

Biologie-inspirierte Grenzflächen- und Materialgestaltung



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Forschungsergebnisse des IPF ermöglichen es immer mehr, komplexe biologischen Mechanismen gezielt durch funktionelle Polymermaterialien zu steuern und damit neue Therapieansätze zu erschließen. So konnte 2017 ein neues Konzept zur Behandlung chronischer Wunden auf Basis von Glykosaminoglykan-Hydrogelen publiziert werden, durch das entzündliche Prozesse abgeschwächt werden können. Die Methode eignet sich prinzipiell auch für die Behandlung anderer entzündlicher Erkrankungen. (Science Translational Medicine 9 (2017) eaai9044)

Die Konditionierung von humanen Stamm- und Vorläuferzellen durch definierte Mikromilieus *in vitro* ist für die Aufklärung von biologischen Mechanismen wie auch für die anwendungsbezogene Entwicklung von Gewebemodellen bedeutsam. Arrays von Mikrokavitäten aus sehr weichem Gelmaterial (Schermodulus 1 kPA) konnten in bisher unerreichten Dimensionen (in Durchmesser bis hinunter zu 3 µm) hergestellt und lokalisiert mit Adhäsionsliganden-Peptiden und Zytokinen funktionalisiert werden. Der Nutzen dieses Ansatzes wurde in parallelisierten Kulturexperimenten mit humanen Blutstammzellen belegt. (Advanced Materials 29 (2017) ID1703489)

Die bedarfsgerechte Freisetzung von anti-koagulant Substanzen durch adaptive Materialien verspricht die Vermeidung von Nebenwirkungen bei der Anwendung von Medizinprodukten im Blutkontakt. Für das hierzu von uns etablierte Prinzip, bei dem Blutgerinnungsenzyme zur Regulation herangezogen werden, konnte durch systematischen Vergleich von verschiedenen Enzymsystemen geklärt werden, wie die Dynamik der Wirkstofffreisetzung maximiert und der systemische Wirkstoffspiegel minimiert werden können. (Biomaterials 135 (2017) 53-61)

Responsive Polymersome sind als zell-analoge Strukturen für neuartige Therapiekonzepte von großem Interesse. Hierzu ist es gelungen, enzymatische Kaskadenreaktionen in artifiziellen Organellen-Systemen zu realisieren, die es prinzipiell erlauben metabolische Prozesse nachzuvollziehen. Die resultierenden Materialien können in der Biotechnologie wie auch in der biologischen Grundlagenforschung genutzt werden. (Angewandte Chemie 2017 DOI: 10.1002/anie.201708826)

Funktionelle Biomaterialien liefern auch für die Untersuchung fundamentaler Eigenschaften lebender Organismen wertvolle neue Möglichkeiten. So konnten durch Mikrofabrikation erhaltene Strukturen für die Aufklärung von Zusammenhängen zwischen Wachstum, mechanischer Charakteristik und geometrischer Form von Bakterien herangezogen werden. (Nature Microbiology, 2, 17115, (2017)).

Aus der fachlichen Verknüpfung mit anderen Instituten der Leibniz-Gemeinschaft ergeben sich neue Optionen für die Entwicklung von adaptiven Biomaterialien. Ein internationales Symposium des Leibniz-Forschungsverbands Gesundheitstechnologien „Biomaterials-based approaches to personalized medicine“ diente der Diskussion von Materialien für individualisierte Therapien.

Die am IPF organisierte 12. Internationale Konferenz „Electrokinetic Phenomena“ war Ladungsprozessen in komplexen Strukturen weicher Materie gewidmet und lieferte insbesondere neue Ansatzpunkte für die skalenübergreifende Kontrolle biomolekularer Wechselwirkungen.

Zu den 2017 gestarteten Projekte des IPF auf dem Gebiet der Biomaterialforschung gehören der Sonderforschungsbereich „Biomaterialien zur Regeneration von Knochen und Haut“ (3. Förderperiode), das Verbundprojekt der VolkswagenStiftung „Self-organization of bacterial cell shape“ und das Forschungstransferprojekt TissueGuard.

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Positively charged surfaces can trigger blood coagulation through Factor VII activating protease

Claudia Sperling, Manfred F. Maitz, Carsten Werner

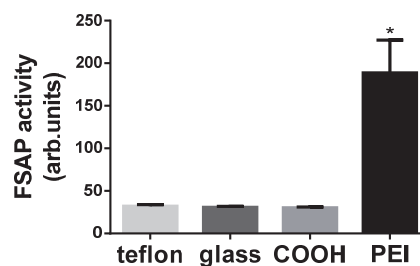
Biomaterials used in stents, artificial vessels, hemodialysis or other blood contacting medical products initiate undesirable thrombotic and inflammatory processes in blood. Patients that rely on these medical devices need systemic anticoagulation permanently or for an extended duration. Knowledge about the role of specific surface characteristics in these blood activation processes is crucial for the design of advanced biomaterials.

Charged material surfaces are known to activate blood coagulation. Autoactivation of the coagulation enzyme FXII has been identified as a main coagulation trigger on negatively charged surfaces. However, the mechanism of coagulation activation on positively charged surfaces remained a matter of speculation. A recently discovered blood coagulation factor with several functions in coagulation and inflammation, Factor VII activating protease (FSAP) [1] was shown to be activated on negatively as well as on positively charged macromolecules. We proposed that this activation can also take place on charged material surfaces and tested its possible contribution to the clotting activation on surfaces.

For that aim, we compared the interaction of human whole blood with four different materials varying charge and hydrophilicity:

- poly(ethylenimine) (PEI, positively charged, advancing contact angle: θ^a $67 \pm 0.5^\circ$),
- poly(tetrafluoroethylene) (teflon, no charge, θ^a $120 \pm 0.5^\circ$),
- a self assembled monolayer with carboxyl groups (COOH) and
- glass [both negatively charged, θ^a $21 \pm 1.2^\circ$ (COOH) / $8 \pm 0^\circ$ (glass)]. [2]

FSAP was only activated on the positively charged surface PEI, not on any of the other surfaces (Fig. 1). Coagulation activation measured as thrombin formation and fibrin formation both were pronounced on glass and PEI, but not on teflon and COOH (Fig. 2). To conclude on the activation pathway we used an inhibitory antibody for FSAP (mab 570). The antibody significantly reduced coagulation on PEI but not on the other surfaces (Fig. 2).



Keywords
hemocompatibility
charged biomaterials
blood coagulation

Fig. 1: Activation of FSAP after incubation of surfaces with human whole blood for 3 hours at 37°C, Statistics: * significantly different to all other surfaces

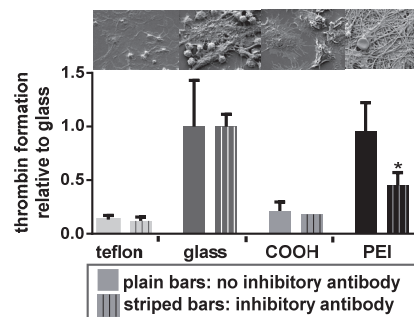


Fig. 2: Activation of blood coagulation after incubation with human whole blood for 3 hours at 37°C. Incubation was performed with (striped bars) or without (plain bars) inhibitory antibody to FSAP, Statistics: * significant effect of the inhibitory antibody; SEM pictures of incubation without inhibitory antibody show surface adherent platelets (teflon), granulocytes (COOH) and fibrin mesh (glass and PEI)

Fig. 3 schematically summarizes the observed surface reactions on the compared materials (COOH is not integrated as the initial activation of FXII is not propagated in this case due to the lack of activated platelets, for details see [3]). On teflon, platelets adhered to adsorbed and denatured fibrinogen. No interaction with FSAP or FXII and no thrombin formation was measurable. On glass, fibrinogen adsorption was activated on the negatively charged surface and effectively supported thrombin activation,

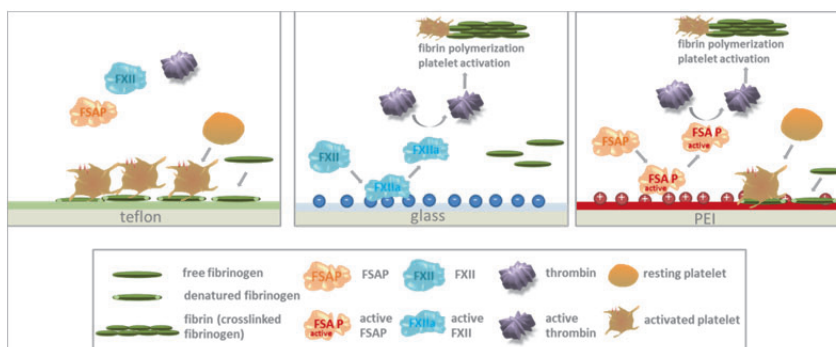


Fig. 3: Scheme of surface initiated reactions on teflon, glass and PEI. (Sizes are not to scale)

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Keywords
hematopoietic stem and progenitor cells
ex vivo culture
hydrogel
3D culture

leading to fibrin formation and platelet activation. On PEI, an intermediate amount of platelets adhered while additionally FSAP was activated on the positively charged surface resulting in strong thrombin formation.

We showed that FSAP can be activated on charged material surfaces, yet activation only takes place on positively charged, not on negatively charged surfaces. We conclude that surface activated FSAP supports thrombin formation on this surface, thus it can act as a primary trigger analogous to the FXII activation on negatively charged surfaces.

Cooperation:

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Deciphering the impact of biophysical and biochemical cues on human hematopoietic stem cells in hydrogel-based 3D cultures

David Gvaramia, Eike Müller, Passant Attalah, Uwe Freudenberg, Carsten Werner

Hematopoietic stem and progenitor cells (HSPCs) are responsible for the production of blood and immune cells throughout the lifetime of an individual [1]. Use of these cells in regenerative therapies has seen a steep rise primarily for the treatment of malignant hematological diseases such as leukemia [2,3]. While currently among the most common stem cell sources used for clinical applications, obtaining of high-quality HSPC grafts is often limited by factors such as scarce availability of the cells, difficulty of engraftment and immune histocompatibility [4]. *Ex vivo* expansion of HSPCs has proven to be complicated due to the failure of replicating the complexity of their native bone marrow (BM) microenvironment, which leads to differentiation of the cultured HSPCs. Although the use of artificial and semi-artificial scaffolds has led to some progress, further advancement of optimal *ex vivo* HSPC manipulation strategies is still limited by the inadequacy of biomechanical and biochemical cues provided by these culture models [5]. In this study, we used *in situ* assembling biohybrid starPEG-heparin (HEP) hydrogels [6] for the three-dimensional (3D) *ex vivo* culture of HSPCs. The hydrogels allow for the independent modulation of biophysical and biochemical culture parameters in a controlled manner and can, therefore, be used to decipher the relevance of biochemical and biophysical parameters in a 3D setting.

Functionalization of the hydrogel scaffolds with early acting hematopoietic cytokines (EAHC) was enabled by the electrostatic, reversible binding of these factors to negatively charged HEP. Sustained release of EAHC from the scaffolds resulted in a higher viability and proliferation of HSPCs in hydrogel cultures compared to the conventional tissue culture plastic (TCP) setting.

Biophysical parameters of the culture were mostly determined by 3D encapsulation, which led to spatially confined round cell shape, with the non-polar distribution of CD133 HSPC-specific marker, as well as restricted contact with the neighboring cells. In contrast, on 2D surfaces, HSPC exhibited heterogeneous mor-

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phology, ranging from round to amoeboid-shaped cells with polarized marker distribution. In addition, on 2D substrates, HSPC segregated together, which was indicative of the high degree of cell-cell communication. Furthermore, an increase of cell density on 2D substrates resulted in an increase in cell proliferation, which was most likely caused by cell-cell contact and paracrine signaling [7]. This effect was strongly reduced upon encapsulation, which points to the functional implications of the reduced cell-cell contact and paracrine signaling in the 3D setting. Finally, upon encapsulation HSPCs formed proliferative clusters and expanded by compression of the surrounding matrix, rather than enzymatic reorganization, thereby being in contact only with their immediate progeny throughout the culture period.

The increase of the matrix stiffness resulted in reduced proliferation of HSPCs in 3D culture due to the decrease in formation and growth of proliferative clusters. Importantly, the decrease of proliferation was not due to the reduced cell viability, which was similarly high in stiff and soft 3D and 2D settings. Further analysis by flow cytometry revealed the higher frequency of quiescent, as well as CD34+ cells in stiff 3D culture settings. This correlated well with the increasing the frequency of long-term culture initiating cells (LTC-IC) – i.e. cells with higher regenerative potential, in 3D stiff cultures, as tested by the *in vitro* functional assay.

Finally, hydrogels containing selectively desulfated HEP were used to evaluate the role of HEP sulfation pattern in the maintenance of LTC-IC in 3D hydrogel cultures. A similar trend of the stiffness-related high frequency of quiescent cells and LTC-IC was observed in stiff 3D cultures with desulfated HEP.

However, the frequency of LTC-IC in stiff gels containing fully sulfated HEP was significantly higher to the desulfated HEP-containing counterparts, indicating that HEP sulfation

(and the resulting affinity for cytokines) contributes to the maintenance of LTC-IC in stiff 3D hydrogel cultures.

Overall, the study demonstrated the successful use of a biohybrid hydrogel system in the elucidation of important biochemical and biophysical parameters of the HSPC culture with relevant functional implications for clinical and research advancements.

Cooperation:

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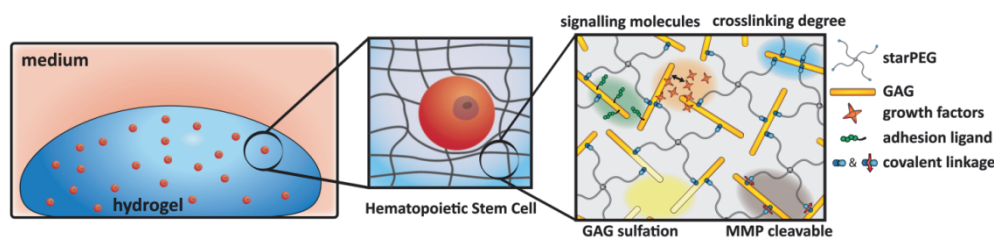


Fig. 1: HSPCs embedded in a biohybrid hydrogel. Hydrogel contains glycosaminoglycan (GAG, typically HEP) and can be functionalized by reversible binding of growth factors to HEP, as well as covalently modified by adhesion ligands. GAGs with different degrees of sulfation can be used to modulate growth factor binding and release, i.e. the biological activity of HEP. MMP-cleavable sequences incorporated in the hydrogel make the scaffold bioresponsive for enzymatic degradation by the embedded cells.

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Keywords
wound healing
sequestration
anti-inflammatory
desulfated heparin

Glycosaminoglycan-based wound dressings to resolve chronic dermal inflammation

Uwe Freudenberg, Lucas Schirmer,
Passant Atallah, Carsten Werner

Wound healing is governed by a wide range of cell signaling molecules regulating cell migration, inflammatory activity, proliferation, angiogenesis and other crucial processes involved in tissue regeneration. Dysregulation of these signals can lead to severe impairment of the tissue regeneration, especially found in chronic wounds.

Scavenging of inflammatory chemokines causing a reduced immune cell influx into the wound area using glycosaminoglycan (GAG) based wound dressing materials was expected to offer a powerful, novel concept to treat chronic wounds. To validate this hypothesis, a modular hydrogel based on end-functionalized star-shaped poly(ethylene glycol) (starPEG) and selectively desulfated derivatives of the glycosaminoglycan (GAG) heparin was customized to sequester inflammatory chemokines from wound tissue [1, 2]. The hydrogels were shown to scavenge chemokines such as MCP-1 (monocyte chemoattractant protein-1), IL-8 (interleukin-8), MIP-1a (macrophage inflammatory protein-1a) and MIP-1b (macrophage inflammatory protein- 1b) in a sulfation dependent manner *in vitro*, in a mouse model *in vivo* and from wound fluids from patients suffering from chronic venous leg ulcers. The consequently reduced migration of the inflammation-fuelling immune cells into the wound area was confirmed in the wild type mouse and in a model of delayed wound healing (db/db mice). When tested in direct comparison to the standard-of-care product Promogran, the current gold standard for chronic wound treatment, our hydrogels formed of StarPEG and N-desulfated heparin were found to be beneficial with respect to reduced inflammation, as well as increased granulation tissue formation, vascularization, and wound closure.

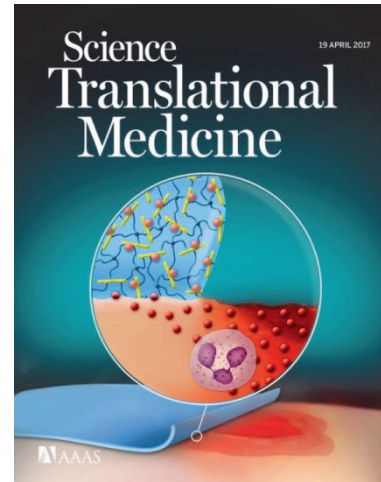


Fig. 1:
Cover Story Science Translational Medicine 19 APRIL 2017,
VOL 9, ISSUE 386: Chemokine capture aids wound healing.

Sponsor:

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Cooperation:

Prof. Jan C. Simon, Dr. Sandra Franz, Leipzig
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Matrix-triggered tubulogenesis for human renal tissue models

Heather M. Weber, Mikhail V. Tsurkan, Valentina Magno, Uwe Freudenberg, Carsten Werner

Currently, the only cure for end stage renal disease is kidney transplantation. Due to the scarcity of organs and the risk of transplant rejection, there is a huge need for better alternative treatments. However, developing renal regenerative medicine remains difficult due to the complexity of the organ. Preclinical studies are often performed with 2D cell culture or animal models, which are not accurate representations of the human physiology. Therefore, we aimed establishing a tunable *in vitro* human kidney model focused on the proximal tubules, a region highly susceptible to renal pathology and toxicity.

To find the optimal matrix to induce proximal tubulogenesis, a set of three hydrogel compositions was examined. Human proximal tubule cells were embedded in hydrogels based on four-armed poly(ethylene glycol) (starPEG) with or without the glycosaminoglycan, heparin (Fig. 1). Matrix metalloproteinase (MMP) cleavable peptide sequences were incorporated as linker units in two hydrogel variants to allow the cells to remodel the matrices.

Interestingly, only hydrogels based on heparin and matrix metalloproteinase cleavable peptide linker units were able to stimulate the morphogenesis of single human proximal tubule epithelial cells into physiologically sized tubule structures (Fig. 2). This approach was shown to be applicable for the modulation of tubulogenesis by adjusting hydrogel mechanics, growth factor signaling, and the presence of insoluble cues (such as adhesion peptides), thus providing valuable new options for applications in regenerative medicine and disease modeling.

In addition, the efficacy of our model was validated as a drug toxicity platform, by incubating a nephrotoxic, chemotherapy drug with the renal tubule model. The tubular structures showed a dose-dependent drug response resembling the human clinical renal pathology. The injured tubular structures also expressed an early *in vivo* renal injury biomarker.

In consequence, the established culture platform is expected to pave the way to more reliable, human cell based nephrotoxicity analyses.

Keywords
in vitro human kidney hydrogel heparin nephrotoxicity

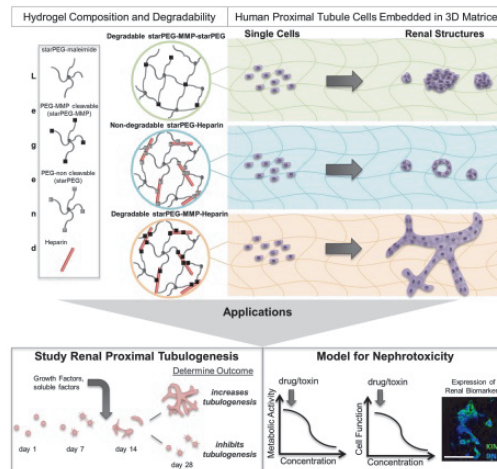


Fig. 1: Experimental design, outcome, and applications for hydrogel-based renal tubulogenesis model. Human proximal tubule cells were embedded in different hydrogel variants to find the optimal conditions to promote tubulogenesis. The established model can be used for renal regenerative medicine or nephrotoxicity applications. Scale bar = 75 μ m.

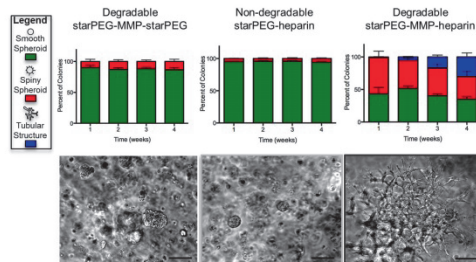


Fig. 2: Quantification of tubulogenesis over 4 weeks (mean \pm s.d.). Representative phase contrast images of structures are below. Scale bar = 100 μ m.

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Cooperation:
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Prof. R. Masereeuw, Utrecht University
Dr. Martijn Wilmer, Radboud University Medical Center

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Keywords
decellularization
macromolecular crowding
kidney

Deposition of kidney-derived matrix enhanced by macromolecular crowding

Valentina Magno, Jens Friedrichs,
Marina C. Prewitz, Carsten Werner

Decellularized extracellular matrices (ECM) are broadly applied in tissue engineering and regenerative medicine. The recent introduction of macromolecular crowding (MMC) for the modulation of ECM deposition by *in vitro* cultivated cells represents a great improvement for better mimicking physiological microenvironments [1, 2]. In our reported work, we used Ficoll 70 and Ficoll 400 (synthetic polymers of sucrose) as macromolecular crowders to explore their effect on the secretion of ECM by primary mouse mesangial cells, ultimately aiming at effective culture conditions to produce kidney specific cell types from human induced pluripotent stem cells (iPSCs).

Confluent cell layers of primary mouse mesangial cells were cultivated either in standard medium (Std) or upon supplementation of a Ficoll70/Ficoll400 mixture (MMC), and then decellularized at day 8 of culture. Scanning electron micrographs of decellularized ECM (Fig. 1) revealed a loose network of thin fibers for matrices produced under standard conditions. MMC enhanced matrices showed a clearly different, tightly packed meshwork of proteins with a peculiar reticular appearance. Immunofluorescent staining of the decellularized ECM (Fig. 1) revealed that cells cultivated under standard conditions secreted discrete amounts of fibronectin and collagen IV, arranged in a rather loose protein network. No laminin deposition was detected under these culture conditions. Conversely, matrices produced under MMC contained abundant laminin, together with increased amounts of fibronectin and collagen IV. Fluorescent lectin staining (Fig. 2) furthermore revealed that MMC resulted in elevated deposition of sulfated GAGs by mesangial cells.

To investigate the supportive capacities of the decellularized ECM, intermediate mesoderm progenitors (IM) derived from iPSCs were seeded on Std and MMC ECM, with or without supplementation of the ROCK-inhibitor Y27632. Geltrex (a commercially available complex mixture of ECM proteins) was chosen as a control. IM progenitors survived and proliferated without the supplementation of the ROCK inhibitor Y27632 only on cell-derived

ECM. In such culture conditions, IM progenitors showed an increased expression of two relevant kidney differentiation markers, SIX2 and FOXD1 (Figure 3). A significantly higher expression of SIX2 and a trend toward higher expression of FOXD1 were observed on MMC ECM compared to Std ECM.

Overall, we demonstrate that MMC protocols can enhance the cell-instructive characteristics of decellularized ECM obtained from mouse mesangial cells *in vitro*, providing valuable new options for the directed differentiation of IM progenitors towards kidney lineages without supplementation of soluble morphogens.

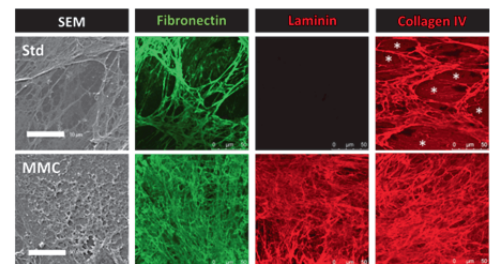


Fig. 1: Mesangial cell-derived ECM produced under Std and MMC conditions.

From left to right: scanning electron micrographs (SEM, scale bar 10 μm) and confocal images of ECM immunostained against fibronectin, laminin and collagen IV.

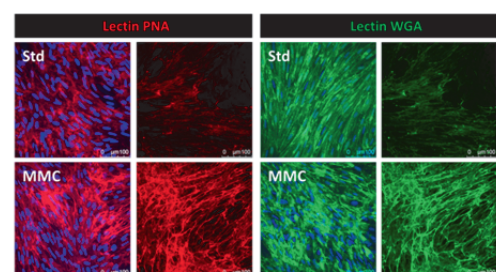


Fig. 2: Detection of GAGs on mesangial cell layers and decellularized ECMs via fluorescent lectin staining.

Red: lectin peanut agglutinin (PNA).
Green: lectin wheat germ agglutinin (WGA).
Blue: nuclei.

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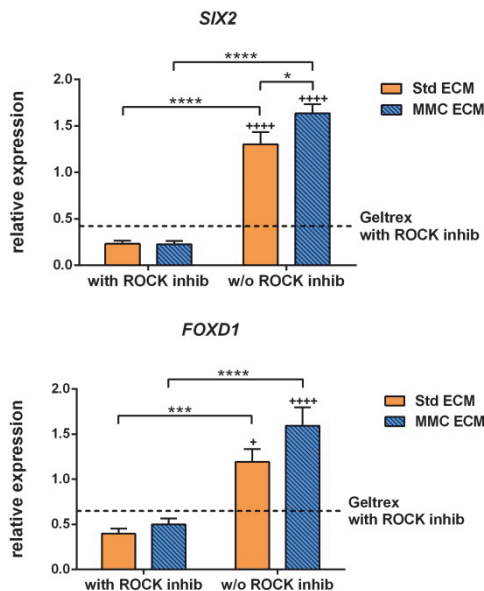


Fig. 3: Gene expression in IM progenitors cultivated for 6 days on Std and MMC ECM. Cells were seeded with or without Y27632 supplementation, with Y27632 being withdrawn after 24h. Dashed line indicates gene expression in cells seeded on Geltrex with Y27632 supplementation (results from 4 independent experiments, n=3. *P<0.05, ***P<0.001, ****P<0.0001, two-way ANOVA. +P<0.05, +++P<0.0001 compared to Geltrex +Y27632, one-way ANOVA. Error bars, S.E.M.).

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Cooperation:
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Charité - Universitätsmedizin Berlin, Berlin-
Brandenburg Center for Regenerative
Therapies

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Cell-size homeostasis in archaea and *E. coli* confined in hydrogel microchambers

Lars D. Renner

For many decades, bacteria cell growth has been studied intensely. Microbes can span size differences of six orders of magnitude [1]. Despite their capability to create different sizes and intricate shapes, a clonal population maintains a precise shape and uniform size over individual cells. In recent studies, several growth mechanisms have been hypothesized with the adder model – for eukaryotic and bacterial cells [2] – being the frontrunner [3]. Archaea share evolutionary features with bacteria and eukaryotes. Given the evolutionary convergence, it was hypothesized that archaeal cells may use a similar mechanism for size regulation. Here, we applied a soft lithography approach [4] to study whether archaeal cells indeed share such a growth control. We grew the archaeal species *Halobacterium salinarum* (specialized to grow in high-saline environments) and compared its growth to the bacterial species *Escherichia coli*.

H. salinarum and *E. coli* were grown in agarose microchambers and their growth behavior was investigated at the single cell level (Fig. 1). Both species grow exponentially at the single cell level and maintain a narrow size distribution by adding a constant volume between cell division events (Fig. 2). However, we observed that *H. salinarum* exhibited a greater variability in cell division placement across individual cells compared to *E. coli* [5]. Our results demonstrate that all three domains of life share a common strategy to control size albeit with varying degrees of noise during cell division.

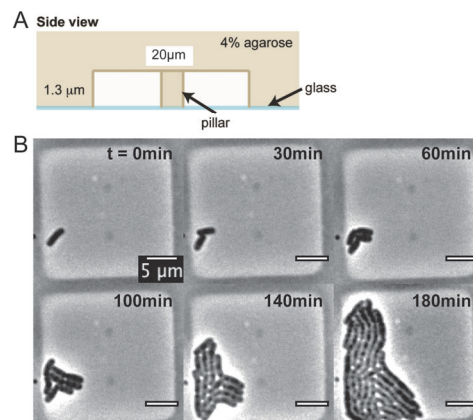


Fig. 1: Experimental setup to study cell size homeostasis. (A) Side view of the developed microchamber to monitor *E. coli* and *H. salinarum* consisting of 4% lysogeny broth agarose hydrogel. (B) Timelapse of growth for a single *E. coli* cell in microchambers (bright-field images) over 5 generations.

Keywords
bacterial shape
growth
archaea

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Keywords
 polymersomes
 host-guest interaction
 docking/ undocking
 process
 surface engineering

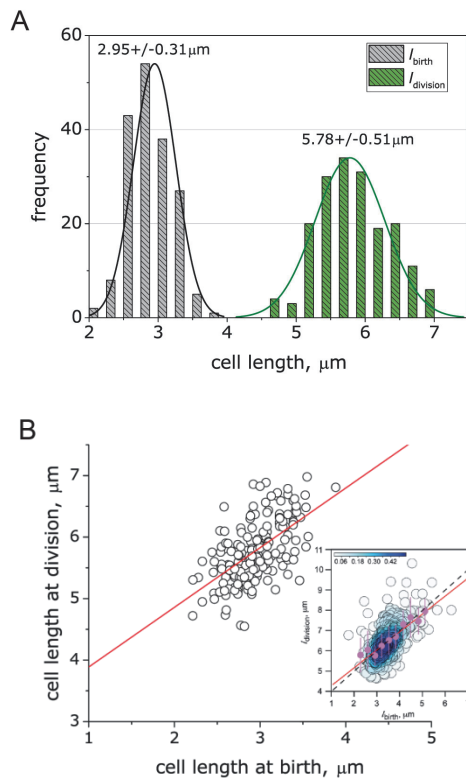


Fig. 2:
(A) Histogram of *E. coli* cell lengths shows narrow distributions around the mean of 2.9 (CV = 17%; n = 178) and 5.8 μm (CV = 13%; n = 178) for birth and division, respectively.
(B) Cell lengths at birth and division are linearly related, and linear regression of the raw data (solid red line) yields a slope of 0.97 ± 0.1 . Inset shows the cell size at birth and division for *H. salinarum* which are also linearly correlated, with a slope of 0.88 ± 0.06 (S.E.; n = 418).

Sponsor:
 VolkswagenStiftung, Program Life? – A Fresh Scientific Approach to the Basic Principles of Life

Cooperation:
 A. Amir, E. Garner, Harvard University

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Shear-force-stable non-covalent conjugation of polymersomes for synthetic and system biology

Hannes Gumz, Anna Charlott Siedel,
 Silvia Moreno, Brigitte Voit, Dietmar Appelhans

Surface engineering of polymeric vesicles (polymersomes, hollow capsules, proteinosomes etc.) plays a deciding role in the design and fabrication of multifunctional nano/ micro-objects for their use in nanomedicine and synthetic biology and as protocells. [1,2] Besides the well-established and intensively studied covalent conjugations on polymeric vesicle surfaces the use of non-covalent conjugation methods (e.g., biotin-avidin, Ni-NTA-His₆-tag) attracted lower interest due to significant problems with determination of the conjugation efficiency. The dynamic character and low stability of the non-covalent bond lead to loss of conjugation during the required purification steps. For the non-covalent conjugation approach our group has validated the use of host-guest interactions as potential platform for well-controlled docking and undocking process of swellable polymersomes. [1] Fig. 1 highlights the implementation of adamantane-β-cyclodextrin interactions outside and inside of polymersomes prepared by the self-assembly of pH-sensitive and photo-crosslinkable block copolymers. By this approach, it is possible to modify the cavity (= inside) of polymersomes with protein mimics. This smoothly indicates that nanometer-sized protein mimics can cross the swollen polymersome membrane from outside to inside and can undergo host-guest interactions. The addition of pure β-cyclodextrin induces the selective displacement of β-cyclodextrin-modified entities at the inner and outer surface of the polymersome membranes. [1] Moreover, control experiments also revealed that the PEG shells of our polymersomes, present outside as well inside, are also able to undergo PEG-β-cyclodextrin interactions. [1] Both kinds of non-covalent conjugation are shear-force stable, as shown when non-conjugated β-cyclodextrin-modified protein mimics or β-cyclodextrin-modified dye molecules are separated from surface-modified polymersome using shear-force driven hollow fiber filtration as purification method (Fig. 1). With this progress, we will facilitate the potential conjugation steps with

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biomolecules/-macromolecules for future experiments in synthetic biology and protocells.

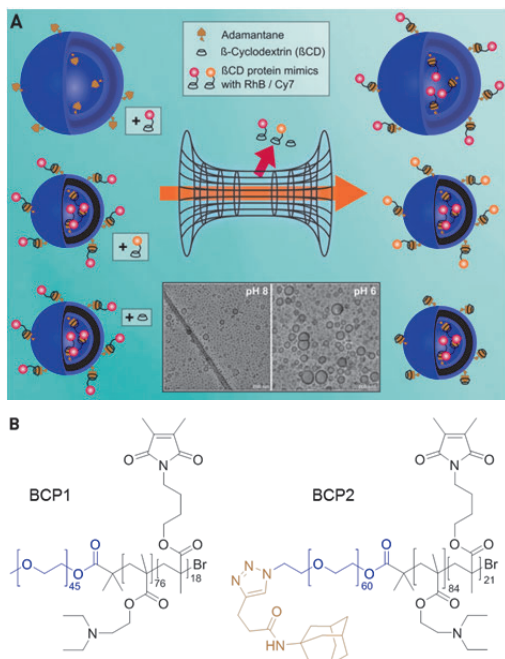


Fig. 1:
(A) Schematic approach for host-guest interaction on the inner and outside surface of polymersome membranes. Purification of non-covalently conjugated polymersomes is carried out by shear-force driven hollow fiber filtration (HFF) method. Cover Image from *Macromolecular Rapid Communication* 2017, Volume 38, Issue 21
(B) Block copolymers BCP1 and BCP2 used for the formation of polymersomes (BCP 1 and 2: blue = PEG units which is the inner outer surface of polymersomes; black = hydrophobic block consisting of pH-responsive monomer and photo-crosslinkable monomer which is the membrane of the polymersomes; brown = adamantane unit which is located at the inner and outer surface of the polymersomes).

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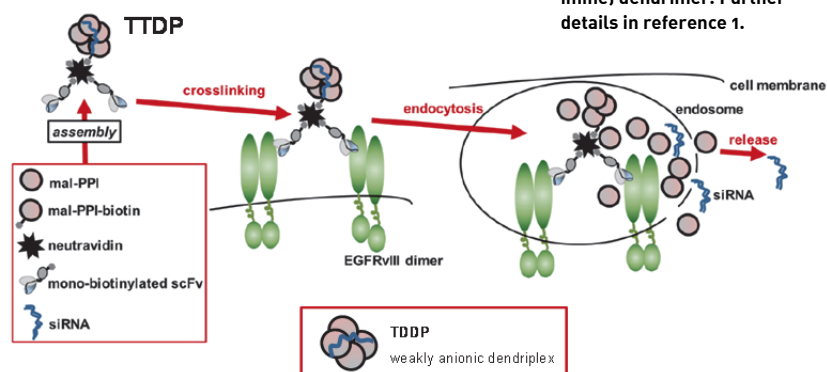
Transfection-disabled dendriplexes for active targeting

Franka Ennen, Andreas Janke, Brigitte Voit, Dietmar Appelhans

The controlled targeting of drugs to specific cells and organs is still a challenging task. One of the key issues is to minimize any side effects due to delivery of toxic drugs to healthy cells destroying them. In line with this we have developed a new concept to minimize the uncontrolled uptake of positively charged dendriplexes due to non-specific ionic interactions with cancerous as well as healthy cells. [1] For the controlled targeting neutral or slightly negatively charged dendriplexes, composed of cationic glycodendrimers and siRNA and further conjugated with antibody, were realized for gene therapy. Importantly, it was shown that these dendriplexes without conjugated antibody are not able to induce apoptosis (cell death) of cancer cells due to the absence of a high enough knock-down efficiency for the delivery of siRNA to the cancer cells. [1] Thus our base materials are characterized as transfection-disabled dendriplexes (TDDP; Fig. 1). Only the decoration of those dendriplexes with cationic antibody-modified neutravidin conjugates (TTDP; Fig. 1) allowed us to validate the desired apoptosis of cancer cells under *in vitro* conditions, while *in vivo* experiments also showed that antibody-decorated dendriplexes, i.p. administered, are predominately delivered to cancer cells in mouse model. [1]

Keywords
 dendrimer
 antibody-neutravidin
 conjugate
 controlled targeting
 dendriplexes
 siRNA

Fig. 1: Simplified process for the fabrication of tumor-targeting dendriplexes (TTDP), composed of transfection-disabled dendriplexes (TDDP) and mono-biotinylated scFv/mal-PPI-biotin/neutravidin conjugate, and their receptor-tailored cellular uptake. Mal-PPI-biotin = cationic monovalent biotinylated glycodendrimer. Mono-biotinylated scFv = monovalent biotinylated single chain fragment variant (antibody for EGFRvIII receptor). Mal-PPI = maltose-modified poly(propylene imine) dendrimer. Further details in reference 1.



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Keywords
lipid membrane
nanoparticles
hydrophobicity
MD simulation

Negative control experiments, using dendriplexes decorated with an incorrect antibody, further confirmed our concept of transfection-disabled dendriplexes as efficient carrier materials to minimize unspecific cellular uptake. [1] The concept will be now extended to modify transfection-disabled dendriplexes with other targeting and/or imaging units for various biological experiments.

Cooperation:
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Dresden, University Hospital Carl Gustav
Carus, Dresden

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Nanoparticles of various degrees of hydrophobicity interacting with lipid membranes

Chanfei F. Su, Jens-Uwe Sommer

Self-assembled lipid bilayers are the protective barriers of living cells but allow for exchange of substances and information between the cell and its surroundings. Identifying the mechanisms of interactions of synthetic NPs with cell membranes is the key for understanding potential NP cytotoxicity [1] and for developing efficient applications such as nanocarriers for targeted drug delivery [2-3]. Particularly, it is of great interest to design NPs to breach the free energy barrier imposed by the membrane and to control the translocation of NPs through lipid bilayers. As it is difficult to obtain microscopic information about NPs interacting with lipid membranes in experiments, molecular simulation provides a valuable physical insight into the interaction of NPs with lipid membranes. However, there still exists a lack of knowledge about the mechanisms behind the translocation process. This concerns in particular the passive transport which does not involve consumption of energy such as ATP-hydrolysis. Using coarse-grained molecular dynamics simulations we have studied the passive translocation of nanoparticles with a size of about 1 nm and with tunable degrees of hydrophobicity through lipid bilayer membranes (see Fig. 1) [4].

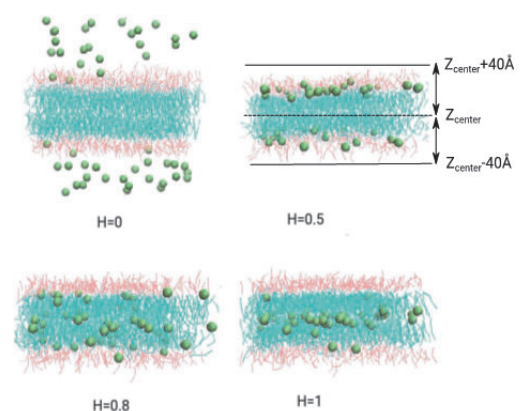


Fig. 1: Snapshots: Lipid membranes interacting with 50 NPs, at different values of the hydrophobicity ($H=0, 0.5, 0.8, 1$). Solvent beads are not shown to improve visibility. The solid lines displayed for $H=0.5$ indicate the thresholds for calculating translocation events.

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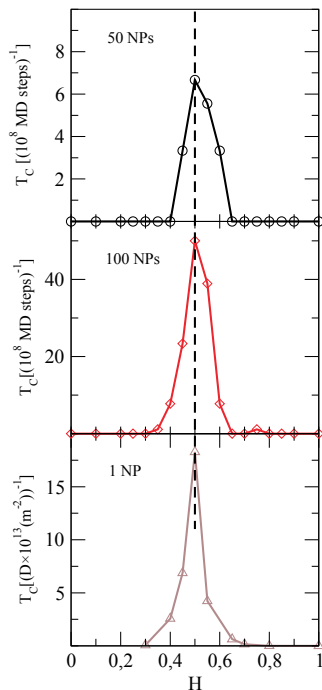


Fig. 2: Translocation rates of NPs as a function of hydrophobicity, in the presence of 50 (black curve, circle symbols) and 100 NPs (red curve, diamond symbols). Brown line with triangle symbols present the results calculated from Kramers theory.

Simulation results shown in Fig. 2 display a narrow window of high translocation rates for NPs with a balanced hydrophobicity close to $H=0.5$, thus verifying the potential model of the lipid membrane. In other words, self-organized amphiphilic membranes in the liquid state may be regarded either as potential traps or potential barriers with respect to NPs, depending on their hydrophobicity.

The directly observed translocation rate of the nanoparticles can further be mapped to the mean-escape-rate through the calculated free energy landscape, and the maximum of translocation can be related with the maximally flat free energy landscape (see Fig. 3). The limiting factor for the translocation rate of nanoparticles having an optimal hydrophobicity can be related with a trapping of the particles in the surface region of the membrane (see Fig. 1). Our studies reveal a generic mechanism for spherical nanoparticles to overcome biological membrane-barriers without the need of biologically activated processes.

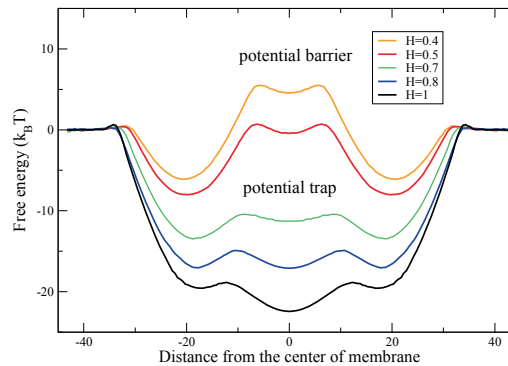


Fig. 3: Free energy profiles as a function of the distance from the membrane center, in the presence of 50 NPs, and at different hydrophobicities, H .

Sponsor:

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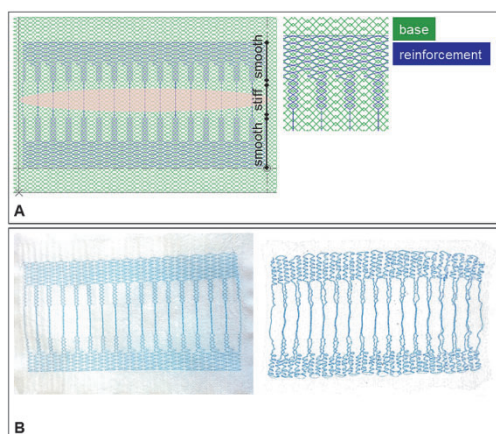
Keywords
 embroidery technology
 stress adapted hernia
 mesh
 anisotropic mechanical
 behavior

Stress adapted embroidered meshes with a graded pattern design for abdominal wall hernia repair

Annette Breier, Judith Hahn, Philipp Kempert, Axel Spickenheuer, Lars Bittrich, Cindy Elschner, Gert Heinrich, Konrad Gliesche

An abdominal wall hernia describes the expulsion of bowels through an opening in the abdominal wall, which is with an incidence of 25 million patients in 2013 one of the most relevant injuries of the digestive system [1]. The margin tissue of the cracked abdominal wall is unable to recover autonomously and untreated hernia can cause complications such as organic dysfunction, intoxication and necrosis of the particular area. Thus, surgical intervention is indispensable. A common method for hernia repair is the implantation of synthetic meshes, usually produced by warp-knitting. Although the relapse rate can be lowered, the application of alloplastic meshes can cause infection, migration, dislocation as well as intraabdominal adherence and fistula [2]. A decisive parameter for triggering malfunction is seen in the structural design of the mesh [2, 3]. Therefore, the adaption of the mechanical behavior of these meshes to the anisotropic stress-strain behavior of native healthy abdominal wall tissue is seen as a promising approach to avoid malfunction and relapse [4]. An ideal mesh should provide an adequate porosity for tissue integration, a mechanical behavior comparable to that of the healthy abdominal wall, and the ability to absorb physiological loads [5]. Embroidery technology provides the required features to design stress adapted hernia meshes [6].

Fig. 1: Mesh design with different functional domains. (A) Stitch pattern design. The hernia mesh includes a base (green) and reinforcement structure (blue) with high stiffness in the area of the opening and graded transition into the mechanical behaviour of the natural abdominal wall in the margin area. (B) Embroidered meshes with (left side) and without (right side) base material.



The aspired embroidered mesh is assembled with two units, a base structure fabricated with a degradable and biocompatible fiber (chitosan) to support ingrowth and regeneration of the natural abdominal tissue and a permanent reinforcing structure to enhance and to mimic the mechanical performance (cf. Fig. 1A). The base structure should not influence the mechanical behavior and thus has to be designed isotropic [7]. The reinforcement unit places the anisotropic behavior into the structure. It is divided into a unit of high stiffness in the area of the opening (center of the mesh) and a graded transition into the mechanical behavior of the natural abdominal wall in the margin area.

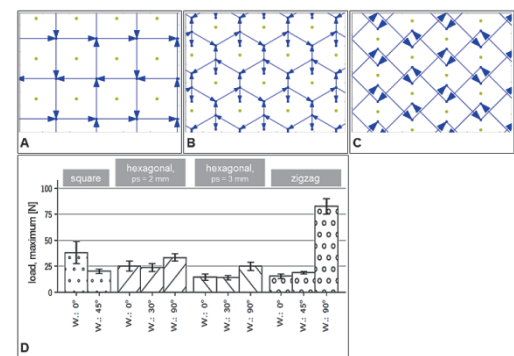


Fig. 2: Three base pattern designs (A) square, (B) hexagonal, (C) zigzag and their influence on the directional mechanical load (D).

To identify a suitable base pattern, three different designs were devised with a square (Fig. 2A), a hexagonal (Fig. 2B) and a zigzag (Fig. 2C) pattern alignment. Uniaxial mechanical testing was performed on a Zwick Roell UPM 2.5 (Zwick/Roell, Germany) approximating the DIN EN ISO 13934-1 in all directions of thread alignment investigating the load-strain behavior as well as maximum load and strain (Fig. 2D). Regarding the maximum load, the hexagonal alignment showed the maximum isotropy. Comparing the strain, an increase of isotropy could be observed from the designs zigzag to hexagonal and square. An optimized interaction of the aspired isotropic mechanical behavior is seen in a combination of the zigzag and the square pattern designs [8]. The reinforcement structure has to perform several tasks, a stiffening support in the area of the opening as well as a smooth transition into the anisotropic mechanical behavior of the surrounding tissue (cf Fig.1A).

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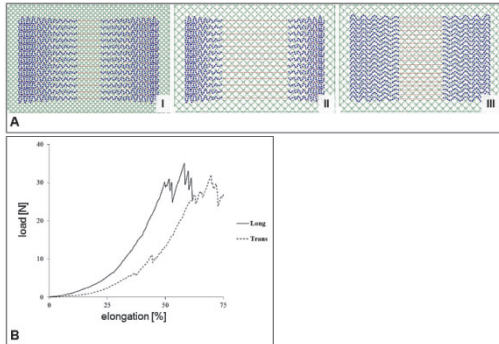


Fig 3:
(A) Examples for the design of the reinforcement structure and
(B) the influence on the anisotropic behaviour. Exemplary load-elongation curves of specimen I, reinforcement pattern longitudinal (Long) and transversal (Trans) to the tension direction.

Thus a high degree of anisotropy is demanded. Several textile parameters given by embroidery technology can be chosen to influence the anisotropy of the reinforcement structure (Fig. 3). The stiffening unit is performed by aligned stitches, so here only the stitch length and the length of the trace can be varied. For the graded transition a wavelike alignment is provided, resulting in the main parameters wavelength, amplitude and gradient. A multitude of different adjustments will be possible enabling the design of custom-made mesh structures [9].

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