxyethyl methacrylate) (pHEMA) with octadecyl isocyanate (C18 ligand) via urethane linkage

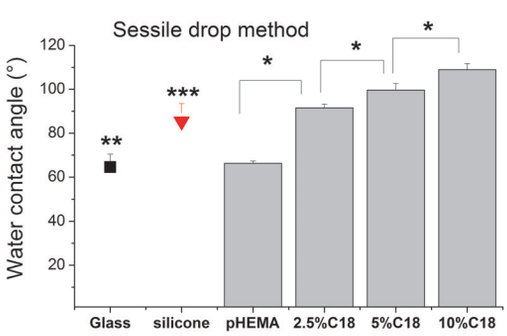


Fig. 1:
Water contact angles (°) of pHEMA and C18--pHEMA dry samples determined by the sessile drop method.
*** Significantly different samples; ** Glass is significantly different from 2.5%, 5% C18 and 10% C18 and silicone; *** Silicone is significantly different from pHEMA, 5% C18, 10% C18 and glass (Mann-Whitney test, $p < 0.05$).**

Specifically, poly (2-hydroxyethyl methycrylate) (pHEMA) was modified with aliphatic octadecyl isocyanates (C18). Derivatization of pHEMA-derived hydroxyl groups with increasing amounts of C18 chains (2.5, 5 and 10%) via urethane linkage (Scheme 1) led to a direct related increased hydrophobicity (Fig. 1) and stiffness.
To evaluate the blood compatibility of the modified surfaces we applied in vitro blood incubation tests, where materials were incubated with fresh human whole blood for two hours at 37°C. This test system enables to determine material related coagulation and inflammation events. Modified and native pHEMA was tested as well as glass and PTFE as reference materials. C18 modification of pHEMA surfaces resulted in reduced coagulation activation (Fig. 2). As anticipated, surfaces modified with C18 chains were shown to induce dynamic and renewable adsorption of non-thrombotic albumin, thus preventing the adsorption of pro-thrombotic blood proteins. In addition, the consumption of pro-inflammatory pHEMA-derived OH surface groups by its linkage to C18 chains further reduced inflammatory responses, reflected by reduced leukocyte adhesion (Fig. 3). C18 modified surfaces thus combine advantageous properties of unmodified pHEMA films (reduced platelet adhesion) with reduced coagulation activation and leukocyte adhesion [2]. The beneficial influence of C18 immobilization on the mechanically weak native pHEMA material may further facilitates its potential use in cardiovascular applications.

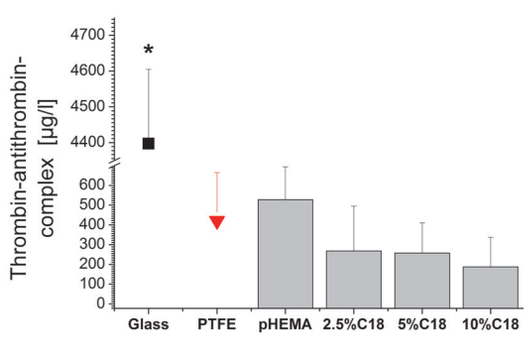


Fig. 2:
Thrombin-antithrombin complex (TAT) levels after 2 hours of incubation with whole blood. * Glass is significantly different from all other surfaces (Dunn's method, $p < 0.05$).

Keywords
haemocompatibility
biomaterials

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Keywords
starPEG
heparin
cryogel
macroporous structure
three-dimensional
scaffolds
human umbilical vein
endothelial cells

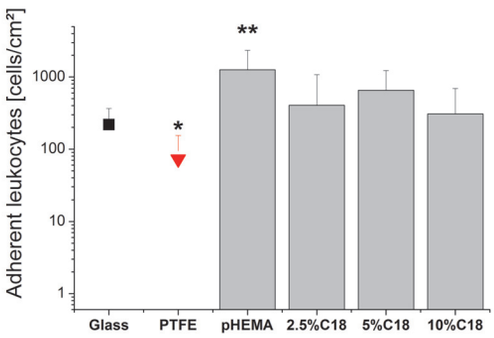

Fig 3:
Surface density of adherent nucleated cells after incubation with whole blood. * PTFE is significantly different from all other surfaces; ** pHEMA is significantly different from glass, PTFE, 2.5%C18 and 10%C18 (Dunn's method, $p < 0.05$).

Cooperation:
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Macroporous starPEG-heparin cryogels

Petra B. Welzel, Milauscha Grimmer, Jens Friedrichs, Claudia Renneberg, Lisa Naujox, Stefan Zschoche, Uwe Freudenberg, Carsten Werner

Several current strategies in tissue engineering rely on hydrogel scaffolds to support and promote the regeneration of tissues. For applications that require rapid cellular ingrowth or expansion, macroporous three-dimensional (3-D) materials with a high open porosity are necessary. Large interconnected pores of 10-100 µm promote cell and tissue ingrowth, vascularization and transport of nutrients and metabolites. In order to regulate cell function and fate, the structural, mechanical, and biomolecular properties of the scaffolds have to be adapted.
To meet this challenge, macroporous scaffolds based on a recently developed modular biohybrid hydrogel material consisting of star-shaped poly(ethylene glycol) (starPEG) and heparin [1] were developed [2]. The well-established network formation via chemical crosslinking (EDC/sulfoNHS chemistry) of amino terminated starPEG and heparin [1] was combined with the cryogelation technology [3]. Sub-zero temperature treatment (-20°C) of the gel forming reaction mixtures and subsequent lyophilization of the incompletely frozen gels resulted in sponge-like materials with a system of interconnected macropores (cryogels) as demonstrated by scanning electron microscopy (SEM, Figure 1).
Mercury intrusion porosimetry revealed total porosities of 89 to 92%. Cryogels swell much faster than non-macroporous hydrogels in aqueous solution due to their sponge-like structure that dramatically facilitates mass transfer. The swollen bulk materials consist of interconnected irregular macropores, ranging between 30 and 180 µm in size, surrounded by rather low hydrated polymer regions (pore walls) of only 10 µm in width (confocal laser scanning microsopy (CLSM) of fluorescence labeled samples). They are rather soft but very tough as shown by uniaxial compression experiments. Increasing the molar ratio of starPEG to heparin in the reaction mixture leads to an increase in bulk Young´s modulus. The pore size can be significantly influenced by

the freezing temperature during cryo-treatment. To control biomolecular properties of the scaffolds, the heparin component can be used for a range of effective secondary biofunctionalization schemes, including the covalent attachment of adhesive ligands (e.g., RGD containing peptides) and the loading of heparin-binding growth factors [4].
The applicability of the starPEG-heparin cryogels as three-dimensional cell carriers for tissue engineering was exemplarily shown by seeding human umbilical vein endothelial cells (HUVECs) onto scaffolds functionalized with the RGD-motif. The cells migrated into the macro-pores and attached to the hydrogel matrix, as shown by representative fluorescence images taken after seven days in culture (Figure 2). MTT-staining (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) indicated a good distribution and penetration of viable cells throughout the scaffold. Thus, we conclude that the properties of the biohybrid cryogel are supportive of HUVEC culture.
Currently, the potential of these customizable cryogels for other clinical applications and as a model system for evaluating cell-cell-interactions in well-defined three-dimensional microenvironments is investigated.

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A. Potthoff, Fraunhofer-Institut für Keramische Technologien und Systeme, Dresden
R. Vogel, J.-U. Sommer, R. Dockhorn, IPF

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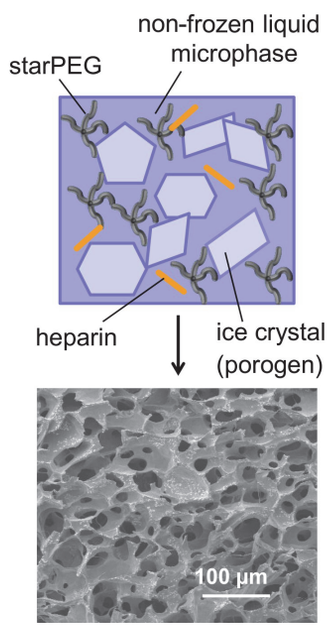

Fig. 1:
Formation of macroporous starPEG-heparin cryogels by cryo-treatment of the aqueous gel-forming reaction mixture. The precursors (starPEG and heparin) are concentrated in the non-frozen liquid microphase (top). Scanning electron microscopy reveals the sponge-like microstructure of the dry scaffold after lyophilization (bottom).

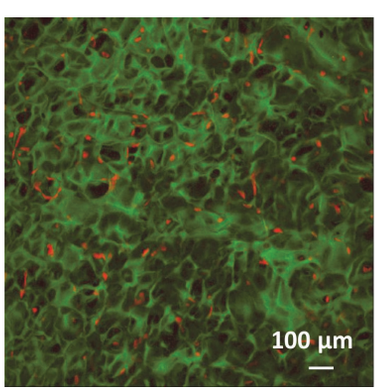

Fig. 2:
Representative confocal microscopy image of human umbilical vein endothelial cell colonization on RGD-modified cryogels after seven days in culture in xy direction (3D-projection) indicating three-dimensional cell growth.
Green: cryogel dyed by Alexafluor488.
Red: actin of endothelial cells dyed by Alexafluor-633-labeled phalloidin.

Biologie-inspirierte Grenzflächen- und Materialgestaltung

Keywords
collembola
cuticle
omniphobic
replication

Exploring the omniphobic characteristics of springtail skin

René Hensel, Ralf Helbig, Julia Nickerl, Carsten Werner

Springtails (Collembola) are wingless arthropods (Fig. 1A) that are impressively adapted to cutaneous respiration in temporarily rain-flooded habitats due to their non-wetting skin morphology.

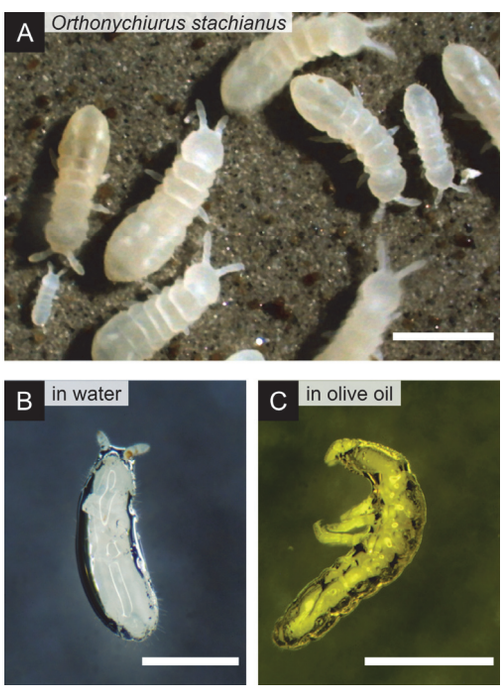

Fig. 1:
(A) Springtail colony of *Orthonychiurus stachianus*.
(B, C) Plastron surrounding the entire animal upon immersion into (B) water and (C) olive oil.
Scale bars: 1 mm.

The skin surface mainly consists of regularly arranged, nanoscopic granules that exhibit characteristic overhangs in a sectional view. These overhangs were proposed to afford the geometrical control over wetting and enable the formation of a stable air cushion (plastron), which surrounds the entire animal, upon immersion into water (Fig. 1B) and even into many low-surface-tension liquids such as oil (Fig. 1C) or ethanol [1, 2]. The spatial arrangement of the nanoscopic granules and the occurrence, shape and density of larger hierarchical elements, such as bristles and papillose topographies, on springtail cuticle correlates with specific habitats and ecology [3].

In order to resolve the impact of the individual structure elements on the omniphobic characteristics of the springtail skin, we developed an adaptive replication process [4] that is illustrated in Fig. 2A. Perfluoropolyether dimethacrylate (PFPEdma) was used as elastomeric mould material that principally ensures high accuracy of the finally replicated polymer structures [5-8]. We produced polymer skin replicas of similar chemical bulk composition, but with distinctive surface morphologies regarding the presence of the nanoscopic granules and surface chemistries. The contact angle data (Fig. 2B) showed a clear correlation with the particular surface morphologies: Polymer replica surfaces containing nanoscopic granules produced contact angles considerably higher than 90° with values up to 150° for both test liquids, reflecting an omniphobic wetting performance irrespective of the polymer surface chemistry. In contrast, the polymer replicas without the nanoscopic surface morphology of the springtail skin were completely soaked by both test liquids resulting in macroscopic contact angles of about 0°. Teflon-AF-coating of the latter replicas afforded water repellence but were soaked by hexadecane. Furthermore, the replicas were analysed by in situ plastron collapse tests and numerical simulations, which allowed for exploring of the high-pressure resistance of the observed Cassie state and the dynamics of the enforced wetting transition from Cassie to Wenzel state.

In sum, it was found that the nanoscopic granules of the cuticle play a decisive role in liquid repellence. Specifically, the overhangs of the nanoscopic granules were concluded to effectively retain air (nanoplastrons) upon wetting, resulting in a solely structural wetting barrier even for liquids with low surface tension. Furthermore, the numerical simulations revealed that the Cassie-Wenzel transition at elevated pressures occurs as a stepwise process. In the first step the macroscopic plastron collapses while air is still entrapped inside the skin nanocavities, which facilitates recovery of the de-wetted state upon pressure reduction.

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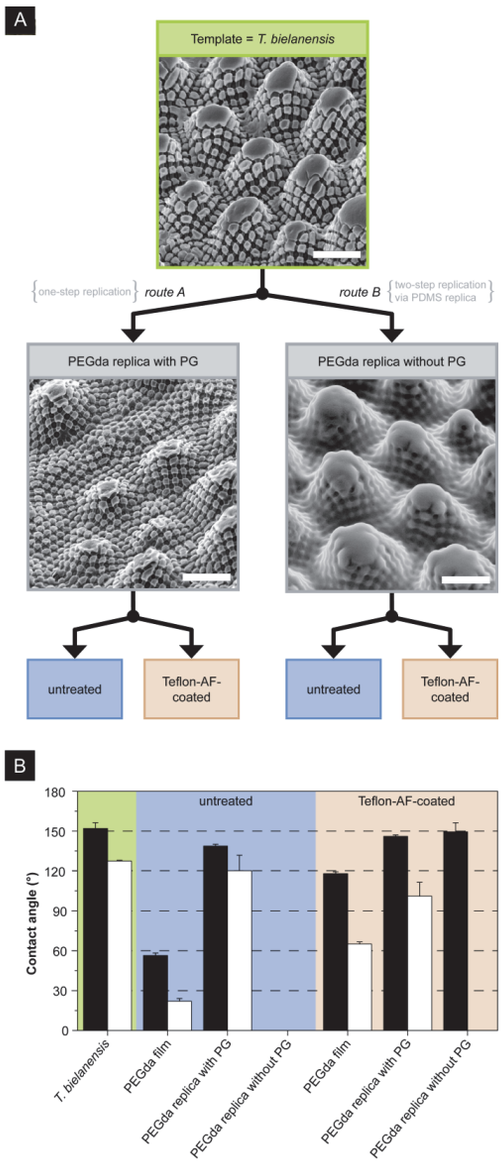

Fig. 2:
Replication process flow and the contact angle measurements.
(A) Schematic illustration of the sample preparation via replication of the natural skin of *Tetrodontophora bielanensis*. Route A generates faithful polymer skin replicas with nanoscopic granules, whereas route B leads to polymer replicas without these granules. The material of the final replicas (in both routes) is poly-(ethylene glycol) diacrylate (PEGda). In a further step, the surface chemistry is varied between untreated and Teflon-AF-coated polymer replicas. Scale bars: 3 µm. (B) Determined static contact angles using droplets of water (black bars) as a polar liquid with high surface tension and hexadecane (white bars) as a non-polar liquid with low surface tension.

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S. Aland, A. Voigt, Technische Universität Dresden, Institute of Scientific Computing

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Keywords
lipid bilayer
membrane
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polymer
adsorption
translocation
permeability

Lipid bilayer membranes interacting with polymer chains and nanoparticles

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Living cells are separated from their environment by biological membranes consisting of a self-organized bilayer of amphiphilic lipid molecules and embedded macromolecules such as pore-proteins. Recent experiments have shown that synthetic amphiphilic polymers may translocate passively [1, 2] through lipid bilayer membranes and may also destabilize the bilayer structure leading to an increase of membrane permeability for other solutes [2, 3]. The theoretical understanding of the mechanisms of translocation by passive transport processes and induced bilayer perturbations would bring forward the development of non-toxic transport agents to deliver cargos into living cells. In this work [4-6] we present a possible physical mechanism of polymer- and nanoparticle translocation and polymer- or nanoparticle induced permeability for smaller molecules.

We use a lattice Monte Carlo model to represent lipids, solvent and macromolecules such as polymers and nanoparticles efficiently on a coarse grained level [5]. The hydrophobic effect is modeled by using short-range repulsive interactions between two molecules with different hydrophobicity, which drive the system from a random start configuration to self-assembled lipid bilayer structures for repulsive tail-solvent interactions in the order of k_BT. Additionally to the bilayer we add polymers and nanoparticles with tunable hydrophobicity to the system. Fig. 1 shows snapshots of individual simulations with single homopolymers [4, 5] and 144 homogeneous NPs (nanoparticles) [6], respectively, where each monomer and NP- segment has the same hydrophobicity. As Fig. 1 suggests, homopolymers and homogeneous NPs show equivalent effects in interaction with the model membrane: for hydrophilic polymers (NPs) the bilayer acts as a potential barrier. Hydrophobic polymers (NPs) are trapped in the bilayer core and diffuse in a quasi-2D solvent of tails. For intermediate hydrophobicities our simulation results indicate an adsorption transition for homopolymers and homogeneous NPs. Here, the solvent phase and the membrane core are equally repulsive for the molecules and the membrane becomes energetically "transparent". In this range of balanced hydrophobicity we detect an increased rate of translocation events of the polymer and of individual NPs. For polymers (NPs) with balanced hydrophobicity both the solvent phase and the hydrophobic bilayer core are repulsive environments. This results in collapsed globular states of the polymer chains and agglomeration of NPs into a cluster. Molecules with hydrophobicities close to the adsorption transition induce perturbations in the bilayer structure, when they are in contact with the membrane or translocate through (see Fig. 1)

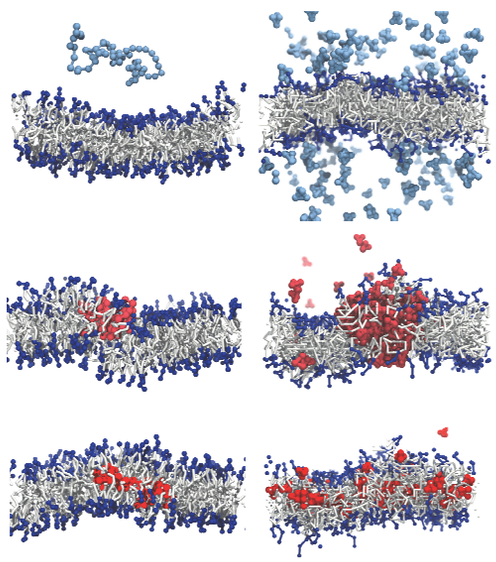

Fig. 1:
Simulation snapshots of homopolymers (left, [5]) and nanoparticles (right, [6]) interacting with a lipid bilayer. The picture shows polymers and nanoparticles, which are hydrophilic (top), hydrophobic (bottom) and of intermediate hydrophobicity (center) close of the adsorption transition (polymer) and slightly above the transition (nanoparticles).

This results in a change of the permeability of the membrane as function of polymer- and NP-hydrophobicity. Close to the adsorption transition, there is a peak increase of membrane permeability with respect to solvent. This demonstrates that the point of adsorption of a molecule with homogeneous hydrophobicity controls molecule translocation as well as induced permeability of the membrane for smaller solutes in our model. Further results carried out for amphiphilic nanoparticles containing both hydrophilic and

hydrophobic sites [6], as well as random copolymers composed of hydrophilic and hydrophobic monomers which indicate similar permeability mechanisms as described above. However, heterogeneous molecules show a more pronounced localization at the membrane-solvent interface and a broader range of membrane activity with respect to their average hydrophobicity as compared to homogeneous molecules.

Our analysis indicates a possible pathway to identify most effective translocation and permeability agents based on amphiphilic copolymers or nanoparticles.

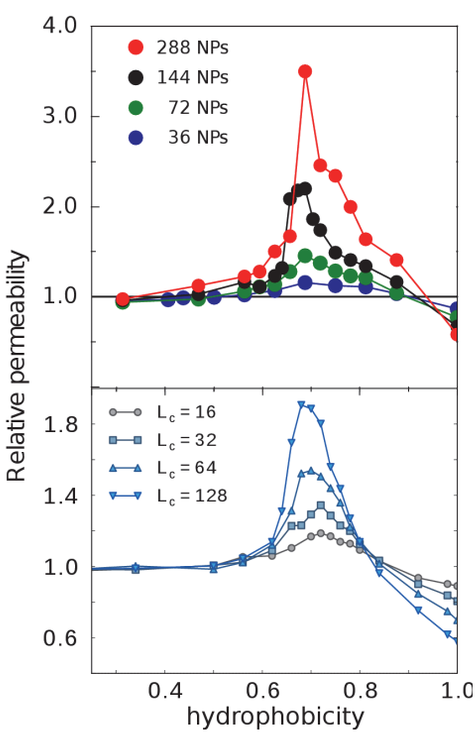

Fig. 2:
Relative solvent permeability of a membrane interacting with nanoparticles (top) and homopolymers (bottom) as function of their relative hydrophobicity as compared to the lipid tails. The upper graph shows the result [6] for the whole simulation box, and the lower graph in the vicinity of the single polymer chain [5].

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