Sensor -based investigations of the role of cellular and viral carbohydrates in modulating the binding and release of herpes simplex viruses from its host

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INTRODUCTION: Many enveloped viruses, including herpes simplex viruses (HSV) attach to susceptible host cells via interaction between their glycoproteins and cell-surface glycosaminoglycans (GAGs). This initial recognition is crucial in the viruses' life cycle as it leads to infection. Equally important, however, is the capability of the virus to overcome these interactions upon egress, to ensure virus propagation. In our work, we study the molecular and physical mechanisms modulating HSV binding and release using an artificial model of the cell's carbohydrate coat [1]. For HSV-1, recruitment of the virus at the host's surface is mediated by interactions between the viral glycoprotein gC and the GAGs chondroitin or heparan sulfate. We therefore investigate how gC glycosylation and GAG sulfation modulate the interaction between virus and cell surface.

METHODS: Artificial cell surface models based on the end-on immobilization of GAGs chains (Fig. 1a) were used to study virus attachment while controlling the GAG's type, degree of sulfation, and surface density. Both native (chondroitin sulfate, CS) and artificially sulfated GAGs (sulfated hyaluronic acid, sHA) were used. Dual wavelength surface plasmon resonance was used to characterize the adlayer, yielding thickness and GAG surface density. The binding and release of individual fluorescently labelled virus particles was visualized under equilibrium conditions using total internal reflection fluorescence microscopy (TIRFM) (Fig. 1b). Analysis of the arrival rate of the virions and of their residence time before release allows for quantification of the viruses' k_{on}, k_{off} and K_D.[2] In this study, the binding behaviour of wild type HSV-1 (KOSc) and mutants (KOSc-Δmuc) lacking the mucin-like region, a highly glycosylated region on the protein, is investigated.

RESULTS: Virus binding was found to be specific to the presence of sulfate groups as a non-sulfated GAG was found to be low binding. This indicates that our platform is functional (Fig. 1b). Analysis of the binding kinetics reveals that KOSc-Δmuc has a lower propensity to bind to CS than the wild type (k_{on} is ~ 15% of the one for the wild type) but that once bound, their release is hampered (mostly irreversible, very small k_{off}). [3] Moreover, KOSc binds to native and artificial GAGs with a similar affinity (K_D), although binding to native CS is characterized by (~2x) larger values of k_{on} and k_{off}.

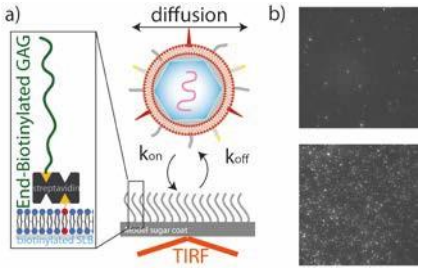

Fig1. Sensing platform to probe virus-GAG interactions. (a)GAGs are immobilized in a brush-like configuration using the biotin streptavidin linkage. (b) Individual GAG-bound virions are visualized with TIRFM. Top: hyaluronic acid (negative control), bottom: chondroitin sulfate.

DISCUSSION & CONCLUSIONS: Our data reveals that both viral protein glycosylation and cell-surface carbohydrate sulfation can significantly impact virus attachment to and detachment from the cell-surface. Our observation of the modulatory role of the mucin-like region is in good agreement with *in vitro* cell experiments which revealed that i) the mutants have a lower affinity to soluble GAGs use as binding inhibitors and ii) that the mutant is trapped on the cell surface upon egress [3]. Experiments on native and artificially sulfated GAGs further show that the attachment of the virus does not only depend on the density of the sulfate groups on the surface (which was about 10X higher for sHA) but also on the type of sulfation, and hence on the possible presence of sulfation patterns resulting from enzymatic sulfation. Our results provide new insights into the mechanisms modulating the binding and release of GAG-binding viruses, which have yet to be fully understood.

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Tunable protein immobilization at interfaces based on polyelectrolyte-protein interactions

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INTRODUCTION: Immobilizing proteins at interfaces is a necessary step in many applications in biomedical science and biotechnology. It is challenging and usually required to control the nature, amount, and activity of immobilized proteins. Since proteins are polyampholytes, they do interact with polyelectrolytes (PE), in a way which strongly depends on the pH and ionic strength of the surrounding medium. It is usually considered that PE provide a mild environment to proteins, which may help keeping their activity unaffected by surface immobilization. Here, we explore two different approaches with the aim to take advantage of the PE-protein interactions for the controlled surface immobilization of proteins.

METHODS: In a first approach, mixed brushes of poly(ethylene oxide) (PEO), a protein-repellent polymer, and of a negatively- or positively-charged PE, respectively poly(acrylic acid) (PAA) and poly(2-(dimethylamine)ethylmethacrylate) (PDMAEMA), were prepared by the "grafting to" approach, using thiol-terminated polymers and a gold substrate. These mixed brushes were used to (i) switch protein adsorption ON and OFF depending on pH and I conditions; (ii) selectively adsorb/desorb a given protein from a mixture of several proteins. In a second approach, PE-protein complexes were prepared then immobilized at interfaces under the form of layer-by-layer (LbL) assemblies. This was in particular performed for poly(styrene sulfonate) (PSS)-lysozyme complexes, which were assembled with poly(allylamine hydrochloride) (PAH). These different approaches are illustrated in Fig. 1. Polymer brush formation and protein immobilization were monitored using quartz crystal microbalance. Surface chemical composition was determined using X-ray photoelectron spectroscopy and time-of-flight secondary ions mass spectrometry. Gel electrophoresis and silver staining were used to determine the nature of proteins collected from the interface. PE-protein complex formation was assessed based on turbidimetry and dynamic light scattering measurements. Lysozyme activity was measured based on a fluorimetric assay.

RESULTS: *Approach 1a*: Adsorption from single solutions of albumin, lysozyme, type I collagen and immunoglobulin G was monitored on mixed PAA/PEO brushes for different pH and I values, to identify conditions triggering adsorption and desorption of these proteins. Each protein was repeatedly adsorbed and desorbed by switching pH and,or I values, by taking advantage of polymer conformation changes, leading to the respective preferential exposure of PAA and PEO[1]. *Approach 1b*: Adsorption was performed from single and mixed solutions of albumin, lysozyme and fibrinogen on PAA/PEO and PDMAEMA/PEO brushes, in variable pH and I conditions. It was demonstrated that the selective adsorption of one protein could be achieved, as well as the sequential desorption of the proteins when the three of them were adsorbed initially, by means of appropriate pH and I triggers. *Approach 2*: PSS/lysozyme complexes were successfully built and characterized for different PE/protein ratios. They were further incorporated in LbL assemblies, by alternate deposition with PAH. A high lysozyme activity was measured on silica particles modified by such lysozyme-containing LbL assemblies.

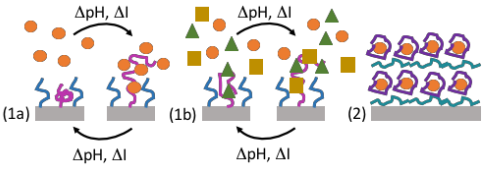

Fig. 1: Summary of the approaches used to achieve a tunable protein surface immobilization.

DISCUSSION & CONCLUSIONS: The obtained results collectively show that polyelectrolytes can be used to enhance and modulate protein immobilization at interfaces, in a very versatile way. This opens great perspectives for the development of biointerfaces with selected, controlled and switchable biological activity.

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A Conformationally Unstable DNA-Based Biosensor for the Fast Detection of Bacteria

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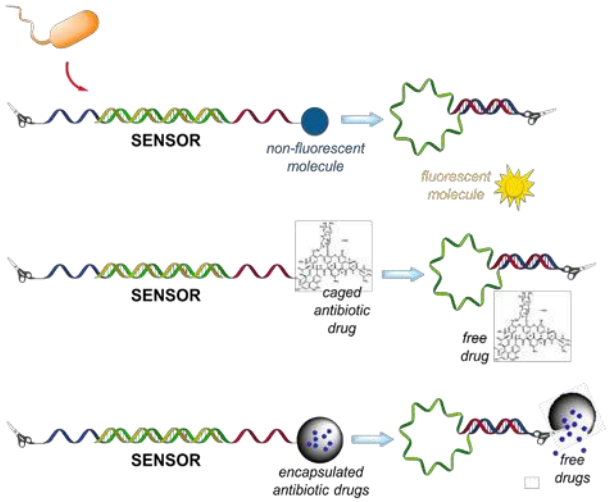
INTRODUCTION: The rapid and precise identification of bacterial species is a fundamental aspect in the treatment of infections. Nowadays the analyses are made by cell culture, but the determination is very imprecise and several different antibiotic treatments have often to be tried before to overcome a disease. DNA-based sensors, such as chips for the DNA microarray analysis[1] or molecular beacons,[2] are nowadays well established techniques that permit the detection of DNA or RNA and hence the recognition of bacterial species. However the signal produced is in most cases purely physical, which limits their application to the simple detection. The main innovation brought by our system is the release of a chemical entity as response to the presence of bacteria. The type of substrate produced by our device can be adapted depending on the targeted application, without changing the detection mechanism. This highly flexible system can be exploited to produce a simple colorimetric device, whose sensitivity could however be improved by the tuning of the released molecule, or to build a system able to release a molecule that acts directly against the bacteria or trigger an indirect response from the human body or from a device coupled to the sensor (Scheme 1).



Sch. 1: Colorimetric and drug release sensors.

The sensing element is a short DNA strand acting as a placeholder to keep open a DNA hairpin, and it is removed by hybridization with specific sequences of bacterial ribosomal RNA, which can be found in relatively high concentrations in bacterial lysates.[3] The closing of the hairpin put in proximity two reactive functions leading to a chemical reaction with release of a substrate.

RESULTS: A proof of concept of the bacterial recognition mechanism was established, by using unmodified oligodeoxynucleotides, with two different techniques – differential scanning calorimetry and polyacrylamide gel electrophoresis – which showed that the sensing process happens in a few minutes and at room temperature.

Several fully functionalized sensors with different leaving groups have been produced. The desired reactivity has however not yet been met. Problems in the stability of the final product and in the reproducibility of the coupling reactions were encountered and are under investigation.

A patent has been deposited in CH, EU, USA,CN, BR, CA, HK, SK.

DISCUSSION & CONCLUSIONS: The results obtained so far showed the high potential of the system, due to the good functioning of the recognition mechanism and to its high versatility. In the future this could be used to synthesize sensors with or without the triggering system coupled with different kinds of nanomaterials.

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I'm Rubber and You're Glue: Soft Robotic Elastomeric Systems for Control of Bioadhesion

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INTRODUCTION: It is desirable to control adhesion of biological materials to synthetic materials in a number of technological contexts. For example, there are over 30 million Foley urinary catheters sold annually, and the greatest problem with Foley catheters is catheter-associated urinary tract infections (CAUTIs). CAUTIs are the most common cause of hospital-acquired infections [1]. Biofilms on urinary catheters not only promote symptomatic CAUTIs, but may also grow to a thickness sufficient to occlude the drainage and lead to dangerous and expensive emergency treatments [2]. Our technology uses a non-antibiotic, mechanical approach to physically remove biofilms and thereby circumvent the failings of chemical and biological approaches. We have shown in several studies that a range of adherent bacterial films and some macroscopic biofouling organisms (e.g. barnacles) can be efficiently removed from elastomeric substrata through cyclic straining of elastomeric substrata. This new approach to the control of bioadhesion has numerous potential applications, including in the prevention of CAUTIs. While our presentation describes several such applications, for brevity in this abstract, we present data only on a prototype of a soft robotic catheter.

METHODS: Proof-of-concept prototypes of on-demand biofilm-removal catheter shafts were constructed using pourable silicone and 3D printed molds to incorporate an additional intra-wall lumen that could be hydraulically inflated. We assessed strain generated by intra-wall inflation and compared against strain numerically calculated using the finite element package Abaqus. Mature *P. mirabilis* crystalline biofilms were then grown on prototypes' lumens using a continuous-flow artificial bladder model. Prototypes were removed from the growth model before rinsing them at 4 mL min-1 for 1 minute. Thirty seconds into the rinse, prototypes designated for inflation were inflated 10 times to 35% strain. Biofilms in control and inflated prototypes were stained with crystal violet and compared.

RESULTS & DISCUSSION: We conceptualized and constructed a catheter shaft configuration with an additional intra-wall lumen separated by a thin wall from the main, urine drainage lumen of the catheter shaft. Inflation of the intra-wall lumen generated strain in the luminal wall without substantially affecting the external dimensions. Mature biofilms formed in the main lumen after approximately 42 hours in the biofilm growth model. The 10x inflation cycles produced visible biofilm debris in the effluent, and subsequent examination of the main lumen confirmed that a large portion of the biofilm was removed ($p<0.006$).

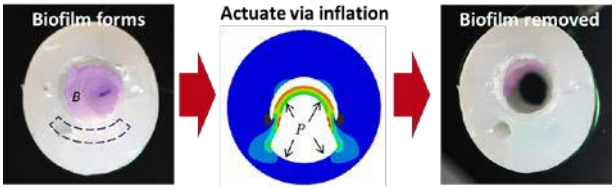

Fig. 1: Biofilm, B, was grown on the main lumen of proof-of-concept prototypes. The intra-wall inflation lumen was actuated to generate substrate strain, which thereby removed biofilm.

CONCLUSIONS: We developed a method for on-demand removal of biofilms from catheters that can be applied in the previously-inaccessible main lumen. Crystalline biofilms were effectively debonded from prototypes using this method, suggesting a promising option for control of catheter-associated biofilms and UTIs. The mechanical biofilm-removal method circumvents the many chemical and biological issues with previous approaches to biofouling control and provides the basis for the development of a new urinary catheter technology. This abstract presents only one example of the use of elastomeric strain to release surface bound biological species. Our presentation will provide a summary of our work to date.

ACKNOWLEDGEMENTS: Funding for this work was provided by the NSF's Research Triangle Materials Research Science and Engineering Center (DMR-1121107), the Office of Naval Research (N0014-13-1-0828), and The Duke/Coulter Translational Partnership.

CMOS High-Throughput Electrophysiology System to Analyze Neurons at Subcellular and Network Levels

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INTRODUCTION: Individual neurons are the primary functional units of the nervous system. Neurons form complex networks that compute and store information. The concerted electrical activity of neuronal ensembles provides the basis for the perception of the world and influences the behavior of the organism. Neural electrical signals can be recorded non-invasively and at high throughput by microelectrode arrays (MEAs). Microelectronics-based MEAs have been realized in the recent years by using standard integrated circuit or complementary metal-oxide-semiconductor (CMOS) technology [1,2]. Here, we present a novel high-density MEA (HDMEA) that enables to interrogate neural networks at unprecedented precision and to extract novel parameters that can be used to accelerate pre-clinical drug discovery and basic academic research.

METHODS: The HDMEA, as depicted in Fig. 1, features an active sensing area of 3.85 × 2.10 mm^2 hosting 26'400 platinum microelectrodes. The electrodes are arranged in a grid-like configuration with a center-to-center pitch of 17.5 µm, yielding an electrode density of 3265 microelectrodes per mm^2. The readout channels consist of three amplification stages, providing a programmable total gain of up to 78 dB for the accommodation of a wide range of different signal amplitudes. The amplified and band-pass filtered signals are sampled at 20 kHz with on-chip 10 bit analog-to-digital converters (ADCs). A 4-layer 1.6 µm-thick passivation stack, consisting of alternating SiO_2 and Si_3N_4 layers was deposited by plasma-enhanced chemical vapor deposition (PECVP). Pt is used as electrode material [1,2].

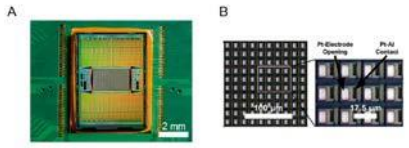

*Fig. 1, **(A)** Chip micrograph of the CMOS device. **(B)** Close-up of the electrode array [1,2].*

RESULTS: We used our HDMEA system for analyzing electrical neuronal signals in brain slices, cortical neural cultures and retinae [3-5]. We demonstrated the possibility to record sub-cellular-resolution electrical signals from neuronal compartments, including action potential propagation along axons. The high quality data generated by our technology allow for precisely detect single-neuron activity in networks of thousands of connected neurons [6]. We also used our HDMEA system for biomarker screening in mouse models of human diseases. We found a mouse model that reproduced symptoms of a human eye disease, congenital nystagmus, and which can be used to develop new treatments for this disease[7]. Finally, we simultaneously recorded from genetically identified neurons in the retina and studied their concerted activity in neural coding[8-10].

DISCUSSION & CONCLUSIONS: Our novel high-throughput technology is accurate, portable, easy to use and scalable. We have demonstrated the capabilities of our system by (I) discovering novel biomarkers of human diseases[7] and (II) by measuring neuronal activity parameters at subcellular and network levels, which was not possible with previous MEA technology[3,4,10]. We intend using our technology to support preclinical drug development in order to reduce the costs and for basic academic research.

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DNA-Surface Technology – Beyond transcript profiling and genotyping

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INTRODUCTION: Beginning with the early observation that denatured DNA binds to glass surfaces in 1979, the advent of DNA microarray technology in the course of the human genome project in the late 1980s has led to the evolution of sophisticated DNA-functionalized solid substrates which are nowadays routine tools for fundamental and applied research in biology and medicine [1]. However, the versatility of these microstructured DNA patterns goes far beyond the etstablished applications in genotyping and expression profiling because their capability for highly parallel, site-directed immobilization of complementary nucleic acids through canonical Watson-Crick base-pairing can be harnessed to assemble complex surface architectures comprised of colloidal materials and proteins [2] (Fig. 1). While these approaches enable novel sensor platforms for protein and small-molecule analysis and even cellular processes, the full potential of DNA surfaces can be exploited by implementation of functional DNA devices and structural DNA nanotechnology.

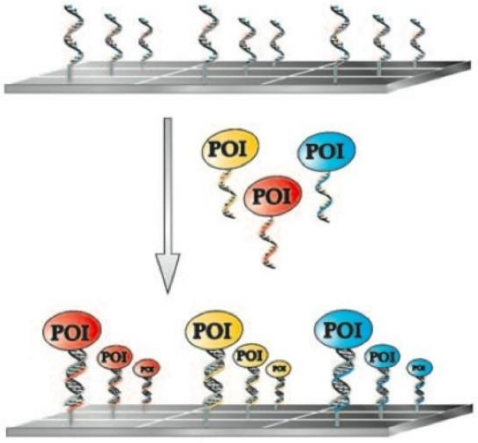

Fig. 1: Principle of DNA-directed immobilization (DDI) of proteins on DNA mircoarrays (POI = protein of interest)

METHODS: Surface chemistry and (bio)orthogonal coupling strategies for site-selective positioning of proteins and other ligands along with bottom-up self assembly, top-down microstructuring and microfluidics are essential methodologies required for state-of-the-art DNA chip applications in biomedical research.

RESULTS: In this survey, I will set the historical development of DNA chips into perspective of current developments. Based on the initial description of oligonucleotide-directed self-assembly of proteins [3], this approach has been refined in order to produce nanoscaled multi-protein arrangements on DNA nanostructures, useful for biosensing and biocatalysis. Likewise, on the micrometer length scale, the DNA-directed immobilization (DDI) of proteins on solid surfaces gives rise to production of protein biochips (Fig. 1). Owing to the lean and highly hydrated double-stranded DNA linker, the proteins are undisturbed by the surface and maintain their biological activity. The DDI method has meanwhile been used in numerous studies of many labs worldwide, primarily in the areas of biomedical diagnostics and proteome research [2]. Due to the high quality of DDI-based protein arrays and because the approach is compatible with bottom-up nanostructuring, this approach becomes increasingly important for advanced applications in molecular cell biology. A recent case study includes the development of a multiscale DNA-based platform to investigate fundamental processes of early cell signaling [4].

DISCUSSION & CONCLUSIONS: The aforementioned case studies of our group along with the results of other laboratories clearly indicate that DNA-surface technology has reached the state of a sturdy and reliable platform for the in-depth investigation of biological processes. The current developments suggest that this approach hold a great potential for applications aiming towards monitoring and controling living systems.

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Slippery interfaces: A new concept in anti-biofouling materials

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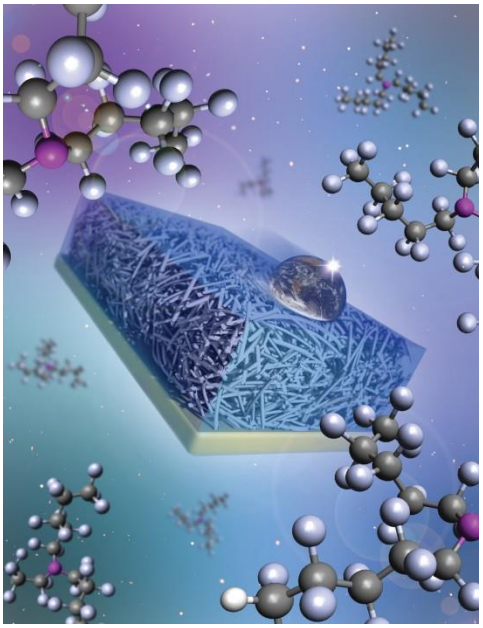
Abstract



Living organisms and biological substances are among the most difficult and persistent sources of surface fouling, particularly in medical and marine settings. The ability of organisms to adapt, move, cooperate, evolve on short timescales, and modify surfaces by secreting proteins and other molecules enables them to colonize even state-of-the-art antifouling coatings, and small surface defects can trigger protein aggregation and blood clotting. Attempts to combat these issues are further hindered by conflicting requirements at different size scales and across different species. Our recently developed concept of Slippery, Liquid-Infused Porous Surfaces (SLIPS) provides a defect-free, dynamic liquid interface that overcomes many of these problems at once. A single surface is able to prevent adhesion of a broad range of genetically diverse bacteria, including many pathogenic species that underlie widespread hospital-acquired infections, as well as marine algae. The same approach resists adhesion of proteins, cells, and blood, preventing clogging and thrombus formation inside medical tubing and catheters. At a larger scale, the slippery interface repels insects, barnacles and mussels, which slide off and actively avoid the coated surface. We are currently developing this strategy to solve longstanding fouling issues in a wide range of medical, marine, and other settings.

Drops: A tool to structure materials

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Abstract

Drops are well-suited templates to produce nanoparticles of a defined size, shape, and composition. For example, they are often used to produce powders in the food and pharmaceutical industries through spray drying. In this case, drops are usually dispersed in air and their size is adjusted to tune the size of the spray-dried particles. In addition, drops can be used to control the structure of these particles by tuning the evaporation rate of the solvent. Indeed, if drops are made sufficiently small, such that they have a high surface-to-volume ratio and therefore dry very quickly, they can even produce amorphous particles from materials that have a high propensity to crystallize. In the first part of my talk, I will present a microfluidic spray drier, a nebulator, that produces 300 nm diameter drops that are surrounded by air that flows at supersonic speeds. These drops dry so quickly that crystallization of solutes, contained in the drops, is kinetically suppressed. Hence, the resulting spray-dried particles are amorphous even in the absence of any crystallization inhibiting additives.[1] This is particularly beneficial for the formulation of hydrophobic substances, such as many newly developed drugs or certain food additives, whose bioavailability is limited by their slow dissolution rates and low solubility.

Drops are also well-suited templates to produce microparticles and microcapsules of a controlled size, shape, and composition. In this case, drops are usually dispersed in a second, immiscible liquid. The resulting liquid-liquid interfaces offer the possibility to tune the surface chemistry of these particles. In the second part of the talk, I will present examples where the liquid-liquid interfaces of double emulsion drops, which are small drops contained in larger drops, are employed to produce self-assembled monolayers of amphiphilic molecules that are used to functionalize surfaces of microcapsules, thereby making them responsive to external stimuli[2] or mechanically very robust.

Influence of polysaccharide chain conformation on friction and adhesion

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Abstract

This presentation will cover research on tribology of various naturally occurring polysaccharide systems. These investigations include the role of hyaluronan in the lubrication of natural cartilage, the effect of chain conformation in mucins on friction and adhesion, and the hydration of glycans in glycoproteins.

Chemical and mechanical analyses of the superficial layer of cartilage led to the conclusion that the surface was coated with a soft gel-like layer consisting mainly of hyaluronan. The extent to which this layer plays a role in lubricating cartilage could not be determined but it may explain the lower friction values that are obtained when intact joints are measured compared to excised cartilage.

Hyaluronan is a very long straight-chained polysaccharide that forms a random structure in synovial fluid, at an approximately three times higherconcentration than the one allowing a fully relaxed conformation. In mucins, the saccharide chains are much shorter and tend to form ordered, brush like structures around the protein backbone. Friction studies on ordered brush-like polymers with dextran as the side-chain showed that there was a dramatic change in friction as load increased. This behaviour was compared to that of random structures of dextran and it was found that the highly ordered structure was optimal for both low friction and high adhesion.

Glycoproteins were found to have a beneficial effect on the lubrication of artificial joint materials. In addition to examining the friction behaviour of alpha-1-acid glycoprotein and alpha-1 antitrypsin, their ability to resist force was also studied using a surface force apparatus. These measurements showed the presence of highly ordered water molecules at the surface of the glycan residues.

From simple diagnostic platforms to genetically-encoded discovery of serological diagnostics

Ratmir Derda

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Abstract

In this presentation, we will describe our efforts towards development of portable diagnostics using a combination of simple tools such as paper based platforms and bacteriophage-based detection. Our efforts build on paper-based devices, which have been proven to be a versatile platform for culture of mammalian or bacteria cells and detection of bacteria in resource limited environment. Bacteriophage offers another opportunity for simplification of bacterial detection, increasing the sensitivity and decreasing the detection time. One of the putative detection platforms combines micro emulsions and phage-based bacteria detection. With the development of portable emulsification solutions, emulsion-based detection platforms offer a practical promise. Finally, we will describe how bacteriophage-displayed libraries can be used for discovery of novel molecular components for simple serological tests for Tuberculosis. Specifically, we will focus on discovery of mimetics of mycobacterium-derived antigen lipoarabinomannan (LAM) using genetically-encoded libraries of glycopeptides

Tissue engineering for medicine and pharma

Ursula Graf-Hausner

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Abstract

Human three-dimensional (3D) tissue models offer new perspectives as R&D tool for regenerative medicine and drug development in pharma industry. 3D tissue models are used as biologically relevant systems for preclinical compound screening and disease research. They help to identify potential toxic liabilities in an early phase of the drug discovery process and enable the reduction of animal experiments. Scaffold-free spheroids are going to be a new standard for preclinical *in vitro* testing. Microtissues produced with patient derived cells reflect the tissue heterogeneity and are potential suitable tools for drug development towards personalized medicine. Scaffold-based tissue models could be used as tool to investigate possible strategies for regenerative medicine. As an example, we established an *in vitro* model of a periodontal pocket to assess an injectable self-assembling peptide system to treat periodontitis by regeneration of the periodontal ligament. Organ-like tissue models should simulate the high complexity of our body. In order to meet this goal, innovative technologies like 3D bioprinting and microfluidics have to be integrated into the production and maintenance process of tissue models.

Bioprinting allows the precise deposition of cells, matrix, and other bioactive molecules in 3D space and is therefore expected to mirror the *in vivo* tissue complexity. We developed a bioprinting solution with the following features: i) micro valve-based inkjet printheads for cell jetting and contact printing with needles to print into 96 well plates, ii) a chemically-defined ECM-surrogate BioInk that is print- and cytocompatible, iii) a photopolymerization unit to crosslink the BioInk with UV-LED (365 nm) and iv) a cell mixing unit to avoid cell sedimentation in the print cartridge during printing. For tissue generation one layer of BioInk is printed and polymerized providing a stable support for the subsequent printed cell layer. This process is alternated to produce a multi-layered 3D tissue construct. In a proof-of-concept study we established robust protocols to print full-thickness skin equivalents [1] for future use in the cosmetic industry. In an industry-driven research project we are developing an *in vitro* tool for drug assessment to treat muscle-related diseases. The idea is to provide an all-in-one solution to produce and analyze printed *in vitro* muscle/tendon tissues in a well plate. The customized 24 well plate harbors two posts in each of the wells. The final goal is to print muscle/tendon precursor cells around and in between the posts to induce tissue formation with tendon around the post and muscle fibers between the posts. First, monocultures of primary human myoblasts and primary rat tenocytes were printed separately in a dumbbell-shape around the posts. After cell differentiation the myoblasts were stained positive for myosin heavy chain (MHC) and myotubes developed and for tendon the characteristic collagen I-distribution around the cell nuclei was detected [2]. The printed muscle tissue is contracting upon electrical stimulation and shows physiological functionality.

Bioprinting is also used in a project to produce tubular structures in a hydrogel. Preliminary experiments show that the proximal tubule of the kidney is printable with proximal tubule epithelial cells to cultivate them under physiological flow conditions.

The development of standardized 3D *in vitro* tissue models combined with read-outs is a prerequisite for the future success of 3D tissues in drug development and substance testing.

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Translational barriers to antimicrobial medical technologies

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Abstract

Increasing placements of medical devices now available for clinical implant use, often in aging populations and increasingly in developing countries, are a significant health care issue due to enhanced infection incidence intrinsically related to implanted medical devices. These devices are also implanted against a background of increasing bacterial antibiotic-resistance. Improvements in the prevention, diagnosis, and treatment of these device-associated infections will remain priority targets both for clinicians and the translational research community charged with addressing these challenges with innovation. Diverse approaches have been historically used to counter device-related infection, including antibiotic lavages, locally tethered or released antimicrobials, device coatings, local electric fields and current applications, and newer approaches targeting bacterial adhesion mechanisms, communication pathways, and virulence factors. Combination medical devices provide new innovative opportunities using local antibiotic formulations released from established classes of implants.[1-3]

Improved methods are required to assess infections and to implement new technologies that reduce implant-associated infections (*see R. Luginbuehl abstract*, this session). A classic problem is lack of in vitro-in vivo correlations, validation or efficacy for any given antimicrobial method or approach.[4] A second issue is the lack of clear regulatory requirements for antimicrobial implant product approval and reimbursement guidelines for antimicrobial products. The end result is substantial translational risk, unknown product outcomes, and a critical lack of industrial or commercial enthusiasm to take any approach forward toward clinical use.[2,5] Introduction of new antimicrobial experimental designs, approaches to endowing medical devices with antimicrobial properties, including on-board antibiotic-releasing depots and device translation in vivo are therefore often caught in a translational impasse between basic science and technology.[2,4,5]

Despite the many biomaterials designs published, innovations and antimicrobial approaches that address infection prophylaxis and treatments, few approaches ever make it to clinical use. Few strategies to date show much efficacy in vivo in humans despite promising in vitro anti-microbial efficacy and even some promise in (largely rodent) animal implant models.[4] Scientific issues involve inadequate evaluation methods, including problematic, non-predictive in vitro assays and also irrelevant animal models of infection with devices. Fewer still are commercialized for global use.[3-5]

Blame for this problem is often attributed to massive, inflexible government bureaucracies and inability to navigate conflicting or complex medical device regulatory policies.[2,3] However, other factors, including ignorant “expert” peer review communities for research grants and scientific journals, capitalistic forces with unreasonable cost-development timelines for commercialization, and inadequate translational mechanisms at academic institutions also loom as barriers.[5] Clinical translation to humans is also stymied by the formidable costs of conducting clinical trials.[5] To better impact device-centered infections, basic research must appreciate requirements of industrial partners required to commercialize these technologies, and regulatory agencies must accept new affordable clinical trial designs and surrogate endpoints defining human success.

Engineering proteins and polymers for immunological tolerance

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Abstract

Vaccines, in which antigen and adjuvant molecules are delivered together to induce effector memory immune responses, have been transformative in human and animal health. In addition to inducing effector adaptive immune responses, so-called inverse vaccination to induce antigen-specific tolerance is of high interest. We are exploring biological and polymer approaches to deliver protein antigens in a tolerogenic manner, including targeting antigen to the surfaces of erythrocytes after injection, based on the premise that aged erythrocytes are cleared tolerogenically, along with exogenous antigen cargo they may carry. We have shown the ability to induce antigen-specific anergy as well as T regulatory responses, working in models of autoimmunity and of immune response to protein drugs. The liver is a target of particular interest, and we are developing polymers that can target antigen, tolerogenically, to particular cell populations in the liver.

Proteins associated with the surfaces of apoptotic cell debris are processed by antigen-presenting cells in a tolerogenic manner, based on the biological activity of a number of apoptotic signals. We have sought to exploit this by expressing antigens as fusion proteins with antibody fragments that target glycophorin A, a marker of erythrocytes, or by conjugating antigens to peptides that target glycophorin A, reasoning that as the cells eryptose, they and their carried antigen will be cleared. For example, using this approach with the therapeutic protein E. coli asparaginase, we were able to escape immune detection over numerous sequential injections [1]. With a diabetes antigen, we were able to prevent onset of diabetes in an adoptive T cell transfer model in the NOD mouse [2].

We are also exploring a second approach, where we use polymers that mimic the glycome of apoptotic debris, which are rich in sugars such as N-acetylgalactosamine residues, as a result of cleavage of terminal sialic acid residues by neuraminidases. Antigen conjugates with such polymers are cleared efficiently in the liver and induce tolerance sufficient to prevent onset of diabetes in an adoptive T cell transfer model in the NOD mouse.

Solubilization of brick dust – Chemistry, physics and processes

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Abstract

With pure synthetic polymer chemistry getting more and more a mature science, interdisciplinary cooperation is gaining increasing importance. In particular with respect to applications in the life sciences, where materials need to be designed to directly interact with living systems, cross-collaboration beyond the borders of traditional chemists is the key to success. This presentation will illustrate three examples from the fields of pharmaceutical drug formulations addressing current industry needs.

One of the biggest issues of the pharmaceutical industry is poor solubility of many newly developed drug candidates leading to poor adsorption in the gastro-intestinal tract and, thus, limited biological availability of the drug.[1] This problem can be overcome in several ways, especially by increasing the apparent solubility of the active by means of additives referred to as solubilizers,[2] or by nanoscale structuring of the solid active formulation leading to an increased dissolution rate of the drug.[3] Also, amorphous drug formulations [4] and co-crystals [5] have gained increased attention in the last years.

Although registration hurdles for new pharma polymers are high, a new polymeric solubilizer has been developed and introduced into the pharmaceutical markets. This new polymer is a graft copolymer of vinyl acetate and vinyl caprolactam on a PEG backbone, leading to an amphiphilic polymer which both in the solid state and after dissolution interacts with the drug and stabilizes the amorphous and the molecularly dispersed state, respectively.[6]

Nanostructuring of organic matter can also be used to increase the bioavailability of poorly soluble actives.[7] These approaches are particularly effective when combined with polymeric solubilizers, as we will present in this lecture (Fig. 1).

Finally, the formulation of poorly soluble drug molecules in tailored co-crystal architectures can break down the crystallization enthalpy of the drug and lead to significantly enhanced bioavailability - as well as IP opportunities.

Development of antimicrobial medical devices: Caught between scientific excellence and industrial constrains

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Abstract

The clinical problem of device associated infection is probably most visible in the pure number of published papers on the topic that exceeds 25'000 published articles in peer-reviewed journals in the past twenty years. Most of that literature is focusing on clinical manifestation and administration of a therapy, which is typically based on systemic antibiotic therapy and eventual removal and exchange of the affected devices. Only a minor fraction, about 3'000 articles, elucidate on novel materials and engineered surfaces that exhibit antimicrobial properties.

The origin of any device associated infection is always that bacteria adhere to the material surfaces or settle in nearby tissue [1]. These bacteria are protected in part from the host immune system since it is compromised near and at the material surfaces. Too often, bacteria escape therefore fast eradication by the immune system, proliferate, and form a protecting biofilm which is evidenced as a persistent infection. Based on mechanism, it is hypothesized that the rate of infection can be reduced by engineering novel materials that either prevent bacterial adhesion or inhibit and kill bacteria at or near the material surface [2]. Many innovative concepts have been suggested and tested demonstrating the potential suppression of bacterial activity at the material surfaces. But unfortunately, only very few and very simple developments have been translated from the bench to a medical device product. On the one hand, it is due to the fact that the concepts have to be specifically tailored and separately optimized for each device application which prevents a mass production. On the other hand, industry has to meet very stringent requirements and prove efficacy and safety of such materials. This puts emphasis on proper and comprehensive characterization and *in vitro* evaluations [3]. Those points are most often neglected by academic researchers that focus foremost on highest impact of their publications and less on adequacy and significance of their testing methods. The evaluation methods rely on a limited set of configurations which all have different prerequisites and yield diverging information. Within the framework of a so-called COST Action, a Pan-European effort supported by the EU Framework Program Horizon 2020, one of the aims entails to advance the topic of best practice for selecting the proper test and evaluation methods [4]. Since there is no such a test method as "one fits all", it requires a combined effort between material engineers, molecular biologist, microbiologist, and clinicians, to categorize the available test methods and discuss them in detail according to their working mechanism. These efforts should yield international standard guidelines and test methods for evaluating the antimicrobial efficacy of materials used in medical devices. In that regard, reference systems, test conditions, and relevant application specific bacterial strains are suggested. Such a best practice will allow for benchmarking the engineered antimicrobial surfaces and thus, for cross-comparison of reported results in literature in the near future.

Nanopores with bio-inspired surface coatings for single protein analysis

Michael Mayer

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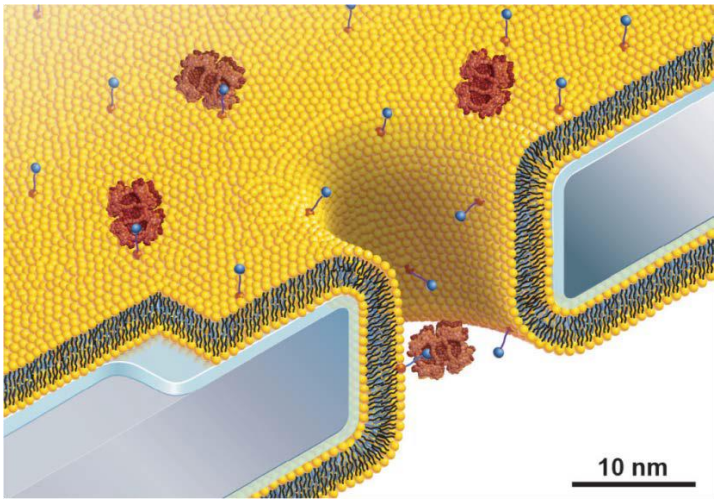
Abstract

Our group is interested in developing novel strategies to explore the structure and function of single proteins in solution. As an example of our advances in this area, this talk describes the use of electrolyte-filled nanopores with self-assembled lipid membrane coatings to determine, simultaneously and in real time, the shape, volume, charge, rotational diffusion coefficient, and dipole moment of individual proteins and protein complexes. It introduces the main concepts for a quantitative understanding and analysis of modulations in ionic current that arise from rotational dynamics of single proteins as they move through the electric field inside a nanopore. The resulting multi-parametric information raises the possibility to characterize, identify, and count individual proteins and protein complexes in a mixture. This approach interrogates single proteins and determines parameters such as the shape and dipole, which are excellent protein descriptors and cannot be obtained otherwise from single proteins in solution. Hence, this five-dimensional characterization at the single particle level has the potential for instantaneous protein identification, quantification, and sorting with exciting implications for protein folding studies, structural biology, proteomics, biomarker detection, and routine protein analysis.



Engineering the microenvironment with biomaterials

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Abstract

The microenvironments of tumors and immune tissues clearly play a number of key roles in cancer. Biomaterials allow one to recreate and engineer, in 3D, highly controlled microenvironments to both study and manipulate cancer in the laboratory and in the body (Gu and Mooney, 2015). We have been developing biomaterials to study how aspects of cancer biology are regulated by the dimensionality and stiffness of the materials (Chaudhuri et al., 2014; Branco da Cunha et al., 2016).We foundthat angiogenic signaling, the transition to malignancy, and cell surface receptors are all controlled by microenvironmental signals. Therapeutic cancer vaccines are being developed with some of these same materials to create new microenvironments in the body, in which immune cells can be programmed to direct both cellular and humoral responses against cancer antigens (Ali et al., 2009; Kim et al., 2015; Bencherif et al., 2015). These biomaterial-based vaccines, based on some of the same biomaterials utilized for 3D cell culture studies, have demonstrated highly potent anticancer activity in preclinical studies. Altogether, these findings demonstrate the potency of biomaterials, and bioengineering more broadly, to address key issues in cancer biology and therapy.

A novel biointerface that suppresses blood coagulation and cell morphological changes by scavenging excess reactive oxygen species

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Abstract

Various materials are employed in blood-contacting implantable and extracorporeal medical devices, such as artificial hearts, artificial blood vessels, hemodialyzers and apheresis columns. Since most of those medical devices have poorly biocompatible surfaces, anticoagulants such as ethylenediamine tetraacetic acid (EDTA), hirudin, heparin, and warfarin are utilized to prevent thrombosis and embolism induced by contact between blood and these medical devices. Given the side-effects of these anticoagulants, such as heparin-induced thrombocytopenia, however, numerous efforts have been made to reduce the activation of blood in response to contact with material surfaces. Suppression of such blood activation can effectively reduce the amount of anticoagulant required. In order to improve the blood compatibility of material surfaces, a number of versatile methods have been applied. One of the most simple and important techniques is polymer coating using biocompatible polymers such as poly(ethylene glycol), zwitterionic polymers, microphase-separated polymers, and poly(2-methoxyethyl acrylate). These approaches can drastically reduce the adsorption of serum proteins. Protein adsorption triggers the activation of blood cells and blood coagulation on material surfaces through a complex series of events, including the activation of platelets, leukocytes, complements, and the fibrinolytic systems. Thus it has long been thought that the suppression of protein adsorption is highly important in the design of blood-compatible surfaces. Nevertheless, even today, all blood-contacting devices cause thrombosis with long-term usage. In fact, synthetic vascular grafts with inside diameters of less than 6 mm cannot be used because they are prone to early thrombotic occlusion. In the case of cardiac stents, drug-eluting stents have been developed to inhibit endothelial cell proliferation and restenosis, but even these stents were found to be thrombogenic. Interestingly, it has been revealed that blood–material interactions cause an increase in the levels of reactive oxygen species (ROS) and inflammatory cytokines, which is brought about by the activation of blood cells; this leads to blood coagulation and whole body inflammation. The excess ROS continuously amplify inflammation, thereby increasing the risk of potentially life-threatening disorders. Indeed, long-term hemodialysis therapy induces cardiovascular disease and atherosclerotic complications, which result in high morbidity and mortality in chronic renal failure patients. However, there have been few reports with experimental evidence that ROS generation is related to inflammation in blood when in contact with the material surface, and the extent of ROS involvement in inflammation is not yet clear.

We have focused on the effect of ROS-scavenging materials in vivo and have used nitroxide radicals, such as 2,2,6,6-tetramethyl-piperidine-N-oxyl (TEMPO), which catalytically react with ROS and can be used as electron spin resonance (ESR) probes in vivo. Core–shell type polymeric micelles containing nitroxide radicals have already been developed for the treatment of oxidative stress injuries and in vivo ESR imaging. It has been confirmed that they not only strongly scavenge ROS in vivo and in vitro but also significantly prevent oxidative stress damage in a cerebral ischemia–reperfusion injury model in rats, renal ischemia–reperfusion injury model in mice and a neuron cell line used as a model for Alzheimer's disease, in which excess ROS is generated. In the process, we have hypothesized that ROS-scavenging characteristics play a crucial role in blood activation and coagulation when blood comes into contact with material surfaces. To validate our hypothesis, we have designed and developed nitroxide radical-containing homo- and co-polymers, viz., poly[4-(2,2,6,6-tetramethylpiperidine-1-oxyl)aminomethylstyrene] (PMNT) and PEG-b-PMNT. In this study, we have investigated a blood compatibility of these nitroxide-radical containing polymers. In addition, we applied this antioxidative biointerface for the separation of hematopoietic stem progenitor cells (HSPCs) from the liver of fetal mice and confirmed that the separated HSPCs prevented undesired non-specific differentiations, possessing high viability tendency.

Because the nitroxide radical-containing polymers prevent blood coagulation in addition to suppression of inflammation as stated above, we applied this mechanism for organ-anticoagulation materials. We designed redox flower micelle, which possesses nitroxide radicals in the core and converts to hydrogel under physiological temperature and ionic strength conditions and named as a redox injectable gel (RIG). When the RIG solution was sprayed to organ surfaces after physical surgery, the formed gel on the organ surfaces strongly prevents their adsorptions due to the elimination of ROS in addition of physical separation. The presented nitroxide-radical-containing polymers are thus promising as new biomaterials.

Polymer membranes decorated with biomolecules: Novel systems with medical potential

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Abstract

Modern medicine is focusing on the development of new concepts that combine multifunctional compounds with stable, safe carriers or membranes in patient-oriented diagnostics or therapeutic strategies. Suitable amphiphilic block copolymers can self-assemble into 3D supramolecular assemblies, such as compartments with sizes in the nanometer range, or membranes mimicking biological membranes.[1] Compared to conventional, low molar mass building blocks (e.g. lipids), membranes based on macromolecular self-assembly have advantages of superior stability, robustness, and possibility to tailor their physical, chemical, and biological properties.[2] Here, we present protein-decorated membranes as selective permeable walls of compartments or as bilayers on solid support that provide distinct spaces for desired reactions at the nanometer scale. Biopores and channel proteins inserted into the polymer membrane of compartments selectively control the exchange of substrates and products with the environment. In this was they support an *in situ* activity of the encapsulated enzymes, and therefore the development of artificial organelles mimicking natural organelles, upon up-take in cells.[3] Biopores and membrane proteins inserted in solid supported polymer membranes serve to mediate a transport of ions/molecules through the membrane, and therefore to induce biofunctionality.[4] The encapsulation and/or insertion of active molecules (enzymes, proteins, mimics) in polymer compartments and membranes provide functionality to the hybrid materials, while the synthetic membranes support their stability and robustness, as essential factors for applications.[5]

The properties of such membranes can be extensively controlled via chemical composition, molecular weight and the hydrophilic-to-hydrophobic block length ratio of the polymers. These nanoscience based concepts open new avenues in protein therapy (artificial organelles) as well as sensing approaches (“active” surfaces).

Lung tissue engineering to study the pulmonary administration of drugs – Opportunities and challenges

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Abstract

The human body has different entry portals for drug administration, such as the lungs via inhalation, the gastro-intestinal tract via digestion, the blood vessels via intravenous injection, and the skin via transdermal passing. The lung with its huge internal surface provides a non-invasive and promising portal of entry for any type of aerosol, *i.e.* micro-, nanoscaled particles or drugs (Mueller et al. 2014). Depending on size and properties, inhaled materials / drugs can enter the lung and interact with the respiratory wall. While larger aerosols are deposited in the conducting airways, smaller ones reach the peripheral gas exchange region and may pass the air-blood tissue barrier to enter the systemic circulation.

There is an urgent need for novel technologies to accelerate the selection of novel drug targets which can be used via inhalation. It has been realized in the past that advanced 3D *in vitro* cultures, *i.e.* scaffold-cell combinations (or different types of cells), close the obvious gap between simple monolayer cultures and animal models, combining the advantages for increased throughput capabilities as well as predictive power (Alepee et al. 2014). In addition, air-liquid exposure systems have been developed allowing a more realistic aerosol exposure in the lung cell system than the commonly used suspension exposure method. Further, several different systems have been developed for the dose-controlled deposition of various aerosols at the air-liquid interface of cultured lung cells allowing a full control over material characteristics and also monitor mass deposition on the lung cell surface on-line, which will allow us to produce a dose-effect correlation (Müller et al. 2011).

In conclusion, there has been a clear trend over the past few years to develop reliable 3D multicellular lung tissue models that allow the investigation of possible effects of materials / drugs under more realistic conditions such as aerosol exposure at the air-liquid interface. The advantages and limitations of the different *in vitro* cell and exposure approaches will be discussed in order to provide a clear overview as to whether or not lung cell cultures are a preferable model for basic mechanism and risk assessment in the field of drug discovery.

Robust and physiologically relevant characterisation methods for contact lens medical devices: A step into better understanding of tribological impact in the eye

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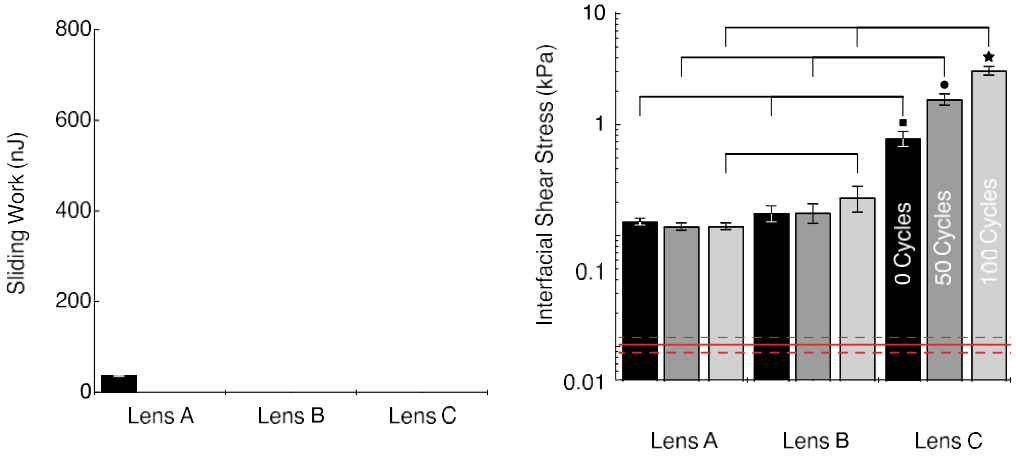
Abstract

Over the last decade, industrial and academic interest in physiologically-relevant tribological measurements of contact lens medical devices has grown significantly. Much of this interest can be attributed to correlations between the measured coefficient of friction (CoF) of a new, unworn contact lens and its level of comfort, measured subjectively in a clinical trial. Generally, for many contact lens materials, a lower CoF value measured on the unworn lens has been associated with a greater level of subjective patient-reported comfort at the end of the day; however, this initial CoF value (*i.e.* that measured after removal from the package) is not always predictive of clinical performance. This is because a lens undergoes exposure to the tear-film, which is a complex biological lubricant, as well as mechanical stress when blinking. Because of the interaction of soft contact lens materials with the tear-film components (*i.e.* through uptake of lipids, proteins, mucins, certain ions, *etc.*), the way in which a given lens is conditioned in the presence of a physiological fluid (*in vitro* or *in vivo*) can substantially affect its tribological behavior. For these reasons, a methodology for assessing several key tribological features of contact lenses in a physiologically-relevant *in vitro* experimental condition was established.[1] This method combines a controlled physiological conditioning step with the measurement of contact lens CoF, frictional energy (E_f), and shear force (F_s), using a micro-tribometer equipped with a mucin-coated glass counter-surface and an optical microscope. The development of this methodology along with the tribological assessment of several leading contact lens materials are presented and correlated with additional confocal fluorescence microscopy measurements showing contact lens/tear-film component interactions.



Engineering of complex vascularized tissues and their application

Heike Walles

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Abstract

Vascularization is a major challenge in creating tissues *ex vivo*. Complex tissue engineered constructs exceeding a thickness of 100-200 µm need a vascular system in order to supply the cells with oxygen and moreover remove waste products. This restricts generation of tissues with an appropriate size for clinical application.

We developed 3D vascularized tissues based on decellularized porcine small bowl segments and preserved tubular structures of the capillary network within the collagen matrix which is functionallly associated with one small vein and artery (**bio**logical **va**scularized **sc**affold - **BioVaSc**). This vascularized matrix enables the generation of a functional artificial vascular network and vascularized tissues such as trachea[1], bone[2], skin[3], tumour[4], fatty tissue and intestine.

These vascularized tissues are applied as tissue models as a preliminary stage, prior to animal experiments, to investigate functional parameters like penetration, distribution, and metabolization of substances in different tissue layers. Further, with the help of suitable markers, issues concerning the proliferation, differentiation, cell death, but also, the initiation and graduation of tumors of the applied cell types can be examined. First clinical application will be shown during the presentation[1].

Non-invasive monitoring of such engineered tissues during their in vitro maturation or post implantation in vivo is relevant for graft evaluation. However, traditional methods to analyses cell and matrix components in tissue engineered constructs such as histology, immuno-histochemistry, or biochemistry requires invasive tissue processing, resulting in scarification of these constructs.

At the end of the presentation an overview of non-destructive methods as impedance[5] and Raman spectroscopy to characterize the complex implants will be given.

Acknowledgments:
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Glycosaminoglycan-based, cell-instructive hydrogels

Carsten Werner

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Abstract

Sulphated and non-sulphated glycosaminoglycans (GAGs) can be instrumental in biomedical technologies . Sulphated and non-sulphated glycosaminoglycans (GAGs) can be instrumental in biomedical technologies beyond. In particular, incorporation of GAGs into biomaterials has been demonstrated to allow for the biomimetic modulation of growth factor signaling, providing control over therapeutically relevant cell fate decisions in various different settings. In an attempt to systematically explore the related options, we have introduced a rational design strategy for biology-inspired hydrogels based on multi-armed poly(ethylene glycol), GAGs and peptides (1,2,3). The theoretically predicted decoupling of biochemical and mechanical gel properties was confirmed experimentally and applied for implementing GAG-based biofunctionalization schemes to afford cell adhesiveness and morphogen presentation. A number of applications of customized GAG-based materials will be given, including inflammation-modulating wound dressings (3), cryogel particles to support cell replacement in Parkinson's disease (4) and gel matrices to enable tissue and disease *in vitro* models for cancer biology (5,6) and nephrotoxicity studies. In sum, our reported approach demonstrates the power of joint theoretical and experimental efforts in creating bioactive materials with specifically and independently controllable characteristics (7).

Biomolecular synthesis of ssDNA and its interactions in solution and with surfaces

Stefan Zauscher

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Abstract

Polynucleotide block-co-polymers show rich micellization behavior in solution, and hold promise as surface-tethered brushes as novel functional materials for nano- and biotechnological applications.[1] To date, however, the enzymatic synthesis of block-co-polynucleotides with high MW is still a challenge. Moreover, little is known about the design rules for the micellar morphologies that are formed by these polymers.[2] Here, we report on our approaches to enzymatically synthesize polynucleotides on surfaces and in solution and their assembly into micelles (Fig. 1) and networks.[3] Specifically, we provide a kinetic model and the design rules for the synthesis of multi-functional polynucleotides that self-assemble into highly modular micellar systems with potential for drug delivery applications, furthermore we show the synthesis of polynucleotide brushes for sensing applications.

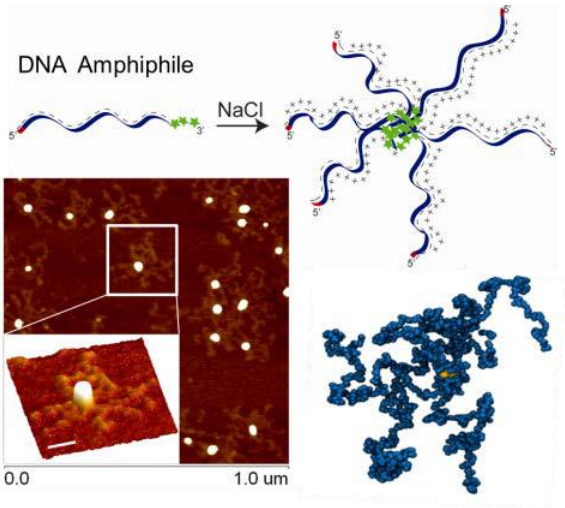

Figure 1: Clockwise, self-assembly of polynucleotide amphiphiles into star-like micelles, a snapshot from the DPD simulations of micellization, and AFM height image of star-like DNA micelles on a mica surface in air.

Visible light –induced living surface grafting Polymerization from organic monolayers

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INTRODUCTION: Controlled synthesis of functional macromolecular architectures at interfaces have attracted great scientific and technological attention.[1] Among the different approaches, living free-radical polymerization can effectively functionalize substrates via the growth of polymer brushes under controlled growth conditions. The technique relies on active moieties that allow a controlled polymerization starting at the surface.[2] Visible light-induced living surface grafting polymerization was used to functionalize low density polyethylene (LDPE)[3]. For the photopolymerization reaction, Isopropylthioxanthone (ITX) was first photoreduced under UV light and sequentially coupled onto the surface of polymeric substrates, and the produced isopropyl thioxanthone-semipinacol (ITXSP) ‘‘dormant’’ groups were subsequently reactivated under visible light to initiate a surface grafting polymerization.[3]

Using visible light-induced living surface grafting polymerization from organic monolayers, we developed a versatile platform for controlled surface functionalization.

METHODS:

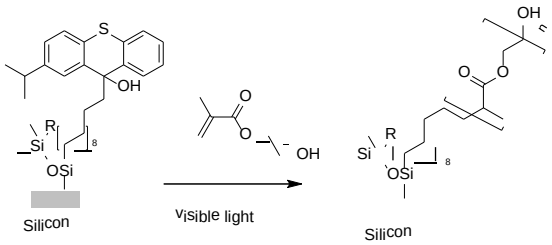

Fig.1: Reaction process introducing ITX to the OTS monolayers based on the ITX photoreduction under UV irradiation and HEMA grafted on the OTS film initiated with ‘‘dormant’’ groups by visible light irradiation.

As starting monolayer, octadecyltriethoxysilane (OTS) was deposited on silicon surfaces. The coupling of the photoinitiator isopropyl-thioxanthone (ITX) was performed under UV light. Using LED illuination, hydroxyethylmethacrylate was polymerized at the surface for the desired time. To determine the thickness of the organic films, spectral ellipsometry (Woollam M-2000) was used. Moreover, sessile drop water contact angles were measured with a custom-built contact angle goniometer. FTIR data for the modified surfaces were recorded at atmospheric pressure with a Bruker Tensor 27 FTIR- spectrometer with MCT detection.

RESULTS: The formation of ITXSP on the OTS layer was verified by photometry and an increase of the layer thickness as determined by spectral ellipsometry. Successful HEMA polymerization was again determined by spectral ellipsometry. The polymerization rate was 3 nm/h and film thicknesses up to 12 nm were obtained. As expected, an increase in HEMA thickness resulted in a WCA decrease. FTIR spectra measured after each modification step provided signals characteristic for ITXSP and HEMA. The spectroscopy results and restart experiments confirm the successful coupling of the photoiniferter and the living polymerization character of the surface reaction

DISCUSSION & CONCLUSIONS: Free-radical grafting polymerization from ultrathin organic monolayers induced via visible light irradiation was demonstrated. The polymerization reaction was based on the reactivation of ITXSP dormant species, which were preimmobilized onto an OTS surface region by a UV irradiation process and subsequently reactivated to initiate the free-radical polymerization of the monomer HEMA under visible light irradiation. This approach provides full spectroscopic access and thus provides a new platform to well characterized polymeric interfaces for biointerfaces research.

ACKNOWLEDGEMENTS: We acknowledge funding by the DFG (RO2524/4-1) and ONR (N00014-15-1-2324).

Multiscale Origami Structures as Interface for Cells (MOSAIC)

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INTRODUCTION: Surface-based bioanalytical methods are of outmost importance for fundamental and applied biomedical research. While micrometer-sized patterns of biomacromolecules can be created by "top-down" methods, such as ink jet and micro-contact printing, nanometer-sized arrays of molecules can be efficiently generated by "bottom-up" approaches, which take advantage of molecular self-assembly. Novel combination of these two approaches would be tremendously useful for bioanalytical applications, which address native biomolecular assemblies at nano-scale regime, such as the supramolecular clusters of membrane-associated molecules found in living cells.

METHODS: Among self-assembly techniques, DNA origami nanostructures (DON)[1,2] can be used as molecular pegboards for the precise arrangement of proteins with a lateral resolution of ~6 nanometers.[3] Site-directed immobilization of DONs on micro-patterned arrays can be achieved via DNA directed immobilization.[4] DON constructs presenting the epidermal growth factor (EGF) as ligand were employed to activate the cellular epidermal growth factor receptor (EGFR) in adhered MCF7 cells. A series of Cy3-labeled DONs were decorated with either 4, 5, 8, or 12 Streptavidin-EGF molecules, which were either arranged evenly distributed ("far") or densely clustered ("close") on the DON´s surface (Figure 1a). EGFR activation was detected by antibody staining (Figure 1b) and the effect of the features´ nanostructure of the immobilized EGF-DONs onto EGFR activation was statistically analyzed.

RESULTS: The obtained box-whisker plots (Figure 1c) clearly indicate that both stoichiometry and spatial arrangement of the DON-EGF constructs quantitatively influence EGFR activation. Increasing numbers of ligands led to an increased activation, which leveled off at 8 ligands/origami. In the case of 4 or 5 EGF-STV entities/DON, constructs with evenly distributed ligands revealed a significant larger receptor activation than their complementary analogues, containing the same number of ligands in densely clustered arrangements.

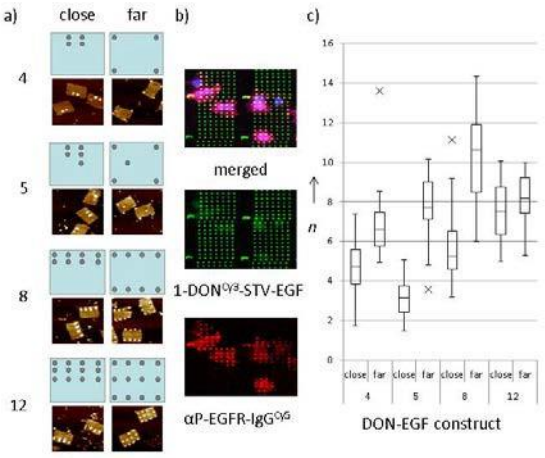

Fig. 1: a) Schemes and AFM images of DON constructs with different numbers and arrangements of EGF-modified STV entities. B) Fluorescence micrographs of representative experimental results, along with statistical analysis (c).

DISCUSSION & CONCLUSIONS: The MOSAIC technology was successfully used to investigate the activation of EGF receptors in living MCF7 cells through distinctive nanoscale arrangements of EGF ligands. Further implementations of this method will deploy the full potential of MOSAIC to create artificial biointerfaces useful to explore and exploit fundamental processes of cellular signaling and communication.

ACKNOWLEDGEMENTS: We gratefully acknowledge financial support of this work by Deutsche Forschungsgemeinschaft (grant NI 399/10-2) and the Helmholtz programme BioInterfaces in Technology and Medicine. This work was partly carried out with the support of the Karlsruhe Nano Micro Facility (KNMF), a Helmholtz Research Infrastructure at KIT.

Bio-sensing using Silicon-nanowire ion-sensitive field effect transistor ISFET

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INTRODUCTION: Metallic and organic ions play a vital role in regulating the activity in the living cells through simple (diffusive) or active transport across biological membranes. This process is achieved by ion channels or ion pumps anchored within the membranes of living cells. The field of molecular systems engineering is growing rapidly and aims to mimic living cells by inserting biological membrane proteins into amphiphilic polymeric membranes. In order to test, characterize, and monitor the activity in the artificial environment, a label-free, highly sensitive, and miniaturized sensor is desirable. ISFETs biosensors, based on Silicon-Nanowires (SiNWs) are promising candidates for the detection of chemical and biochemical species. ISFET devices have been proved to be highly sensitive and low noise pH sensors in numerous biological applications. [1]

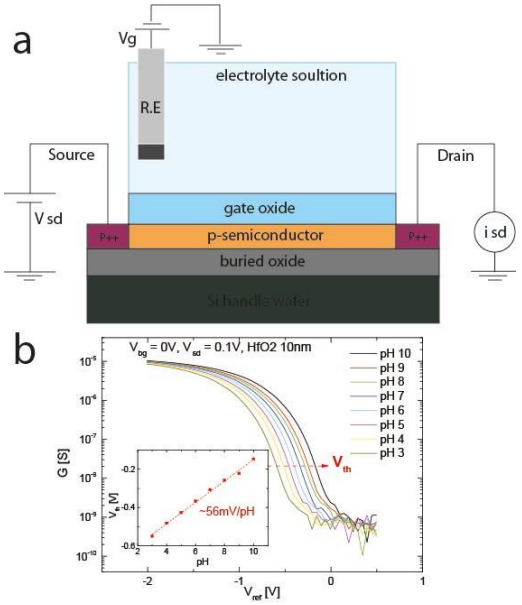

Fig. 1: Sensing using Silicon Nanowires ISFETs. ***a)*** *Cross section of a p-type SiNWs ISFET* ***b)*** *Transfer-curves at different pH (conductance* G *vs. liquid gate potential* V_{ref}*) of a SiNW with* HfO_2 *as gate oxide.*

A first approach for sensing ions is using the covalent or non-covalent functionalization of the SiNWs with highly selective receptors, such as DNA or RNA oligomers, antibodies, enzymes and so on. We have demonstrated the selective sensing of species other than protons using SiNWs, decorated with ionic receptors for Na^+, K^+, Ca^{2+}[2] and F^-[3] we have modified the individual gold coated nanowires by forming self-assembled monolayers (SAMs) of 15-crown-5 Na^+ ligand, an 18-crown-6 K^+ ligand, a F^- specific ruthenium complex and a 4-carboxyl terminated K^+ receptor, making our setup an ideal candidate for the measurements of different ions, utilizing organic surface modification.

PMOXA-PDMS-PMOXA is an amphiphilic block co polymer, capable of assembling vesicular structures or planar membranes in aqueous conditions. It is further known for its ability to host biological membrane proteins.[4] The combination of our unique sensing platform with such polymeric membranes allows to characterize the divers molecular transport processes across artificial membranes. Furthermore, the metabolic cross-communication between the molecular factories and cellular systems is expected to be monitored.

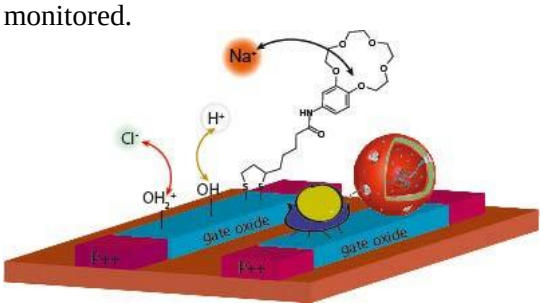

Fig.2: Biosensing using Silicon Nanowires ISFETs

ACKNOWLEDGEMENTS: We gratefully acknowledge Swiss Nanoscience Institute (SNI), RECORD-IT project, NCCR-MSE and the University of Basel .

Novel Switchable Microstructured Surface as a Tool to Investigate Cell Response in Real Time

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INTRODUCTION: Infertility is a disease and represents a global health problem, estimated to affect as many as 186 million people worldwide [1]. The current live birth rate from Intracytoplasmic sperm injection (ICSI) averages around 30% and one of the reasons for it to be so low is the sperm quality. Since ICSI was developed, there has been little progress in obtaining a robust, clinically significant, cost effective tool for determining selection of the best sperm cell. Therefore, we are currently developing a device for detection and selection of high quality sperm cells for use in ICSI. Sperm response to progesterone (Ca^{2+} signaling) is a good marker of sperm quality but current techniques to assess such responses are cumbersome and subjective. We propose that being able to assess Ca^{2+} responses and flagellar activity in a large number of attached cells before and during brief, reversible, exposure to progesterone, may be a novel, simple, rapid and potentially cheap high-throughput assessment of sperm quality and fertilizing potential. This approach will lead to select the best cell to be injected into the egg.

METHODS: Our smart surface is based on oligopeptide mixed self-assembled monolayers on gold, showing electrically tunable properties. Orthogonal chemical functionalisation was optimised for cells immobilization on glass and to create the switchable surface on gold. In order to locally expose progesterone to a single cell, micropatterned surfaces of gold and glass were fabricated using a standard photolithographic process.

RESULTS: We developed a smart switchable surface which by means of an applied positive or negative electrical potential exposes or sterically shields progesterone, undergoing transitions between fully extended (ON state) and collapsed (OFF state) conformations. This behavior was demonstrated by SPR (Fig.1a) and fluorescence microscopy, exposing the switchable surface to progesterone antibody. Switching efficiency of 70% was achieved. Micropatterned devices for single cell controlled and monitored exposure to progesterone were fabricated (Fig.1b). Studies are currently being performed to correlate progesterone stimulus on demand to Ca^{2+} signaling and motility responses of individual cells.

a

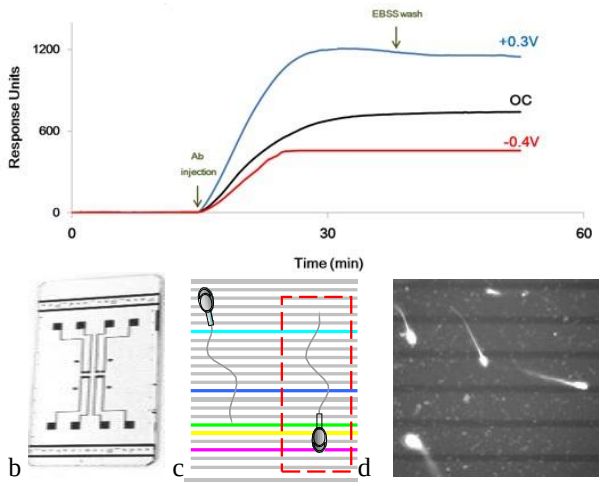

Fig.1 a)SPR sensorgrams showing binding of progesterone antibody to switchable surface; b)Photo of fabricated micropatterned device for studies with cells; c)Schematic representation of sperm cells attached to a micropatterned surface where different stimuli are applied; d)Fluorescence image of cells attached to switchable surface.

DISCUSSION & CONCLUSIONS: We have previously demonstrated the feasibility of this kind of smart switchable surfaces [2,3]. When exposed to progesterone, high quality cells initiate a rapid calcium transient and generate a burst of motility, causing reorientation or spinning of the cell. This might be detected by phase contrast microscopy and assessed by real-time image analysis, providing instant identification of responsive cells - those where Ca^{2+} signalling is expressed and functional. This system could be integrated into use with simple light-microscopy of cell motility to enable selection of sperm for ICSI.

Graded organisation of fibronectin on polymer surfaces to control growth factor presentation and cell response

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INTRODUCTION: Cells receive and respond to signals from their native environment, the extracellular matrix (ECM). Fibronectin (FN), an essential component of the ECM, is involved in the control of important cellular processes; it contains recognition sites for other ECM and FN molecules, growth factors and cell receptors. Elucidating the cell-ECM interplay is critical to the design of bioinspired material systems for regenerative medicine applications. In particular, controlling the delivery and activity of growth factors (GFs) has become a key aspect in material-based approaches to drive stem cell fate and tissue regeneration[1]. In our work we explore the potential of surfaces with similar and defined chemistries in the regulation of FN adsorption, GF (specifically bone morphogenetic protein 2, BMP-2) presentation and human mesenchymal stem cells (hMSCs) response.

METHODS: Copolymers with controlled ratios of ethyl acrylate, EA and methyl acrylate, MA were studied: 100/0, 70/30, 50/50, 30/70, 0/100. AFM imaging and ELISAs were carried out to characterise FN conformation upon adsorption, the availability of important FN domains and BMP2 adsorption. Cell adhesion was analysed after 3 hours and 1 day of culture by staining against vinculin. Cell differentiation was explored by staining against osteogenic markers like osteocalcin after 21 days of culture.

RESULTS: AFM images revealed a network-like conformation of FN on PEA. With decreased concentration of EA, the FN network appears less connected. Finally, FN forms globular aggregates on PMA.

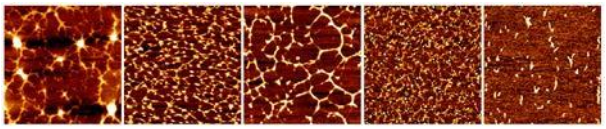

Fig. 1: Phase AFM images of FN distribution on spin coated samples. The size of the images is 1x1 μm^2.

ELISAs demonstrated that the FN binding domains for integrins and growth factors are differentially exposed upon adsorption. Additionally, similar amounts of adsorbed BMP2 were found. AFM was used to assess the specificity of BMP2 binding on the FN coated surfaces. BMP2 particles were observed on PEA whereas only few particles were found on PMA.

The morphology of hMSCs was studied by analysing immunofluorescent pictures of focal adhesions. Well-formed focal adhesions were observed, however no differences were found with respect to focal adhesion size.

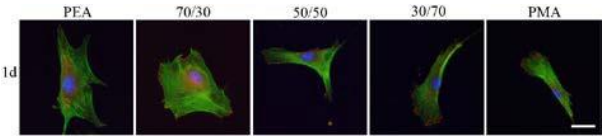

Fig. 2. Cell adhesion of hMSCs on protein coated samples 1d after seeding. Fluorescent staining of focal adhesions (red), actin cytoskeleton (green) and nuclei (blue).

In order to identify how the surfaces influence osteogenic differentiation of hMSCs, osteocalcin staining was carried out. After 21 days of culture, osteocalcin aggregates were visualised indicating higher osteocalcin expression when BMP2 was adsorbed onto the FN-coated surfaces.

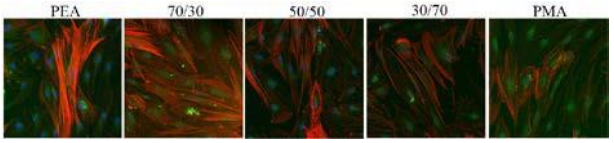

Fig. 3. Cell differentiation of hMSCs on FN and BMP2 coated surfaces. Fluorescent staining of osteocalcin (green), actin (red) and nuclei (blue).

DISCUSSION & CONCLUSIONS: This work provides a way to direct FN fibrillogenesis and GF presentation by controlling the EA/MA ratio. Such changes ultimately affect cell behaviour, allowing, e.g., to control osteogenic differentiation using BMP2.

ACKNOWLEDGEMENTS: The support from ECR through HealInSynergy 306990 is acknowledged.

Self-Healing Spongy Coating for Drug Delivery

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INTRODUCTION: On-site release of therapeutics from medical devices has proven to serve as a general strategy to combine device function and pharmacotherapy. Recently, layer-by-layer (LbL) self-assembly has emerged as a striking tool for engineering surface-mediated drug delivery systems (DDSs) with controlled structure and composition. Developing a simple and versatile approach to efficiently incorporate drugs into LbL films remains to be largely unexplored.

METHODS: A polyelectrolyte multilayer coating was assembled from polyethylenimine (PEI) at pH 9.0 and poly(acrylic acid) (PAA) at pH 2.9. The formation of spongy structures within the coating was achieved by acidic treatment and freeze-drying. The loading of hydrophobic drug was enabled through wicking drug solution, followed by solvent removal under vacuum. The self-healing of those micropores was triggered at 100% relative humidity (RH). Aqueous solution of hydrophilic drug(s) was also wicked into the spongy coating. After the wicking process, the films were simply placed on the countertop under ambient condition to enable the spontaneous healing of microporous structures (Fig. 1).

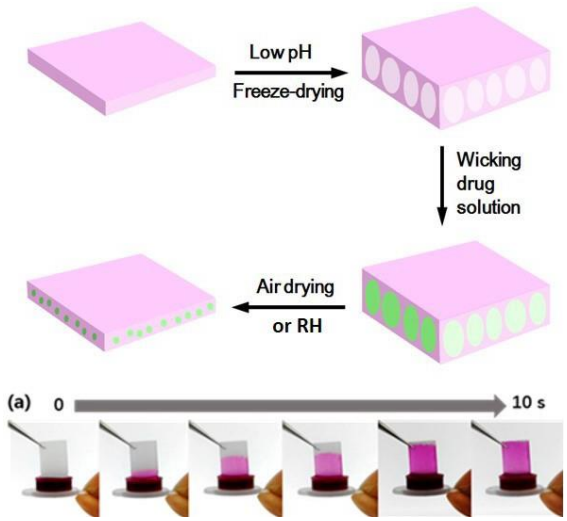

Fig. 1: Schematic illustration of the drug integration of a self-healing spongy coating.

RESULTS: The high porosity of spongy coatings enabled the high loading of a hydrophobic drug (triclosan in this case) to be a one-shot process, and the drug loading could be adjusted according to the concentration of the loading solution.[1] After drug loading, exposure to RH healed those original micropores and triclosan aggregated into many microdomains within the coating. The self-healing of a drug-loaded microporous coating reduces the diffusion coefficient of triclosan by two orders of magnitude, which is favorable for the long-term sustained release of the drug.

The loading capacity of this spongy coating for hydrophilic drugs was investigated using positively charged methylene blue (MB) and negatively charged crocein orange G (COG).[2] Simultaneous integration of MB and COG can be readily achieved by utilizing a mixed drug solution. The ratio between two drugs within the coating can be precisely customized according to the ratio of the loading solution over a wide range of dual-drug ratio.

DISCUSSION & CONCLUSIONS: In this study, a self-healing spongy coating was described to facilitate the surface-mediated delivery of various drugs. The formation of spongy structures within the coating was achieved by acidic treatment and freeze-drying. The high porosity of this spongy coating enables the loading of hydrophobic or hydrophilic drug to be a one-shot process via wicking action, thus dramatically streamlining the processes and reducing complexities compared to the existing LbL strategies. What's more, various combinations of hydrophilic drug can be readily integrated into this coating. We believe that embedding other species such as macromolecules and particles might also be possible utilizing this platform. Also, this dynamic strategy could provide a new concept for construction of drug releasing polymeric coatings since self-healing of a damaged structure is a common phenomenon of many polymers.

ACKNOWLEDGEMENTS: This research was supported by Zhejiang Provincial Natural Science Foundation of China under Grant No. LR15E030002, the National Natural Science Foundation of China (51333005, 21374095, 51573162).

Hybrid Structure Sensor Design for Biomedical Sensor Applications

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INTRODUCTION: The evolution of sensor technologies has been largely based around hard materials, but for body-mounted designs, the evolution of soft condensed matter sensors (SCMS) offer conformability to the body surface, ensuring good compliance between the body surface and the sensing mechanism. Electrically conductive hybrid sensor and compliant meta-materials offer a way to optimize sensor physical designs to a variety of body-mounted applications, allowing their mechanical properties to be tuned as desired. In the current investigation a conductive piezoresistive hybrid sensor based on thermoplastic elastomer (TPE) combined with Carbon Black (CB) particles [1] has been combined with a printed meta-material structure and soft silicone structure. The hybrid sensor has been designed as a platform to be tailored for body-mounted applications such as vector deformation of the skin surface of the human body and vital function monitoring (blood pulse wave mechanics).

METHODS: Hierarchical structures were based on an auxetic unit cell, and produced using the fused filament fabrication (FFF) 3D printing method. FilaFlex, a printable TPE material was used to allow for large bending of the structure. A Velleman K8200 printer was used with a 0.25 mm extrusion nozzle to print hierarchical arrays with a wall thickness of ~0.4 mm. A test procedure for evaluating motion capture of the wrist as well as blood pulse wave detection was established in a previous work [2], and employed in the current study. A band sensor with a 10 mm sensor length and elastic band was mounted on the wrist and a 10 position hand position was evaluated for motion capture, and additionally, with the wrist at rest, the pulse wave profile was measured.

RESULTS: A sensor design based on printed TPE and casted silicone was produced and tested on the body. With the hybrid sensor mounted on the wrist, it was possible to capture motion by combining the results of different sensor positions. The pulse wave of the blood could be seen and then used to calculate the beat frequency.

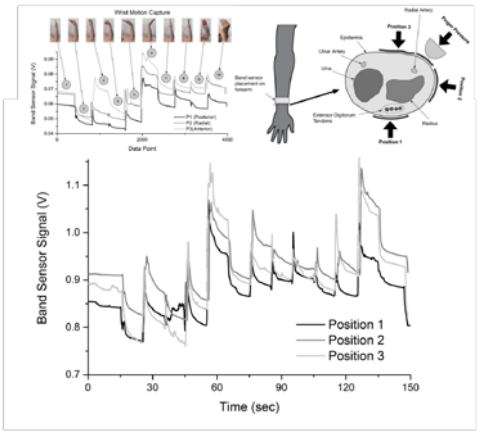

Fig. 1: Description of motion capture of sensor on human wrist, placing sensor around wrist allows for capturing motion profile.

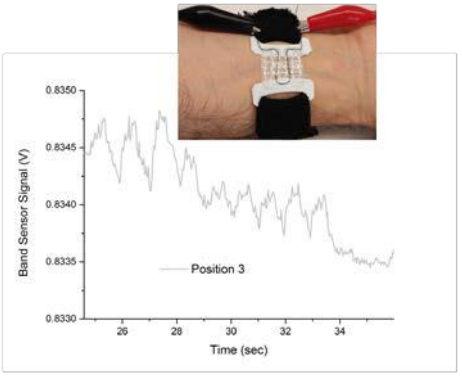

Fig. 2: Hybrid sensor mounted on the wrist, with pulse wave monitoring with raw data.

DISCUSSION & CONCLUSIONS: The current sensor design was effective in capturing raw data for body motion and pulse wave. Future designs can refine and improve the sensor signal quality and improved performance.

ACKNOWLEDGEMENTS: This work was possible through a grant by the Swiss Commission for Technology and Innovation (CTI Medtech 17614.1 PFLS-LS).

Immobilization of cell adhesion peptides and extracellular matrix proteins on synthetic surfaces for anti-coagulant blood interfaces

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INTRODUCTION: The main challenge when designing implants that are in blood contact such as the membrane in a ventricular assist device (VAD), is to prevent the blood from coagulating. Nature however provides us with a perfect blood-tissue interface, the endothelium. When engineering such endothelium on top of artificial materials, the success depends on the stable adhesion of the cells to the substrate and their functional state. It is known that by modifying surfaces biologically, e.g. with proteins or specific peptides, cell adhesion behaviour can be manipulated.[1] Studies have shown that peptides can be immobilized onto various materials via a mussel inspired oxidation of tyrosine.[2,3] However, although peptides are known to promote the adhesion of cells, they cannot guarantee their phenotypical functionality.[4]

Our aim is to enhance the adhesion behaviour and functionality of endothelial cells by covalently introducing biological features. For this, we explore the possibility of using mussel inspired oxidation of tyrosine to functionalise substrates with both cell adhesion peptides as well as biologically more relevant extra cellular matrix proteins.

METHODS: Green fluorescent protein with tyrosine label (Y-GFP) was used as model protein for the mussel-inspired immobilization. Tyrosinase was used in order to oxidise the phenol of the tyrosine into an o-quinone. O-quinones can covalently bind to radicals formed on the surface by oxidation. The reaction was carried out on poly-stryrene (PS) and alginate hydrogels (AL).
Success of the reaction was characterised by measuring the GFP signal on the surface.

RESULTS: Covalent immobilization of Y-GFP onto PS was confirmed by fluorimetry and microarray imaging. With increasing tyrosinase used for the mussel-inspired oxidation reaction from 0 up to 0.6 Kilo Units (KU)/mL the signal from the immobilized protein on the surface increases by 35%. However, the same reaction carried out on alginate hydrogels, did not show the same trend. The signal obtained is high, does not

significantly change with increasing amount of enzyme and shows high standard deviations.

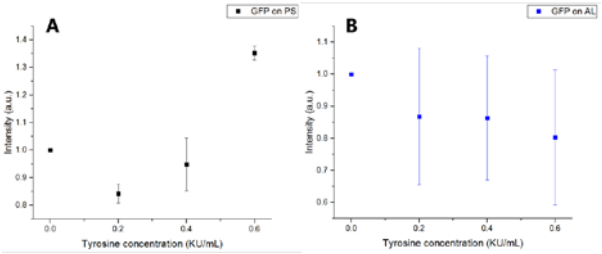

Fig. 1: (A) Y-GFP immobilized on PS. Signal from GFP increases with increasing amount of tyrosinase used. (B) Y-GFP immobilized on AL. No clear trend in the fluorescent signal with increasing amount of tyrosinase detectable. (Values are normalised to 0KU/mL; n=2)

DISCUSSION & CONCLUSIONS: We were able to achieve controlled covalent immobilization of GFP as model protein via a mussel inspired oxidation method on flat PS surfaces. However, when applying the method to alginate hydrogels, the reaction seems to be less controllable. This might be due to an unspecific side reaction: the chelation of the his-tag of the protein in the hydrogel by calcium ions. Therefore in the next step peptides without a his-tag will be immobilized on alginate.
We will explore this approach further to immobilize adhesion peptides, extracellular matrix proteins and their combinations onto polymeric and hydrogel surfaces for enhanced adhesion and functionality of endothelial cells.

ACKNOWLEDGEMENTS: This work is part of the Zurich Heart project of Hochschulmedizin Zürich.

Interfacial Polymerization Systems for Screening of Multi-enzyme Complexes

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INTRODUCTION: Interfacial polymerization systems relying on enzyme-mediated production of free radical initiators are capable of amplifying biological signals and converting them into interfacial polymer coatings. Here I provide an overview of a new application area for this technology, specifically as a screening tool for the optimization of enzyme formulations[1,2,3]. Bioconversion of cellulose into sugars *en route* to renewable fuels and chemicals requires potent enzyme formulations. Directed evolution and screening campaigns are mandatory for enzyme optimization, however, current methods for screening carbohydrate-active enzymes are incompatible with low-volume high-throughput detection platforms. To address this need, we developed a system which allows us to detect the formation of hydrogel in response to enzymatic conversion of biomass. The amount of gel can be quantified optically, and provides a rapid readout that is easily miniaturized and parallelized. We demonstrate the ability of this system to quantify endo/exoglucanase synergy, measure thermostability, and quantify substrate targeting effects within multi-enzyme carbohydrate active complexes (i.e., cellulosomes).

METHODS: Plant matter (napier grass, miscanthus) was mechanically processed to produce coarse powder, and punched into discs. The reagent systems comprised the following components: glucose oxidase (1 mg mL^{-1}), $FeSO_4$ (250 µM), ascorbic acid (250 µM), PEG diacrylate (M_n 575, 150 mg mL^{-1}), NaAc buffer (pH 4.5, 20 mM). Varying amounts of cellulase enzymes, or recombinant multi-enzyme complexes with or without a carbohydrate binding module were tested. Autofluorescence of cellulose (λ_{ex} = 365, λ_{em} = 430 nm) provided a strong signal which was attenuated upon gel polymerization (**Fig. 1A)**.

RESULTS: Time-resolved autofluorescence attenuation on cellulose substrates was found to be a strong indicator of cellulolytic enzyme potency. The assay format relying on biomass discs deposited into 96-well plates was straightforward to implement and highly parallel, while relying on small volumes of reagents (~100-300 µL). This represents a significant improvement on the standard reducing sugars assays which use several mLs of sample. The assay also had predictive power for analyzing the synergistic effect between endo– and exoglucanase enzymes (**Fig**. **1B**).

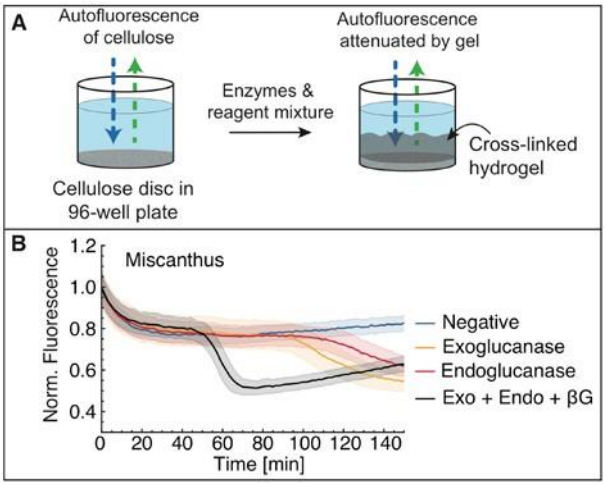

Fig. 1: Interfacial polymerization quantifies cellulolytic enzyme activity. (A) Polymerization of the gel coating in response to sugar production by the enzymes results in turbidity/light scattering and reduces substrate autofluorescence. (B) Endo/exoglucanase enzyme synergy is observed as an earlier onset of polymerization time for the formulation containing all synergistic combinations of enzymes (black trace).

DISCUSSION & CONCLUSIONS: The hydrogel polymerization system quantifies enzyme activity in a parallel, rapid format that is amenable to high-throughput screening.

ACKNOWLEDGEMENTS: Society in Science, Branco Weiss Fellowship, ETH Zurich.

Effect of 3D microgrooved and 2D micropatterned substrates on vascular endothelial cell cytoskeletal organization

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INTRODUCTION: In many cell types, cellular function, morphology, and cytoskeletal orientation are intricately intertwined. In the specific case of arterial endothelial cells (ECs), cell shape and orientation correlate with predisposition for atherosclerosis with elongated cells that are aligned in the direction of blood flow remaining largely protected while cuboidal cells are prone. It has long been known that flow acting on the apical EC surface regulates cell cytoskeletal organization; however, recent work has demonstrated that biophysical cues on the basal surface form the extracellular matrix also play a critical role (1). Indeed, presentation of topographic signals in the form of 3D microgrooved or 2D micropatterned substrates is a powerful tool to control and guide cell behavior in vitro. However, the optimal way to deliver topographic signals in order to target a particular cytoskeletal architecture remains to be established. Furthermore, it remains unknown if 3D microgrooves and 2D micropatterns modulate cytoskeletal organization via similar mechanisms. In the present work, we have compared the effect of 3D microgrooves and 2D micropatterns of similar dimensions on EC cytoskeletal organization.

METHODS: 3D microgroove substrates were obtained by replica molding of polydimethylsiloxane (PDMS) on a silicon master containing parallel and straight channels with a groove and ridge width of 5 micrometre and depth of 1 micrometre. 2D micropatterned substrates containing 5 micrometre-wide adhesive stripes spaced by 5 micrometre gaps were produced using the deep UV light method (2) on flat PDMS substrates. Prior to cell seeding, microgroved, micropatterned and unpatterned (control) substrates were incubated for 1 hr with a 50 microgram/mL fibronectin solution. Bovine aortic ECs (BAECs) were seeded on the substrates at low density in order to avoid cell-cell contact. Cells were fixed either 2 or 24 hrs after seeding and stained for actin with TRITC-phalloidin. Actin organization was determined using the MomentMacroJ v.1.4 script run in Fiji.

RESULTS: 3D microgrooved substrates had a profound effect on the organization of BAEC cytoskeletal organization. In particular, actin stress fibers in cells seeded on 3D microgrooved substrates were highly oriented along the pattern direction at both the 2- and 24-hr time points (Fig. 1). On the other hand, actin stress fibers in cells seeded on unpatterned (control) surfaces did not exhibit a preferred cytoskeletal orientation at either time point. Interestingly, stress fibers in cells seeded on 2D micropatterned substrates displayed an average orientation that was intermediate between the 3D microgrooved and the unpatterned cases, with a slight increase in actin fiber alignment from 2 to 24 hrs after cell seeding.

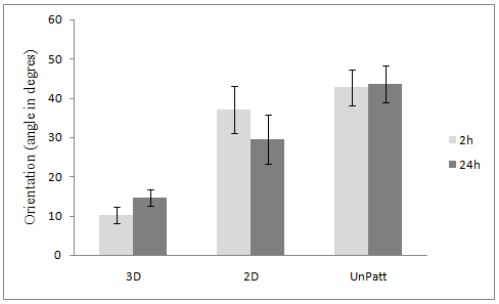

Fig.1: Orientation of the cell overall actin organization relative to the pattern direction (Error bars represent the standard error of the mean).

DISCUSSION & CONCLUSIONS: Substrate topography is a potent signal that has proven to be very effective in regulating cell adhesion, proliferation, and migration. In this work, we studied the effects of 3D microgrooved surfaces and 2D micropatterned substrates on EC cytoskeletal organization. We found that 3D microgrooves are much more effective in organizing and aligning actin stress fibers in the direction of the pattern than 2D micropatterns. We are currently exploring the mechanisms by which 3D and 2D topographies regulate cytoskeletal orientation. This work provides novel insight into the effects of topographic signals on EC behavior and promises to inform strategies aimed at optimizing the performance of the surfaces of implantable cardiovascular devices.

Au-nanostructured surface exhibits antibacterial properties

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INTRODUCTION: Antimicrobial strategies by conventional approaches rely on biocidal substances such as silver and antibiotics, which are problematic due to potential chemical toxicity and the potential for antimicrobial resistance to occur. A biophysical approach to directly modify surfaces is an attractive alternative. By taking Au as a proof of concept metal material, we present here a novel cost-efficient micro/nanofabrication platform based on self-organization for engineering various nanotopographies and study the structural effects on the antibacterial performance

METHODS: Au nanotopographies were fabricated by electrodeposition of Au in nanoporous templates (1). Nanopillars with 50 nm in diameter and various heights were fabricated. Methicillin-resistant *Staphylococcus aureus* (ATCC 6538), a Gram-positive bacterial strain responsible for many infections, was used as the model strain in this study. Bacteria adhesion was analyzed by epi-fluorescence microscope after 2h incubation of bacteria with surfaces.

RESULTS:

Fabrication of nano-structured surfaces

Nanopillars with 50 nm in diameter and various heights were fabricated.

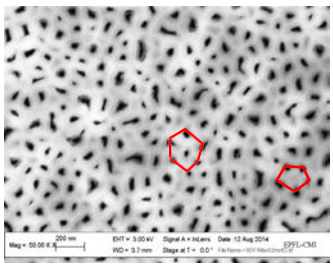

Fig. 1. Top view of alumina nanoporous template after plasma etching of W. The nanopores are self-organized in a near-hexagonal pattern as indicated.

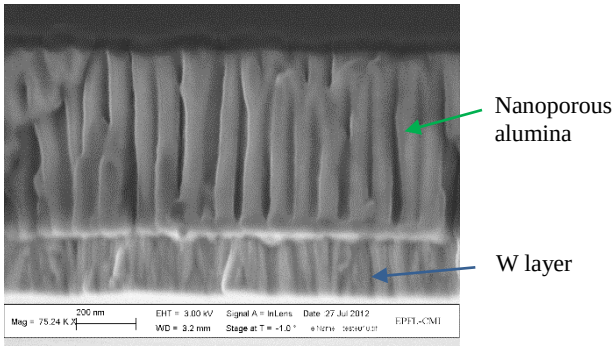

Fig. 2. Cross section image of the alumina nanoporous template.

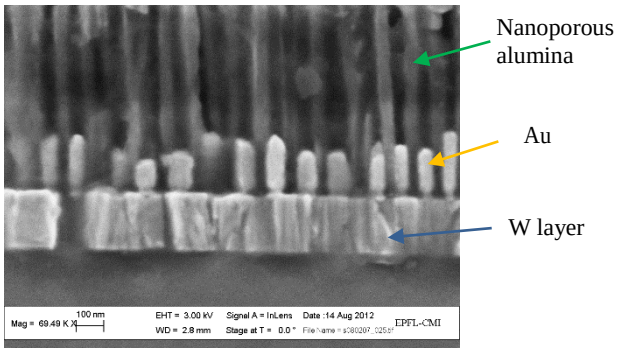

Fig. 3. Cross section image of Au nanopillars in the nanoporous template by electrodeposition.

Comparison of antibacterial properties for nanopillar structures

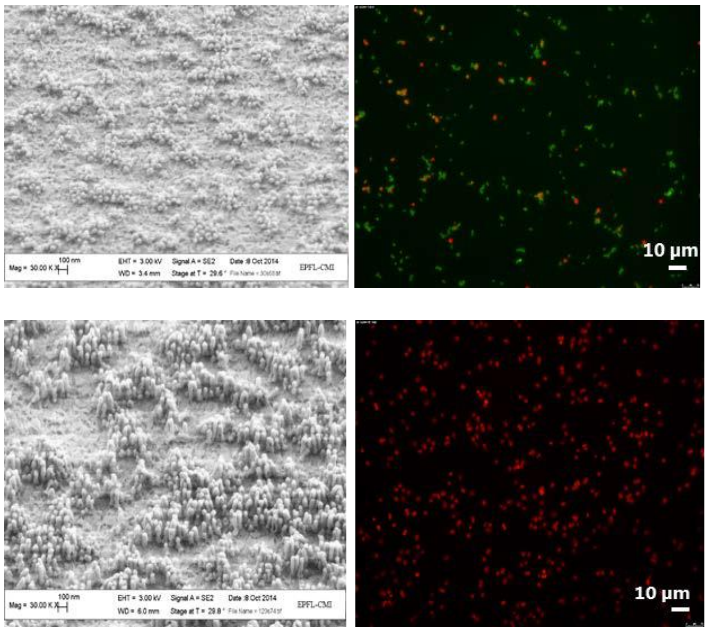

Fig. 4. Au nano-dots on W reference substrate obtained by deposition time of 30 s (upper panel) and Au nano-pillar by 120 s (lower panel). Most of the S. aureus cells appear alive (in green) on nano-dots surface. Nearly all cells appear dead (in red) on nano-pillar surface.

CONCLUSIONS: Au nanopillars with aspect ratio larger than 2:1 exhibited promising antibacterial property.

Implementation of a PDMS microdevice for the improved purification of circulating microRNAs

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INTRODUCTION: The relevance of circulating microRNAs (miRNAs) as non-invasive biomarkers for several pathologies is nowadays undoubtedly clear, as they have been found to add fundamental diagnostic and prognostic information to patients' clinical picture[1]. In light of this, considerable effort has been made in developing Lab-on-a-chip devices able to speed up and standardise the bench work. In this context, a very promising polydimethylsiloxane (PDMS)-based microdevice which integrates the processing of the biological sample, i.e. purification of circulating miRNAs, and reverse transcription was previously developed in our lab[2,3]. In this study we aimed at analysing in depth the best microdevice manufacturing conditions, acting on PDMS mold casting, curing and functionalisation, with the aim of improving the performances of its surface to capture extracellular miRNAs from biological samples.

METHODS: In order to act on the intrinsic PDMS properties, we focused on the modulation of roughness and charge. PDMS surface roughness was modulated by casting with several templates (terminated with silicon oxide coated by a thin anti-adhesion aluminum layer), followed by a panel of curing conditions. Atomic force microscopy (AFM) was employed to estimate changes at the nanometric scale. To introduce modifications in surface charge we functionalized PDMS with different mixes of positively charged 3-aminopropyltrimethoxysilanes (APTMS) and neutral poly(ethylene glycol) silane (PEG). The surface chemical composition was characterized by X-ray photoelectron spectroscopy (XPS) and the number of exposed primary amines was quantified with the reagent sulfosuccinimidyl-4-o-(4,4-dimethoxytrityl) butyrate (s-SDTB). As our final end point, the adsorption rate of all these different conditions was assessed by fluorescence microscopy by incubating a synthetic fluorescently-labeled miRNA.

RESULTS: Our preliminary analysis identified functionalisation of PDMS as a necessary step to significantly increase the amount of microRNA captured by the surface. The modulation of the surface charge provided a much more relevant improvement in miRNA capture over roughness modulation (i.e. mold casting strategies). 0.1% APTMS and 0.9% PEG was found to be the best silane mixture to obtain a functional positively-charged coating, able not only to capture miRNAs but also to make miRNA available for further analysis steps as on-chip reverse transcription. PDMS properties were stable for over two months and PDMS functionalisation lasted up to 48 hours.

DISCUSSION & CONCLUSIONS: Our analysis highlighted that surface charge has a higher impact than roughness in improving PDMS suitability for the selective capture of microRNAs. Therefore, functionalisation of PDMS with 0.1% APTMS and 0.9% PEG is a indispensable step for ameliorating miRNA capture on the surface, in order to enhance the purification ability of our PDMS microdevice and provide the template for further on-chip reactions, i.e. reverse transcription and amplification. Furthermore, PDMS properties were found to endure during aging for over two months and functionalisation procedure, which takes about an hour to be performed, was tested to be stable for at least 48 hours. These observations are very encouraging in the perspective of implementing a disposable microdevice for the purification and the analysis of circulating miRNAs from body fluids. The extraction efficiency of our PDMS microdevice would be substantially improved by the charge modulation, ultimately leading to an important step forward in the development of a innovative, easy-to-use and integrated system for the direct purification of less abundant circulating microRNAs.

ACKNOWLEDGEMENTS: This work was accomplished in the framework of NEWTON (Advanced nanosystems for a new era in molecular oncology) funded by MIUR (RBAP11BYNP) Grants-FIRB2012-2016.

Customizable bioadhesive micropatterns for cell-culture

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INTRODUCTION: Bioadhesive micropatterns, capable of laterally confining cells to a 2D lattice cover a basic and growing demand in several life science applications. According to current literature [1, 2] on cell micro-patterning, it appears that the common dimension of the patterns covers a size interval going from hundreds of nanometers up to tenths of microns. It also becomes clear that micropatterns have typically been prepared by means of photolithography. Fabricating the same by inkjet-printing is a new approach and renders lithography masks redundant. There are advantages in offering micropatterns on demand, as this will cover an established need of cell laboratories demanding high-quality, customized chips for research and diagnostic applications.

METHODS: Surface coatings are generated by the use of the Perfluorophenylazide coating technology as presented by Serrano et al. [3] on homogenous surfaces (Fig. 1). The suitability of this technology for surface patterning has been shown recently by Sterner et al. [4]. In this case, the patterns are generated using a photolithography mask in combination with spin-coating of the adhesive and the non-fouling polymer.

In case of printing, an industrial inkjet printer capable of dispensing only one picoliter of solution is used. This approach allows printing the hydrophobic, cell-adhesive polymer only in the zone of interest and therefore reducing processing time and costs.

RESULTS: Preliminary results obtained with a 1 picoliter printer are presented in Figure 2. The size of a single polymer dot on the functionalized substrate is 25 µm in diameter.

DISCUSSION: Within the present project we aim at the preparation of structured surfaces showing smallest feature sizes with inkjet printing in combination with a thin film coating technology based on photochemical linkage of polymers onto various surfaces such as glass, plastic and metals.

Current results indicate that it is possible to easily obtain 25 micron sized features. Current efforts are aimed at further decreasing this limit and at testing the stability of the coating in presence of cells.

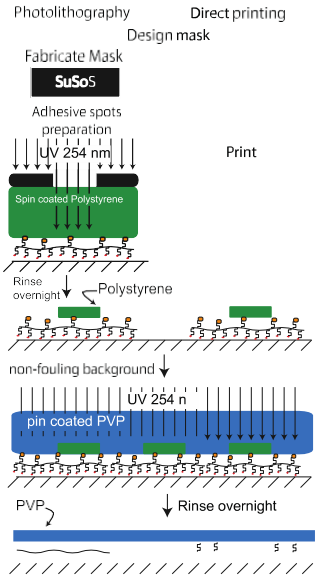

Fig. 1: Schematic comparison of the patterning generation processes based on the PFPA-Technology.

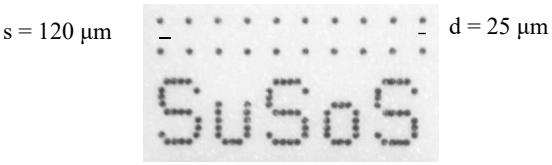

Fig. 2: Preliminary printing results showing the current standard resolution obtained with a standard ink formulation (dot volume 1 pl, dot diameter 25 µm, micropattern spacing 120 µm).

ACKNOWLEDGEMENTS: The present project has been supported by the SATW Transferkolleg initiative (www.satw.ch).

Deposition kinetics of bioinspired polyphenol nanocoatings and their effect on osteogenic differentiation on titanium surfaces

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INTRODUCTION: Flavonoids and other polyphenol-based surface coatings have recently attracted increasing interest as surface functionalisation strategies for medical implants due to their diverse functional properties: these coatings have been shown to possess antioxidant, anti-inflammatory, antibacterial, and antifibrotic properties [1-4]. The objective of this study was to assess the coating deposition kinetics of two different phenolic compounds, tannic acid (TA) and pyrogallol (PG), and the effect of these coatings on human primary osteoblast adhesion and differentiation.

METHODS: TA and PG coating deposition on titanium (Ti) surfaces was monitored in real time using quartz crystal microbalance with dissipation monitoring (QCM-D). Coating morphology was imaged using atomic force microscopy (AFM). The chemical properties of the coatings were characterised with Fourier transform infrared spectroscopy (FTIR) and x-ray photoelectron spectroscopy (XPS). Human primary osteoblasts (NHOs) were cultured on mirror-polished Ti samples with polyphenol coating deposited for 2 h (TA2, PG2) and 24 h (TA24, PG24), while uncoated samples served as control. Cytotoxicity was evaluated after 48 h, whereas metabolic activity, alkaline phosphatase (ALP) activity, and calcium content were measured after 7 and 14 d. Gene expression of osteocalcin (OC) and ALP was assessed with quantitative polymerase chain reaction (qPCR)

RESULTS: The deposition kinetics of both TA and PG on titanium surface is shown in Fig.1. TA deposition was characterised by rapid increase in thickness up to 5 h, after which a plateau in mass increase was reached. PG deposited continuously on the surface throughout the 24 h observation time. The maximum dry thickness of the formed coatings after 24 h was 53.0 ± 3.7 nm and 49.9 ± 9.7 nm for TA and PG, respectively. FTIR and XPS characterisation of the formed coatings revealed changes in their chemical composition, particularly in their hydroxyl groups, in comparison to the precursor molecules. Metal ions from the buffer solutions were also detected on the coating surface, and therefore, also play a role in the coating formation.

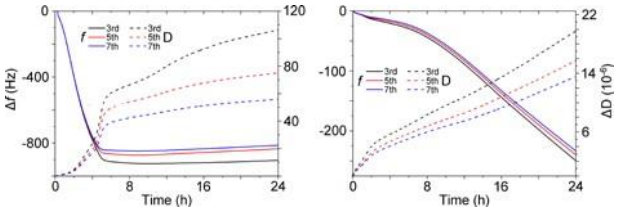

Fig. 1: Tannic acid (left) and pyrogallol (right) coating deposition kinetics on titanium surfaces.

Regardless of their thickness, both tannic acid and pyrogallol coatings were found to be non-toxic to human primary osteoblasts and allow cell adhesion to the coated implant surface. Thinner coatings deposited for 2 h (TA2 and PG2) were shown to be more osteogenic with higher calcium content and ALP activity at 14 d in comparison to both the uncoated Ti surface and coatings deposited for 24 h (TA24 and PG24). Gene expression of OC and ALP showed similar results with TA2 and PG2 showing increased mRNA expression in comparison to the corresponding 24 h polyphenol coating.

DISCUSSION & CONCLUSIONS: Tannic acid and pyrogallol formed homogeneous coatings on titanium surface by auto-oxidation mediated polymerisation. Both TA and PG coatings on Ti promoted osteogenic differentiation of osteoblasts adhered onto the samples surface, particularly on coatings formed at short deposition times resulting in thinner and more rigid phenolic coatings. The reduction in osteogenic differentiation on coatings deposited for 24 h may result from their higher hydration ratio and altered mechanical properties. Furthermore, possible release of the phenolic molecules from the coated surface may influence cell behaviour with higher concentrations resulting in delayed growth and differentiation of the cultured cells.

ACKNOWLEDGEMENTS: This study was supported by the Research Council of Norway (Grant no. NFR 230258).

Directing Cell Adhesion via Integrin-specific Photoresponsive Antagonists

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INM – Leibniz Institut für Neue Materialien, Saarbrücken

INTRODUCTION:

Interactions between specific integrins with the adhesive proteins of the extracellular matrix (ECM) play important role in adhesion, migration, and survival of most cells. In pathological states like cancer, the expression levels of particular integrins become up- or downregulated and the ECM changes in composition and morphology. As a consequence, the interaction pattern between the cell and the ECM changes and gains a crucial though not yet elucidated role in the progression of the disease. This creates the need to understand the particular role of each integrin subtype and receptor in cell-ECM communication, and the hope to identify new disease markers or therapeutic targets.

Engineered interactive scaffolds with the ability to expose integrin-specific antagonists allow reconstruction of individual signaling factors of the cellular microenvironment and elicit desired cellular responses. We will present how photoactivatable integrin-specific ligands incorporated into biomaterials can be used to study and trigger integrin-mediated cell response in physiological and pathological states.

METHODS:

The photoactivation of the caged peptidomimetics after coupling to biomaterials will be tested and analyzed by UV spectroscopy. UV spectroscopy in combination with measurements of the layer thickness of the material will allow estimation of the ligand concentration in the biomaterial. Once the photoactivatable integrin-specific antagonists will been developed, the biomaterials platforms for the light-directed adhesion and migration will be attempted. Combination of photoactivatable molecules, biomaterials and cell biology is anticipated to effectively reduce non-specific interactions, provide spatio-temporal control with reduced chemoresistance.

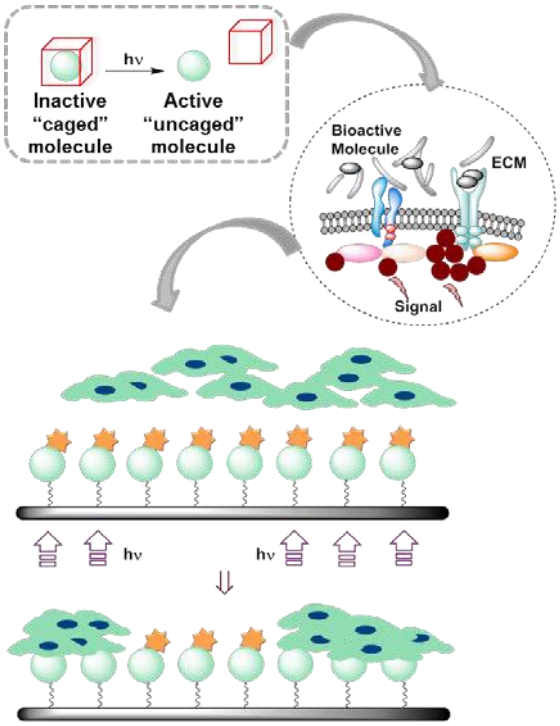

Fig.: General working mechanism of caged compounds, activation of signaling pathways leading to the proposed phototriggered cell adhesion onto micropatterns of caged integrin-specific antagonists.

ACKNOWLEDGEMENTS: INM - Leibniz-Institut für Neue Materialien gGmbH and Universität des Saarlandes.

Mussel-Inspired Dendritic Polymers as Universal Multifunctional Coatings

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INTRODUCTION: Surface modification of solid materials plays an increasingly important role in modern physical, chemical, biological, and materials science. Here we report on a heteromultivalent catechol and amine functionalized dendritic polymer that mimics not only the functional groups of mussel foot proteins (mfps) but also their molecular weight and molecular structure, for a rapid and universal surface coating by both covalent and coordinative crosslinking. The generated coatings can be further functionalized for versatile applications.[1]

METHODS: Dendritic polyglycerol (dPG), which has a highly branched architecture, exhibits a relatively distinct "interior", and exposes functional groups on its surface like folded proteins, was used as a scaffold for multivalent anchoring and crosslinking. The hydroxyl groups present on the dPG scaffold were converted to amine groups, 40% of them were further functionalized by catecholic groups (Figure 1). The average molecular weight (Mn) of synthesized mussel-inspired dPG (MI-dPG) is about 10 kDa, which is in the same range as the mfp-5, ~9 kDa. The coatings can be prepared by either covalent or coordinative crosslinking of the MI-dPGs during a dip-coating approach.

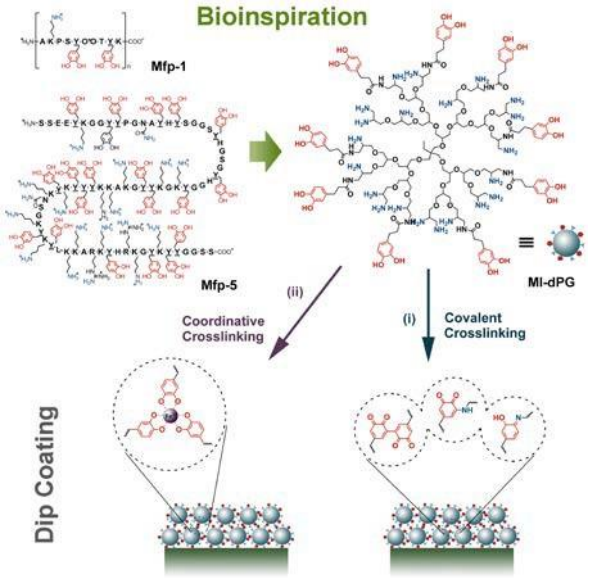

Fig. 1: The chemical mimicry of synthesized mussel-inspired dendritic polyglycerol (MI-dPG) and the related universal surface coatings.

RESULTS: The MI-dPG can form a highly stable coating on virtually all types of material surfaces within 10 min or a micrometer scale coating in just hours. Three types of repellent surfaces were achieved based on the secondary modification of the MI-dPG coatings (Figure 2). A designed hierarchical dPG coating prevent the protein adsorption and cell adhesion.[2] A perfluoroalkyl functionalized surface with two-tier (micro and nano) roughness shows superhydrophobic properties. A slippery surface can be achieved after infiltration with sunflower oil.

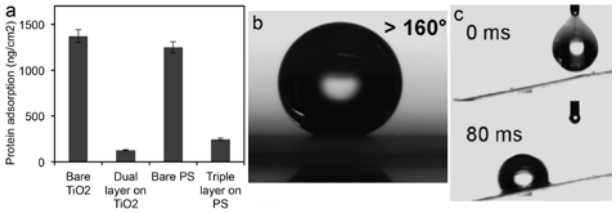

Fig. 2: (a) Bioantifouling surface. (b) Superhydrophobic surface. (c) Slippery surface.

DISCUSSION & CONCLUSIONS: This MI-dPG benefits from its heteromultivalent character and molecular weight, which provide a strong adhesive power. Moreover, the general rules for developing universal coatings can be proposed that: (1) there must be some interaction between the coating materials and the substrate surfaces, even though the interaction might be relatively weak; (2) lateral crosslinking, either covalent or noncovalent, must be present; (3) the coating should be prepared with the available functions or it can be further functionalized. Stronger interfacial interaction and a higher degree of crosslinking can result in more stable coatings, especially on chemically inert surfaces, such as Teflon.[3]

ACKNOWLEDGEMENTS: This work was supported by the Helmholtz Virtual Institute on ''Multifunctional Biomaterials for Medicine''.

Mimicking Paracrine Cell Communication in Fibrillar 3D Scaffolds *in vitro*

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INTRODUCTION: Cells within organisms experience a range of influences which guide their cell fate decisions. Among these environmental cues are cytokines secreted by neighbouring or distant cells determining cell fate of receiving cells. Studying the complex signalling processes of the various mediators *in vivo* is hard to achieve and goes hand-in-hand with unnecessary pain to the animal under investigation. That's why it is pivotal to establish simplified *in vitro* models to mimic these processes. But traditional 2D cell culture with end-point analysis is insufficient to mimic *in vivo* situations and to study highly dynamic processes of heterogeneous cell populations. We therefore set off to establish 3D cell culture environments containing localized cytokine sources. In combination with high resolution microscopy it allows for comprehensive study of cell fate decisions under influence of defined parameters. Especially we are interested in the fate of stem cells because they enable life-long regeneration and tissue repair. Furthermore cell-cell interactions between various cell types including fibroblasts and macrophages in wound healing are addressed within such model systems.

METHODS: For *in vitro* models we used 3D fibrillar networks made of collagen I. By adjusting reconstitution parameters network topology like pore size or elastic modulus were tuned in a defined manner. Cell-cell communication was mimicked by inserting glycosaminoglycan-modified agarose microbeads inside the networks. These microbeads can be laden with proteins of interest to study interactions between cytokine source and a responding cell. Protein release was quantified using fluorescently labelled proteins and confocal microscopy as well as unlabelled proteins using ELISA. Resulting short-range protein gradients were estimated from release data within a diffusion model or determined directly with fluorescence correlation spectroscopy (FCS). Cell types of interest were fibroblasts and haematopoietic stem cells (HSC). Delivered proteins were TGFb1, IL10 and SDF1, Dkk1 respectively. Cell behaviour was studied in terms of proliferation, differentiation and migration.

RESULTS: Determination of cytokine release from the microparticles showed dependence on protein loading concentration and affinity, which is modulated by the choice of glycosaminoglycan inside the microparticle. After high release within the first 24 h, release declines to slow steady state delivery over several days. This release is comparable to physiological levels released by cells. Co-culturing cytokine-laden microparticles with cells led to changes in cell fate. HSC exhibited chemotactic migration towards SDF1 releasing microparticles [1]. A similar release characteristic was observed for TGFb1. TGFb1 seems to act only globally on fibroblasts in the collagen matrix and not locally confined next to the releasing microparticle. Increasing TGFb1 concentration led to an enhanced myofibroblast differentiation. Importantly, local, sustained TGFb1 release caused myofibroblast differentiation at much lower TGFb1 levels in comparison to systemic TGFb1 presentation in the cell culture medium. We propose that TGFb1 is conserved within the glycosaminoglycan-modified microparticles potentiating its impact on fibroblasts.

DISCUSSION & CONCLUSIONS: We present an *in vitro* model based on 3D collagen gels and cytokine-delivering microparticles mimicking secreting cells. This system enables investigation of paracrine cell communication. Our approach comprised HSC niche communication as well as fibroblast fate decision during wound healing.

ACKNOWLEDGEMENTS: We acknowledge the support of grants from Deutsche Forschungsgemeinschaft (DFG, grant: SFB-TRR67/B10 and INST 268/293-1 FUGG), from EFRE and Free State of Saxony (SAB, grant: 100144684), from ESF 'European Social Funds' and Free State of Saxony (SAB, grant: 100140482), and from Human Frontier Science Program Organisation (HFSPO RGP0051/2011). Collaborations with groups of Carsten Werner, Annette G. Beck-Sickinger, Matthias Schnabelrauch, and Michael Cross are gratefully acknowledged.

Breakable silica nanoparticles for the *in-vitro* delivery of biomolecules

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INTRODUCTION: Among the various types of drug delivery systems, mesoporous silica nanoparticles (MNPs) have attracted enormous attention. The interest rises from their advantageous structural properties, biocompatibility, high loading capacities and the possibility to selectively functionalize the material both on the external surface and within the pores[1]. However, silica MNPs, especially when there are functionalized to prevent aggregation, tends to accumulate into the different organs in the body hindering their commercialization as a medical tool. To overcome this issue, we developed different stimulus responsive porous nanoparticles that can deliver drugs and breaks in small pieces after cell internalization. Amongst them both hybrid silica MNPs and capsules containing the redox responsive disulfide (S-S) bridges (*ss*-NPs) have been developed in our group[2,3]. These nanoparticles are able to break in small pieces upon their exposure to a reducing agent, which transforms the S-S group in 2 SH moieties causing the destruction of the systems. Using these responsive nanomaterials we demonstrate how deliver drugs[4] but also bigger macromolecules such as proteins, and induce cell apoptosis[5].

METHODS: The breakable mesoporous silica nanoparticles and the breakable silica shell were synthetized by a modified Stöber process and water-oil emulsion procedure respectively. In both cases, tetraethyl orthosilicate and bis[3-(triethoxysilyl)propyl]disulphide were mixed to form the breakable silica framework (Fig.1). Morphological characterization of the particles were performed by Scanning Electron Microscopy showing round shape particles around 70 nm The breakability properties of the materials were then monitored in solution and upon internalization in Glioma C6 cells by TEM.

RESULTS: The MNPS show a good degree of monodispersity. The pore size of mesoporous silica nanoparticles is 2.5 nm. Breakability tests were performed in presence of glutathione (10 mM) and inside the cells. TEM images show (Fig. 2), that the particles were broken after 48h and are eliminated through an exocytosis process. Cellular uptake followed by confocal microscopy reveals that the particles were localized in the lysosomes after 24h of incubation. They did not cause cytotoxicity up to 0.15mg/mL. However, when the particles were loaded with cytotoxic drugs/proteins, a decrease of the cell viability has been observed.

DISCUSSION & CONCLUSIONS: We created a simple method to synthetized stimuli responsive silica nanoparticles able to break in small pieces upon an external stimulus (here reducing agent). The mechanism of destruction of the particles relies on the reduction of the disulphide and can be controlled with different amount of the organic vs inorganic precursor. We demonstrated that the responsive silica network can also be used for the creation of capsules able to entrap and release proteins inside cells (Fig. 2).

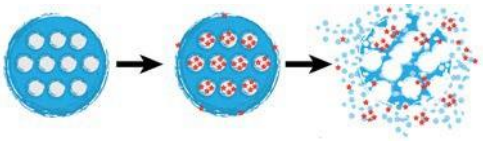

Fig. 1: General methodology to construct stimulus responsive nanoparticles

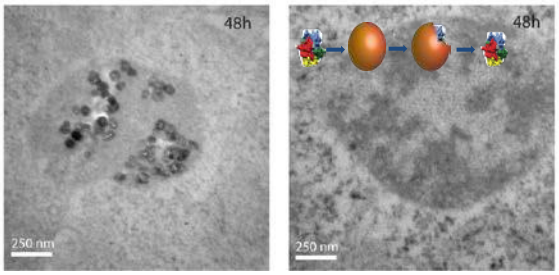

Fig. 2: Transmission electron micrographs of internalized non breakable (on the left) and breakable (on the right) silica shell in C6 glioma cells after 48h.

ACKNOWLEDGEMENTS: We would like to thank, the ARC through the project "TheraHCC" (grant N° IHU201301187).

Designing Targeted PLGA Nanoparticles with a Peptide Antagonist of VLA-4 as a Smart Drug Delivery System for Dysfunctional Endothelial Cells

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INTRODUCTION: Dysfunctional endothelium is the initial and critical step of atherosclerosis. Rapid regeneration of damaged endothelial cells is expected to have a very important role in preventing of the disease. Discovery of smart drug delivery systems, directed against endothelium is essential due to its low access to imaging and pharmacological agents in the blood stream.

METHODS: Fluorescein isothiocyanate-loaded poly (DL-lactic-co-glycolic acid, PLGA) nanoparticles (NPs) were fabricated using double-nozzle electrospraying (at flow rate, DTAB salt concentration and polymer concentration values of 0.08 ml/h, 0.8 mM, and 0.7 w/v%, respectively). NPs were targeted directed against VCAM-1 with a peptide antagonist of VLA-4 (mZD7349 with sequence (cyclo(MePhe-Leu-Asp-Val-D-Arg-D-Lys)). Size, polydispersity index (PDI), zeta potential, and encapsulation efficiency (EE) of NPs and mZD7349-NPs were determined by Zetasizer. Rate of binding and internalization of mZD7349-NPs and NPs to activated human umbilical vein endothelial cells (HUVECs) by TNF-α (10 ng/ml, for 6h) were compared using fluorometry at 4ºC and 37ºC for incubation times of 30, 60, and 120 min.

RESULTS: Characteristics of NPs and mZD7349-NPs are shown in Table 1. The rate and the extent of binding of mZD7349-NPs were greater than NPs in all the three incubation times (Figure 1). Internalization of NPs and mZD7349-NPs decreased at 4ºC than to 37ºC.

Table 1. *Characteristics of NPs (n=3, mean± SD).*

Type	Size (nm)	PDI	Zeta potential (mV)	EE (%)
NPs	225± 14	0.47± 0.06	-11.7± 0.8	86.3± 6.5
mZD7349-NPs	229± 12	0.46± 0.07	-11.6± 1.1	69.6± 7.6

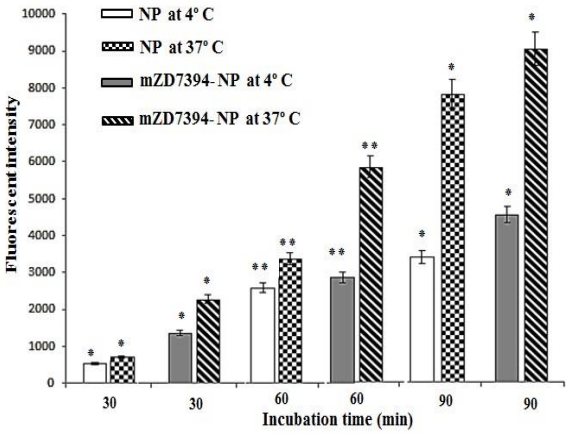

Figure 1. *Rate of binding and internalization of mZD7349-NPs and NPs to activated HUVECs at 4ºC and 37ºC for times 30, 60, 120 min. mZD7349-NPs interacted faster and more with HUVECs compared with NPs at all time-points. Data are presented as mean ± S.D. (n=3), ⋆, ⋆⋆ indicate p < 0.05, p <0.01, respectively.*

DISCUSSION & CONCLUSIONS: Encapsulation efficiency in electrospraying is high and in this technic separation procedure of particles from the solvent is not required. Take up of ligand conjugated- NPs occurs by receptor-mediated endocytosis that occurs faster than the unconjugated NPs endocytosis. Internalization reduction of NPs and mZD7349-NPs at low temperature suggested energy dependent endocytosis of NPs. To conclude, mZD7394- NPs directed against VCAM-1 is suggested as a suitable carrier for atherosclerotic lesions upregulating VCAM-1.

ACKNOWLEDGEMENT: This research was supported by the grant No. 92-03-30-23890 from Tehran University of Medical Sciences.

Interaction between a model lipid layer and the neuroactive drug propofol

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INTRODUCTION: General anesthesia is an induced physiological state of unconsciousness when the patient becomes amnesic, totally relaxed and loses the response to pain. Even though there is more than 150 years since general anesthesia was introduced in medical surgery practice, the molecular mechanism by which drugs can produce this state is still under debate. One of the main reasons is the chemical diversity of the existing general anesthetics.

To gain more understanding of the interaction between anaesthetics drugs with phospholipid membranes, we focused our work on one particular drug molecule, propofol, which is predominantly hydrophobic but contains a hydrophilic OH-group located in the middle of the molecule. Propofol is probably most well-known for inducing general anaesthesia, however it has antioxidant, anxiolytic, analgesic, immunomodulatory and anticancer effects too.

METHODS: The *Vibrational Sum Frequency spectroscopy* (VSF) is a second-order nonlinear optical technique capable of providing molecular information at interfaces. Surface specificity steams from its nonlinear nature, being able of giving sub-monolayer information even in the presence of the same molecule in the bulk. In this method, two laser beams, a fixed visible beam and a tuneable infrared beam, are overlapped on the samples surface which generates a third beam that carries interface information [l]. *Langmuir trough.* A KSV MiniMicro trough was used in order to investigate phospholipid films at various surface pressures.

RESULTS: We have used Langmuir through together with the surface specific laser technique vibrational sum frequency spectroscopy (VSF) to investigate propofol-induced changes in the molecular order in both the hydrophilic head group region and the hydrophobic chain region of three saturated phosphatidylcholines of different chain lengths. In addition, the propofol itself has been targeted, as well as the water molecules hydrating both the lipids and the propofol. The studies have been performed at 25 mNm^{-1}, to mimic a biological membrane, as well as at 5 mNm^{-1}, in order to gain understanding of how the surface pressure affects the ability of propofol to interact with phospholipids.

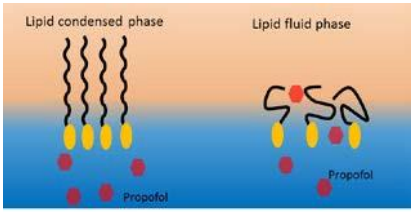

Figure 1. Graphic representation of propofol interaction with the monolayer as a function of pressure.

DISCUSSION & CONCLUSIONS: We found that propofol has the ability to enhance the fluidity of the phospholipid monolayers by penetrating them, a process that was enhanced for phospholipid monolayers possessing more disorder as depicted in Figure 1. The effect of propofol on the head group region depends on the area per molecule, which is affected by the tail length. The water surrounding the head groups of a disordered monolayer is affected by the presence of propofol, while the water hydrating well-ordered monolayers is not. The effect of propofol on monolayer decreases with increasing surface pressure.

It is hoped that the efficiency of inducing anaesthesia can be better understood adopting these observations, and that methods of enhanced drug delivery can be developed to promote better targeting of the anaesthetizing agent.

ACKNOWLEDGEMENTS: This work was kindly supported by Omya International AG, Switzerland.

Stimuli-responsive hybrid organic/inorganic mesoporous silica nanoparticles for biomedical applications

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INTRODUCTION: Mesoporous silica nanoparticles (MSNPs) have attracted a lot of attention in the biomedical field due to their low toxicity and biocompatibility.[1] Indeed their versatile synthesis lead to the tuning of the particle size, shape and to the functionalization of their inner and outer surface. Thus many *in vitro* studies have been reported about the MSNPs and recently, *in vivo* investigations have started since the oral administration of such nanoparticles have been approved by the American Food and Drug Administration (FDA).[2,3]

However, some concerns are related to the intravenous administration of MSNPs, since the materials accumulate in vital organs and often their biodegradability is too slow or absent, preventing clinical translation.

In the attempt to solve this issue, we developed stimuli-responsive hybrid organic/inorganic mesoporous silica nanoparticles based on the incorporation of cleavable organic linkers in the silica framework (Fig.1 (a)).

METHODS: The organic linkers based on imine derivatives have first been synthesized, purified and characterized by spectroscopic and spectrometric methods (NMR, IR, ESI-MS). The transformation upon a pH change, was followed by NMR. Using the well known sol-gel process, the linkers and the silica precursors have been reacted in order to obtain spherical shape and nanometer size particles (150 nm) with pores sizes of 2.3 nm diameter. The particles have been characterized by electron microscopy (SEM, TEM) (Fig.1 (b) and (c)), spectroscopy (XPS, IR) and X-ray light scattering techniques (SAXS, DLS) while the pores diameter has been determined by nitrogen adsorption/desorption method.

RESULTS: The porous particles have been clearly evidenced to be breakable by different techniques (TEM, SEM, NMR spectroscopy). They afford very small fragments releasing soluble aldehydes from the original imine derivative linker upon its cleavage. *In vitro* experiments showed that the particles are uptaken by cancer cells (Hs 578T) after only 5 minutes of incubation. Furthermore, staining and analysis with confocal microscopy enabled us to observe that when the nanomaterials are internalized by the cells, they go into the cytoplasma (Fig.1 (e)). Cell viability assays demonstrated that these particles have a very low cytotoxicity (Fig.1 (d)).

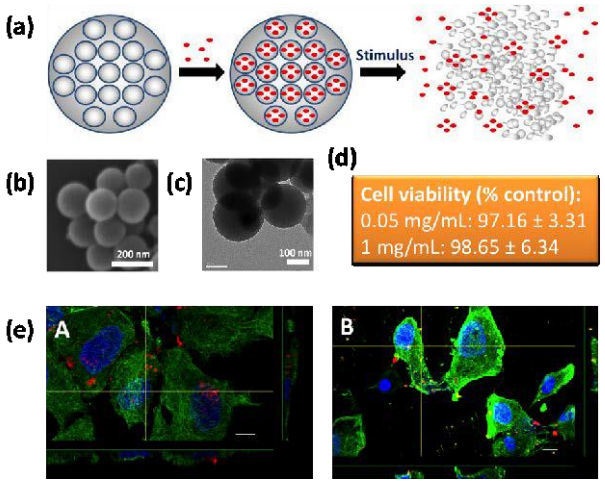

Fig. 1 (a) representative scheme of the investigated system. (b) Scanning electron micrograph of the particles. (c) Transmission electron micrograph of the particles. (d) Cell viability conditions and results. (e) Confocal microscope images of cells after (A) 4h and (B) 24h of incubation with the particles. Cell line: Hs 578T.

DISCUSSION & CONCLUSIONS: Due to the presence of the cleavable linker in the silica framework, a novel type of stimulus responsive silica material has been obtained. Preliminary *in vitro* experiments showed encouraging results and the kinetics of the release as well as evidence of destruction inside the cells will be presented. This is an important step towards the realization of novel smart drug carriers, capable to keep the payload in the circulatory system and to release it only when arrived at the tumor site where the more acidic pH can trigger the release of the content. The elimination of the fragments from the animal body will then be studied.

ACKNOWLEDGEMENTS: We thank the European Research Council (ERC) grant agreement N° 2009-247365, for financial support.

Mechano-responsive Vesicles for Targeted Drug Delivery

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INTRODUCTION: The treatment of an acute heart attack patient requires rapid intervention already on the way to the hospital. A smart, targeted drug delivery of a vasodilator to the site of the arterial blood clog will lead to an immediate relieve of pain symptoms and reperfusion of the heart muscle with oxygenized blood, leading to a lower morbidity burden compared to the current treatment regime.

A stenosed artery is not targetable with current chemical and biological targeting ligands because of lack of specificity. In general, inflammations frequently occur in the entire human body. The average wall shear stress, however, changes by at least one order of magnitude when the blood passes from a healthy into a constricted artery segment. Such a purely physical trigger could be used to target stenosed arteries in a specific manner.

METHODS: We have developed a shear-stress responsive phospholipid vesicle that releases its cargo upon mechanical stimulation [1]. The artificial phospholipid Pad-PC-Pad, see Fig. 1, self-assembles into non-spherical vesicles that show no spontaneous trans-membrane release but do release their aqueous contents when the vesicle is mechanically stressed. The contribution contains recent advances using small-angle X-ray scattering in microfluidic devices, cryogenic transmission electron tomography of the vesicle structures, and first *in vitro* and animal toxicity testing.

RESULTS: 1,3-diamidophospholipids form interdigitated bilayer membranes with overall zero spontaneous curvature [2]. Forced into three-dimensional structures the membranes form facetted vesicles with membrane defects that are possibly attenuated by mechanical stimuli. This should lead to the release of vesicle cargo. A microfluidic device was modelled after the hemodynamic forces found in imaged and characterized diseased human artery segments. This device was placed into the X-ray beam of a synchrotron radiation source. From spatially resolved measurements in the modelled artery occlusion, a picture emerged of vesicle deformation according to the predicted force field applied.

The vesicles were tested *in vitro* (ELISA) and *in vivo* (pigs) for their propensity to activate the complement system. A surprisingly low immune system activation was triggered by Pad-PC-Pad vesicles even when they were dosed one hundredfold higher than the FDA approved Doxil or Ambisome [3]. The Pad-PC-Pad vesicles rapidly form aggregates. Therefore, a PEGylated phospholipid was incorporated into the vesicles. This incorporation does retain the mechano-responsiveness of the vesicles but leads to the low activation of the complement by polyethylene glycol.

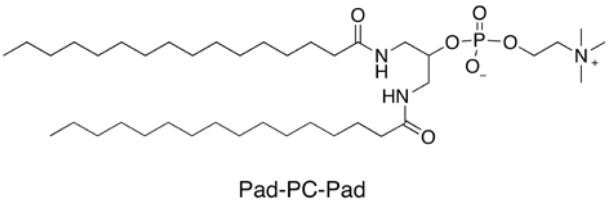

Fig. 1: Structure of the synthetic 1,3-diamido-phospholipid Pad-PC-Pad.

DISCUSSION & CONCLUSIONS: Single component natural phospholipid vesicles are not mechano-responsive [1]. Vesicles formulated from POPC release their cargo both spontaneously or when shaken on a vortex shaker. Contrary, DPPC vesicles do not release their cargo in either case. Therefore, the class of Pad-PC-Pad vesicles show unprecedented mechano-responsiveness and allow a variety of attractive applications in the field of smart drug delivery.

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Face to Face Interaction of Bacteria with Bio-surfaces

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INTRODUCTION: The interface between disease-causing bacteria and bio-surfaces are the microenvironment where bacterial virulence factors can be sensed by certain receptors on the host cells. Upon exposure to bacterial virulence factors including their surface antigens downstream inflammatory can be developed. In this presentation two dissimilar model bacteria, *Treponema denticola* and *Neisseria gonorrhoeae*, was used to discuss the consequences of exposure to their surface proteins.

METHODS: The major outer sheath protein (Msp) of the periodontal pathogen, *Treponema denticola* was enriched through a two-step isolation procedure using non-ionic detergents. Disassembly of actin cytoskeleton upon exposure to Msp was investigated with a barbed-end fluorescent labeling method. The functional impact of actin cytoskeleton disorganization was determined with an *in vitro* scratch wound migration assay in fibroblast monolayers and a videomicroscopy migration assay in neutrophils. The role of opacity protein (Opa) of *Neisseria gonorrhoeae*, the causative agent of gonorrhea was studied in a humanized mouse model. A vaginal infection model and an air pouch model were used to study the interaction between the surface protein Opa with the carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) on the host cells.

RESULTS: Msp-treated fibroblasts became oval in shape and lost their actin cytoskeleton in the center while the control vehicle-treated cells maintained their stellate shape (Fig.1). Msp pretreatment had a significant inhibitory effect on the migration of the fibroblasts across a collagen substratum and inhibited the neutrophil chemotactic migration towards a chemoattractant. To test if CEACAM expression in transgenic mice can make a difference in terms of gonococcal colonization we conducted a vaginal infection with Opa-expressing *N. gonorrhoeae* strains. On day 3 post-infection, gonococci were able to colonized CEACAM transgenic mice to a higher degree compared to wild type mice. To assess the *in vivo* capacity of human CEACAM-expressing neutrophils to be recruited by *N. gonorrhoeae*, and consequent production of pro-inflammatory cytokines we took advantage of an air-pouch model of infection. When CEACAM-expressing mice were injected sub-dermally with *N. gonorrhoeae*, a substantially increased number of neutrophils and pro-inflammatory cytokines could be observed in the air pouch as compared to wild-type mice.

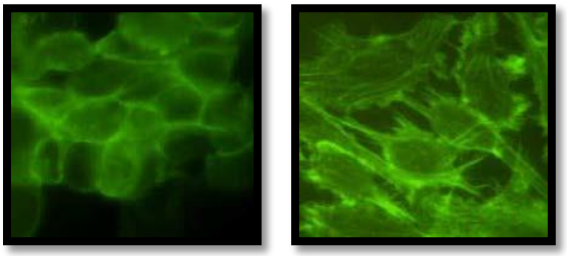

Fig. 1: Effect of major outer sheath protein (Msp) of T. denticola on fibroblasts: Stressed Msp-treated fibroblasts (left) vs. normal (happy) fibroblasts (right).

DISCUSSION & CONCLUSIONS: Both bacterial surface proteins, Msp and Opa can mediate mucosal damages through either disturbing fibroblast function[1] or recruiting neutrophils and productions of pro-inflammatory cytokines. CEACAMS are believed to be target molecules for various bacterial surface proteins including Opa[2].

Flow cell detection of Biofilm formation on a Novel Antimicrobial Antithrombogenic Surface

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INTRODUCTION: Evolve™ is a novel antimicrobial and antithrombogenic material. It is a 3rd generation coating developed by BioInteractions Ltd. AvertPlus™ uses the same antimicrobial technology as Evolve™ and has non-thrombogenic properties, but is not antithrombogenic. Here we show Evolve's™ antithrombogenic properties and how we adapted a biofilm formation assay, the flow cell system, to test the antimicrobial properties of BioInteractions Ltd non-leaching antimicrobial coatings on the surface of medical devices.

METHODS: Platelet adhesion study: blood was taken from a heathy donor, platelets were recovered and diluted with platelet poor plasma to obtain $1x10^5$ platelets/µl. Polystyrene coverslips coated with Evolve™ and uncoated (UC) ones were incubated with the suspension, washed and observed under the microscope.

Flow cell biofilms: The flow cell system[1] was set up to contain two 4 cm pieces of polyurethane catheter in each chamber. After sterilization, the chambers were incubated with plasma overnight, washed and challenged with bacteria for 2h to allow attachment. Then a constant media flow was set up to ensure that bacteria had access to nutrients during the assay. The system was kept at 37°C for 2 days. Biofilm formation on the catheter surface was evaluated under the microscope.

RESULTS: Platelet adhesion studies are often used as an index of haemocompatability. The comparison of Evolve™ coated and UC coverslips shows that the coating strongly reduces platelet adhesion to the surface (Fig. 1).

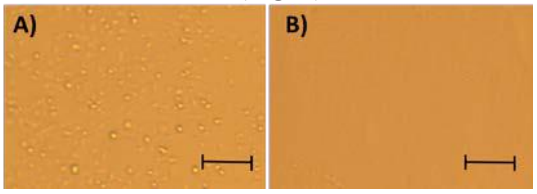

Fig. 1: Platelet adhesion assay on UC (A) and Evolve™ coated (B) surfaces. Images were taken using x40 objective and a Moticam 2000 camera.

Evolve™ antimicrobial properties are not affected by plasma incubation. Polyurethane catheters coated with Evolve™ were incubated with human plasma or saline solution overnight, washed and challenged with bacteria for 24h. The bacteria recovered from the catheters in both conditions were similar on catheters challenged with *S. aureus* ATCC6538 and *E. coli* ATCC8739.

Flow cell biofilm formation technology is being adapted to test the antimicrobial properties of BioInterations Ltd coating. The optimizations were made by comparing biofilm formation on UC catheters versus AvertPlus™ coated catheters. The optimizations done included introducing a sample of the medical device under test in the system without affecting its flow; testing different media for each bacterial species according to its nutrient requests; test media flow to avoid bacteria starvation or wash-out; and the influence of catheters exposure to plasma prior to bacteria challenge. The results obtained clearly show the antimicrobial properties of AvertPlus™ treated catheters (Fig. 2). Evolve™ is currently being evaluated in this system.

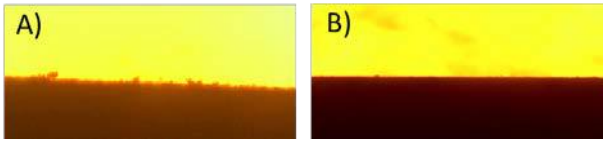

Fig. 2: S. aureus biofilms on the surface of a polyurethane catheter after 48h of growth in a flow cell system. A) UC catheter and B) AvertPlus™ coated catheter. Images were taken using objective x4 and a Moticam 2000 camera.

DISCUSSION & CONCLUSIONS: Platelet adhesion tests on Evolve™ coated polystyrene clearly confirm its antithrombogenic properties. Preliminary antimicrobial tests done on Evolve™ show that plasma exposure does not change its antimicrobial properties and flow cell studies done on AvertPlus™ coated catheters, that shares similar antimicrobial technology, clearly indicate a strong biofilm reduction on AvertPlus™ coated catheters. We have adapted the flow cell systems to test biofilm formation on the surface of medical devices and we are currently using this to test Evolve™ properties against *E. coli*, *S. aureus* and *P. aeruginosa* species.

ACKNOWLEDGEMENTS: To all members of the BioInteractions Ltd team.

Characterizing copper incorporated into the oxide layer of anodized titanium

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INTRODUCTION: In several studies we have introduced the approach for modifying the surface of titanium implants with antibacterial copper [1]. Recently we showed that copper is not only be deposited onto the titanium oxide layer but can also be incorporated into the oxide layer when appropriate process parameters are used [2]. The nature of the copper species in the oxide layer is characterized by XPS spectroscopy.

METHODS: Discs (∅12 mm, 2 mm thick) of cpTi (grade 4) were anodized according to the spark-assisted anodizing method. For Cu functionalizing this method was modified in such a way that Cu deposition and oxide layer formation is performed in a combined deposition-anodizing process using proprietary process parameters (KKS TioCel^{TM}) [1]. After the electrochemical process the surface of the discs were Laser-marked by squares (1 mm x 1 mm) to facilitate local assignment for the surface analysis. Each square was analysed by SEM and EDX (Hitachi TM3000, BRUKER EDX) and mapped for the element Cu. Based on the SEM/EDX surface analysis few squares with clearly detected Cu were selected for XPS depth profile analysis using argon ion sputtering (PHI Quantum 2000; 15keV, 200µm spot, 40W, E_{pass} 117.4 eV with 0.5 eV/measure point for depth profil and 23.5 eV with 0.2 eV/measure point for spectrum fit; Al-Kα, monochromatic; sputter conditions: Ar^+ ions; 4 keV, raster 1.0 x 1.0 mm^2, sputter rate 70nm/min, sputter depth ~ 3 µm).

RESULTS: SEM/EDX analysis show that copper is incorporated as amorphous phase into the oxide layer (Figure 1).

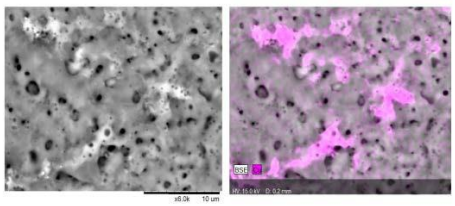

***Fig. 1**: SEM image of the anodized titanium with Cu in the oxide layer (white areas) (left) and Cu mapping by EDX (right).*

Copper signals are observed in the XPS spectrum (Figure 2). The fit of the Cu2p3/2-peak in the spectrum from the surface reveals the copper species as mixtures of Cu_2O&Cu(0) (22.8%), CuO (68.8%) and $Cu(OH)_2$ (8.4%) (Figure 3).

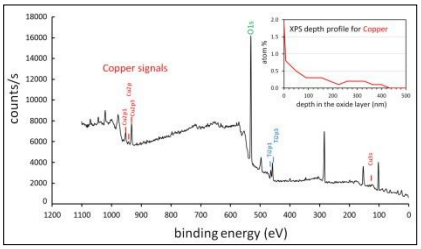

***Fig. 2**: XPS spectrum from the disc surface and depth profile for copper (Cu is seen to a depth of 400nm).*

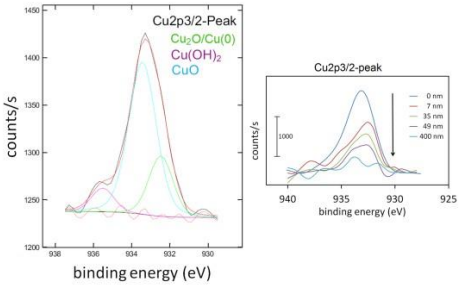

***Fig. 3**: Cu2p3/2-peak of the surface spectrum curve-fitted (left) and at different sputter times (depth) (right).*

DISCUSSION & CONCLUSIONS: The different data obtained so far for titanium samples with copper deposited as crystal clusters onto the surface indicated copper release [4], antibacterial effect [3] and acceptable *in vitro* human cell viability [5]. For the copper incorporated into the oxide layer shown here such studies must still be performed. A delayed Cu release and a prolonged antibacterial effect might be expected.

ACKNOWLEDGEMENTS: XPS measurements have been done as paid service by SGS Institute Fresenius, Dresden. Collaboration with the University of Applied Sciences Northwestern Switzerland and CTI funding are acknowledged.

Reduced Adhesion of *E.coli* on Nanostructured Polycarbonate Surfaces

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INTRODUCTION: Recently a novel concept for the design of antibacterial surfaces based on nanostructuration has gained widespread interest. Inspired by insect wing structures antimicrobial nanostructured surfaces could be generated from materials as diverse as silicon, titanium and graphene ([1]and references therein). However to our knowledge, nanostructured polymer materials investigated so far exhibit only minor, if all, bactericidal activity. Here we present investigations of the antibacterial properties of structured polycarbonate surfaces.

METHODS: Polycarbonate (PC) sheets were nanostructured by treatment with oxygen plasma[2]. To reduce chemical differences between structured polymers and unstructured reference samples references were also plasma treated but with a reduced power. *E.coli* ATCC 8739 were grown in nutrient medium, pelleted by centrifugation and resuspended to an OD_{600} = 0.1 in PBS. 14 mm round polymer samples were incubated ON with 1 ml of bacterial suspension in 24 well plates at 80 rpm. Following incubation the samples were washed at 110 rpm and the polymer adhered living bacteria were quantified by incubation in synthetic growth medium with resazurin metabolic indicator. Bacterial survival was controlled by fluorescent dead/live staining.

RESULTS: We investigated the effect of polycarbonate (PC) nanostructuration on the adhesion and survival of *E.coli* cells. Upon incubation in PBS for either 3 hrs or ON under shaking or resting conditions we consistently observed strongly diminished resazurin reduction on structured versus unstructured PC. Fewer bacteria were observed on structured substrates and the ratio of dead:live bacteria was not obviously changed as compared to unstructured substrates. Taken together these results demonstrate a reduced bacterial adhesion on the structured surfaces. Next we investigated the effect of chemical surface modifications on the survival and adhesion of *E.coli* to structured and unstructured PC by creating either a hydrophobic surface (via silanization) or a surface coated with the known bacterial membrane weakening agent polyethyleneimine (PEI). Both modifications increased the number of live bacteria on structured as compared to unstructured surfaces.

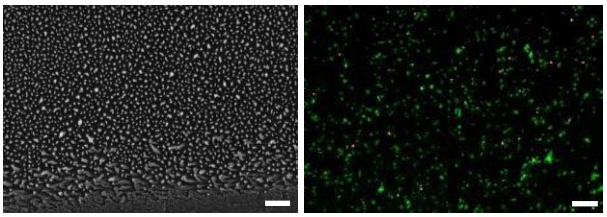

Fig. 1: Effect of surface structuration on E.coli survival. Scanning electron microscopy (SEM) image of plasma treated PC (left) and dead/live staining of E.coli on structured PC (dead bacteria red, live green; right).The length of the PC nanopillars can be seen on the artificially scratched area at the bottom of the SEM image. Scalebars: 500 nm (left), 20 µm (right).

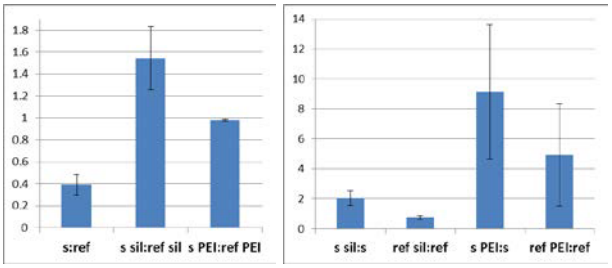

Fig. 2: Resazurin assay ratios of E.coli adhesion experiments. Presented are the signal ratios of structured (s) to unstructured (ref) of pure PC and hydrophobized (sil) or PEI modified surfaces.

DISCUSSION & CONCLUSIONS: Polymer surfaces can be easily nanostructured by plasma treatment. We observed strongly reduced adhesion (factor 0.39 ± 0.09) of *E.coli* cells on nanostructured polycarbonate surfaces. The modification of the surfaces with a hydrophobic silane doubled the adhesion of living *E.coli* to the structured surfaces while a coating with PEI resulted in a nearly 10 x increase of the resazurin assay signal. Future experiments will analyse other polymers to investigate the generality of the observed reduction in bacterial adhesion on plasma treated nanostructured surfaces.

ACKNOWLEDGEMENTS: The presented research was kindly funded by the Swiss Nano-science Institute under the Nano Argovia program.

Preventive, antibacterial properties of superhydrophobic coatings in liquid

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INTRODUCTION: Superhydrophobic coatings have recently emerged as a new strategy to prevent biofilm development by acting on the adhesion of pioneer bacterial cells. They can be divided in three categories, partial wetting coatings described by the Wenzel theory, non-wetting coatings described by the Cassie-Baxter theory and mix of the two first states. Here, by using surface coatings with tunable superhydrophobic properties, we attempt to specify conditions of use of superhydrophobic surfaces, especially in liquid surroundings, for preventing bacterial colonization. Various superhydrophobic states are provided for two distinct chemistries, by series of separate surfaces with a range of six different levels of wettability (I) or on single surfaces revealing a gradient of wettability (II).

METHODS: Coatings (I) and (II) were produced by plasma (co)polymerization (I) and by deposition of nanoparticles and further overcoating by plasma polymerization (II) respectively.[1,2] Chemical composition and surface topography of the coatings were thoroughly described by XPS and AFM analyses and further specific data treatments. Superhydrophobic properties were characterized by an innovative water droplet analysis. Live imaging with microscopy was conducted for evaluating effects of superhydrophobicity levels on surface colonization (from 1h to 48h) by GFP-modified *Escherichia coli*. Acquisition of fluorescence micrographs was performed *in situ* with coatings (I, II) immersed in bacterial suspension in mineral medium or lysogeny broth (LB). Quantities of adhered bacteria were determined by ImageJ® software and plugins.

RESULTS: Microbiological investigations in liquid show that notable reductions of colonization by bacteria compared to reference surfaces (i.e. silicon wafer) can be achieved on coatings revealing Cassie-Baxter or Wenzel regime, both with coatings (I) (fig.1) and (II) (fig.2). This effect is accompanied by retention of air at the coating surface as demonstrated by co-localization of bacteria-free areas and air bubbles on coatings (II). When increasing incubation time or in LB medium, inhibition of colonization highly decreases, which is attributed to surface clogging by biomolecules-related films.

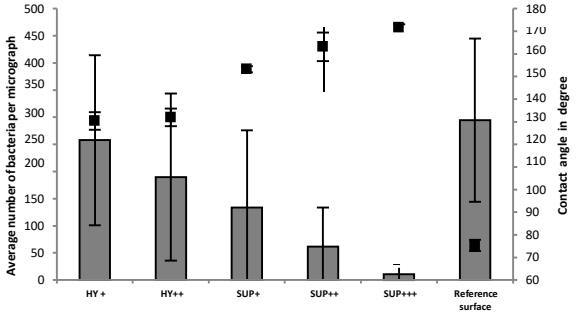

Fig. 1: Colonization by bacteria in mineral medium (1 h incubation) and water contact angles on a serie of from hydrophobic to Cassie-Baxter superhydrophobic coatings (I).

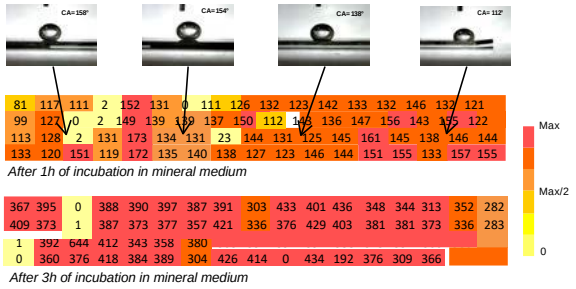

Fig. 2: Water contact angle values and bacteria quantity at various locations of gradient-based coatings (II).

DISCUSSION & CONCLUSIONS: Significant differences in preventive, antibacterial efficacy of superhydrophobic surfaces in liquid have been shown to depend on Cassie-Baxter and Wenzel regimes, surface chemistry and potential supplementary surface conditioning by biomolecules. Superhydrophobic thresholds associated to specific preventive levels will now be compared to those determined in air surroundings after inoculation by bacterial suspension droplets. Investigations under hydrodynamic conditions are also conducted to complete the specification of use of superhydrophobic surfaces materials in health-related fields.

ACKNOWLEDGEMENTS: The authors thank Fonds National de la Recherche Luxembourg (FNR), Region Alsace and Centre National de la Recherche Scientifique (CNRS) for their financial support.

Dynamic Contact Assay of a Novel Antimicrobial and Antithrombogenic Surface

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INTRODUCTION: A dynamic contact assay was used to evaluate the surface activity of a novel non-leaching antimicrobial surface coating, Avert*Plus*™, and the second generation antimicrobial, antithrombogenic surface coating, Evolve™, developed by BioInteractions. Their efficacy was evaluated against *S. aureus*, *E. coli* and *P. aeruginosa* using a dynamic contact assay. Incubation conditions were optimised to achieve a 3-log reduction in the presence of a polyurethane surface coated with the antimicrobial and anti-thrombogenic materials.

METHODS: Antimicrobial activity tests carried out using a dynamic contact assay. This assay is a modified version of the American Association of Textile Chemists and Colorists test method (AATCC 100), known as ASTM E2149-01 [1].

Based on a study by Cady *et al.* 2001 this assay was further optimised for the testing of the antimicrobial activity of Avert*Plus*™ and Evolve™, developed by BioInteractions, against *S. aureus* ATCC 6538p, *E. coli* ATCC 8739 and *P. aeruginosa* ATCC 9027.

RESULTS: Initial experiments using the dynamic contact assay showed that a significant reduction in bacterial viability could be detected in the presence of BioInteractions antimicrobial coatings, see table 1. Significantly enhanced antibacterial activity of Evolve™ against *P. aeruginosa* is observed.

Table 1. Bacterial log reductions in the presence of AvertPlus™ and Evolve™ after 72 hours incubation with 10^7 c.f.u/ml bacterial suspension.

Bacterial Strain	Avert*Plus*™ Log Reduction	Evolve™ Log Reduction
S. aureus	2.83	3.49
E. coli	1.48	1.99
P. aeruginosa	1.78	6.07

Optimisation of the incubation conditions including the methodology of agitation, assay incubation time, concentration of the bacterial inoculum, sampling times, ratio of catheter length (surface area):volume of bacterial suspension ratio resulted in significant improvement of the detected antibacterial activity of BioInteractions coatings. Since the initial study (Table. 1) these optimization steps have been trailed using Avert*Plus*™ challenged with *E. coli* as a model system. A significant improvement in the detected antibacterial activity has been observed with greater than 3-log reduction in the presence of Avert*Plus*™ detected for all samples tested. This improvement was then confirmed on challenge of Avert*Plus*™ with *S. aureus* and *P. aeruginosa.* Perhaps most significant was the reduction in incubation time required to achieve this from 72 hours down to 24-48 hours and in some cases only 5 hours of incubation with coated catheter sections was required.

Using this methodology dose dependent killing could also be observed with an increase in the surface area of the coated surface resulting in increased antibacterial activity.

Further testing is now being carried out for Evolve™ with similar improvements expected. Then both coatings will be tested after a pre-incubation of the catheter sections in plasma.

DISCUSSION & CONCLUSIONS: BioInteractions has developed novel antimicrobial surface coatings targeted for the medical device market. In this study we focused on the determination of the antimicrobial activity of Avert*Plus*™ and Evolve™ using a modified version of the AATCC 100, ASTM method E2149-01. This assay was found to be both durable and reproducible with effectivity demonstrated using a variety of coated catheter types.

Of particular interest is the enhance antibacterial activity of Evolve™ against *P. aeruginosa*. This is thought to be due to the *P. aeruginosa* binding the anti-thrombogenic component of Evolve™, thus resulting in increased contact with the antibacterial agent.

ACKNOWLEDGEMENTS: BioInteractions Team.

Quasi-instantaneous in vitro bacterial inactivation on Cu-Ag uniform catheters

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INTRODUCTION: The first evidence for Cu-Ag (50%/50%) nano-particulate coated catheter is presented leading to an almost instantaneous bacterial inactivation in the dark (≤ 5 min). Bacterial inactivation times on Cu-Ag (50%/50%) were observed in the dark were similar with the times required when irradiating with actinic light. This provides the evidence that the bimetal Cu-Ag coating inactivates bacteria and not CuO/Cu_2O or Ag_2O presenting semiconductor behavior. The release of Cu/Ag-ions during bacterial inactivation was followed by inductively coupled plasma mass-spectrometry (ICP-MS). The amounts observed were in the ppb-range well below the cytotoxicity levels permitted by the sanitary regulations. By stereomicroscopy the amount of live/dead cells were followed within the bacterial inactivation time

METHODS:

Sputtering of Cu-Ag Films on catheters: The coating of the cylindrical 3D catheters was carried in the mean of a magnetron-sputtering. Uniform and adhesive 3D-film coverage was attained on the catheter surfaces. The residual pressure P_r in the sputtering chamber was set at $P_r \leq 10^{-4}$ Pa. The substrate target distance was 10 cm. During the sputtering the sample was rotated to insure a uniform distribution of the coating on the catheter surface. The Cu/Ag atomic ratios sputtered during the course of this study were: 25%-75%, 33%-67% and 50%-50% %Cu-%Ag respectively.

CFU Evaluation and Live/Dead cells on the coated catheters: PU-Catheters were sterilized by keeping them in an oven at 70°C overnight. 100 µL culture aliquots with an initial concentration of ~10^6 CFU mL^{-1} in NaCl/KCl (pH 7) were placed on coated and uncoated (control) catheters. Serial dilutions were made in NaCl/KCl solution. A 100µL sample of each dilution was pipetted onto a nutrient agar plate and spread over the surface of the plate using the standard plate method. Fluorescence stereomicroscopy was used to monitor the live or dead cells on the coated catheters as a function of time.

RESULTS: Figure 1 shows that the bacterial inactivation time of *E. coli* was accelerated to 5 min on a 50%/50% Cu-Ag PU-catheter under actinic light irradiation or in the dark compared to Cu or Ag deposited by itself on PU-catheters [1,2].

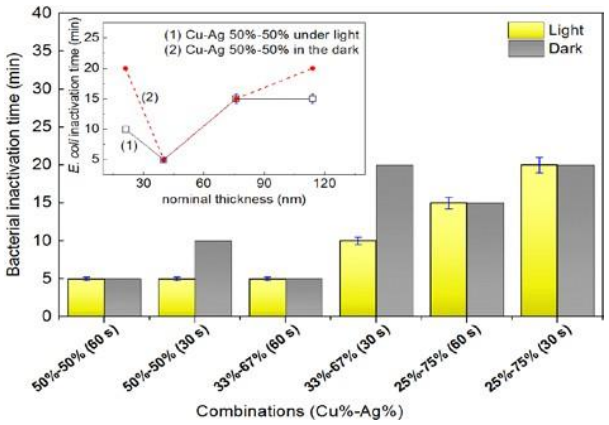

Fig. 1: E. coli inactivation on Cu-Ag PU-catheters sputtered for different times showing the effect of the atomic ratio of Cu:Ag on the bacterial inactivation time. The applied light is a low intensity actinic light (3 mW/cm^2).

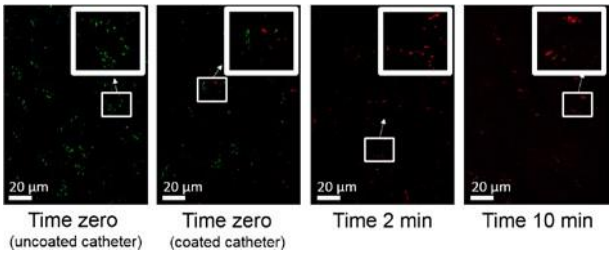

Fig. 2: live/dead E. coli inactivation on Cu-Ag (50%-50%) sputtered PU-catheters with respect to uncoated catheter.

DISCUSSION & CONCLUSIONS: A quasi-instantaneous inactivation kinetics was obtined ≤ 5 min within the time of bacterial plate counting. The bacterial inactivation kinetics was seen to be a function of the Cu:Ag ratio film coating the PU-catheters. Unexpectedly, the inactivation times were seen to be similar on sputtered Cu-Ag films in the dark and under light.

Hygienic coatings with active ingredient controlled release

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INTRODUCTION: Current hygienic coatings (applied in food processing areas, hospitals...) are vulnerable to bacterial and fungal contamination.
Today, coatings formulators are using dry film biocides ideally achieving broad-spectrum and long-lasting protection of the paint film. One of the challenges is that relatively few biocide agents are available, and those actives are also toxic for non-target organisms, at the content levels required in coating.
The present approach proposes a high tech hygienic coating containing "micro-reservoirs" (i.e., microgels, microparticles) of biocides, which are able to release the antibacterial active ingredients in a sustained way, to avoid microbial surface colonization in biomedical environments.

METHODS: To study the potential of microgels as biocide reservoirs for dry film paints, several couples of industrially available microgel particles and typical biocides for dry film paints have been prepared. Dry as-delivered microgels were loaded with biocides [either N-octyl-isothiazolinone (n-OIT); 5-chloro-2-methyl-4-isothiazolin-3-one (CMI) and 2-methyl-4-isothiazolin-3-one (MI) 3/1; 1,2-benzisothiazolin-3-one (BIT); zinc pyrithione (ZnPt) or didecyldimethylammonium chloride (DDAC)], by swelling in biocide aqueous solutions. The biocide-microgels interactions, the biocide level in dried microgels and the kinetics of biocide release from the microgels were analyzed by elemental analysis.

Biological activity of dried biocide loaded-microgels and model waterborne paints containing biocide micro-reservoirs has been investigated, in presence of bacteria (gram positive *Staphylococcus aureus)* or a mixture of ten *fungi (*including *Aspergillus niger).*

The model waterborne hygienic paints were then submitted to an accelerated ageing (48 hours at 50°C, continuous water flow at room temperature during four days) and their biological activity was evaluated once again, according to JIS Z2801, ISO 22196:2007 and NFX 41-520 standards, respectively.

RESULTS: The microgel/biocide pairs shown different types of interactions, depending on the nature and intrinsic properties of each partner: (i) BIT was homogeneously distributed between the swollen gel and biocide solution; (ii) n-OIT was only partially loaded inside the swollen gel; the ammonium quaternary biocide DDAC was irreversibly absorbed by the polyacrylate microgels (with negative charges). The best loading biocide levels were obtained for BIT (1.5%w in dry gel particles) and n-OIT (4.0%w).

All hygienic waterborne paints containing the new micro-reservoirs with either BIT or n-OIT shown strong biostatic to biocide effects against *Staphylococcus aureus* or the ten *fungi* mixture including *Aspergillus niger*.

The biological effectiveness of the new high tech hygienic coatings was maintained even for biocide levels in paints 5 to 30 times lower than in existing industrial paints for biomedical and food-processing environments, and even after intensive and accelerated ageing of the dry-paint films.

DISCUSSION & CONCLUSIONS: Commercial polyacrylate microgels were efficiently used to prepare "biocide micro-reservoirs" for dry-paint films. The best microgel-biocide pairs allowed high level of biocide loading and a sustainable biocide release from microgels under moisture effect.

The new hygienic waterborne paints containing microgel-based biocide reservoirs fulfilled all the biological requirements for hospital and food-processing environments, even for biocide levels much lower than in actual commercial paints.

Entrapping the toxic, biologically active agents inside hydrogel microparticles allowed a sustained release of biocide over a long period of time and an important decrease of the overall biocide level in paints, and consequently safe handling and cleaning procedures.

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A microfluidic platform to study bacterial interactions with surfaces

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INTRODUCTION: Bacteria adhering to surfaces and forming biofilm are responsible for infections such as catheter-associated urinary tract infections (CAUTI), which are a common problem in catheterized patients and can lead to severe health concerns. Although tremendous efforts have been dedicated to develop new catheter materials and coatings to decrease infection occurrence, so far there is no success. For this reason, a better understanding of the initial bacterial adhesion mechanism on surfaces and influencing factors is of upmost importance. A microfluidic approach was used in this project to quantify the initial adhesion rate of *Escherichia coli* cells on glass under different conditions, namely medium composition, fluid flow rate, and pre-treatment of the glass surface with proteins.

METHODS: An *E. coli* suspension (DH5a pGFPuv) was injected inside a microchannel (PMDS bonded on glass with rectangular cross-section: height: 50*μm*, width: 200*μm*). Three parameters were tested: medium (minimum medium M9 and tryptic soy broth medium (TSB)), perfusion flow rate (0.5, 1, 2, 4 *μl/min*), and pre-treatment of the microchannel with protein (10% bovine serum albumin). The surface coverage (i.e. the ratio between the surface covered by bacteria and the surface of the channel) was computed from fluorescent micrographs of the microchannels taken at several time points during the bacterial solution perfusion.

RESULTS: Fig. 1 shows the dependence of adhesion rate to medium composition. In this experiment bacteria suspended in M9 medium showed a higher adhesion rate. After 4 h the surface coverage was more than twice as high for bacteria in M9 in comparison to TSB. The influence of the perfusion flow rate can be seen in fig. 2 for cells suspended in TSB. A perfusion rate of 1 µl/min led to the highest surface coverage. A perfusion rate of 0.5 µl/min led to highest increase of the adhered cells from 1 h to 2 h of perfusion. Although bovine serum albumin supports growth of planktonic cells, it was observed that pre-treatment of the glass surface with it completely abolished *E. coli* adhesion (data not shown).

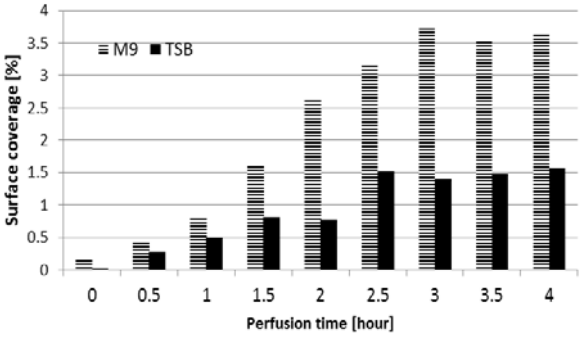

Fig. 1: Surface coverage evolution with time. Microchannels were perfused with bacteria suspended in M9 and TSB media at 1 µl/min. The surface coverage increased with time as more bacteria adhered to the surface and adherent ones divided.

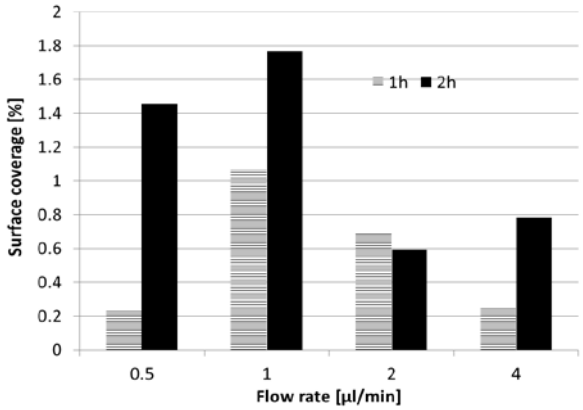

Fig. 2 Influence of the flow rate on the surface coverage. Microchannels were perfused with bacteria suspension in TSB medium at 4 different flow rates. For each flow rate the surface coverage was measured after 1 and 2 hours of perfusion.

DISCUSSION & CONCLUSIONS:

This project highlights the importance of developing appropriate *in vitro* models in order to achieve *in vivo* relevance.

The platform established here is useful in examining the effect of different physiological conditions and material surfaces on bacterial adhesion and biofilm formation. Further improvement of the microfluidic device to allow direct comparison of various settings is needed.

ACKNOWLEDGEMENTS: Authors would like to thank Stefanie Altenried for her technical assistance.

Antibacterial effects induced by mechanical properties of HEMA hydrogel

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INTRODUCTION: Different strategies are described in literature to limit bacterial adhesion without any use of biocide, mainly consisting in modifications of topographical, chemical or hydrophobic properties of the surface. Recently, a new approach was proposed, based on a judicious design of surface mechanical properties. [1] Here, we investigate the potential of a plasma polymer functionalization to provide antibacterial properties by adjusting hydrogel properties for finely tuning the mechanical properties of material surfaces.

METHODS: Hydroxyethyl methacrylate (HEMA) was selected as a plasma polymerization precursor for its capability to undergo hydrogen or van der Waals bondings in plasma polymerized thin film, allowing adjustment of the film hydrogel properties. Deposition conditions were varied by varying power and duty cycle of the plasma discharges. This allowed i) elucidation of the HEMA growing mechanisms and ii) determination of the experimental window in which hydrogel properties can be tailored to obtain different mechanical properties. [2] Plasma polymer thin films were characterized by atomic force microscopy (AFM), Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), contact angle measurements and capillary rise.

Antibacterial properties were evaluated after various time of incubation with 5 10^6 mL^{-1} of GFP-expressing *Escherichia coli* K12 SCC1, in a selective medium (M63G, pH 6.8), at 30°C, under or without any hydrodynamic flow. Bacteria at the hydrogel surfaces were *in situ* observed by fluorescence confocal microscopy. Quantities of mobile and adhered bacteria were determined by using ImageJ® software and home-made plug-ins. Bacterial quantities were determined by both direct enumeration of bacteria and measurement of the surface fraction covered by bacteria.

RESULTS: Physical-chemical characterization of the thin films showed that playing with plasma parameters allows achievement of series of plasma polymer surfaces with the same chemistry but a wide range of mechanical properties (from few hundred kPa to a few MPa).

Microbiological results show that up to a ten-fold decrease of bacterial retention can be achieved on the softest of these hydrogel surfaces against the hardest ones for short-term and static bacterial cultures (fig.1; thin films are identified by the input energy provided to plasma (in $[J.cm^{-3}]^{-1}$). Moreover, more than 70% of bacteria present on the softest surfaces did not adhere but were mobile (less than 5% for the hardest surfaces). In addition, long-term experiments conducted under hydrodynamic flow showed a strong reduction of biofilm formation on soft hydrogel surfaces compared to hard ones.

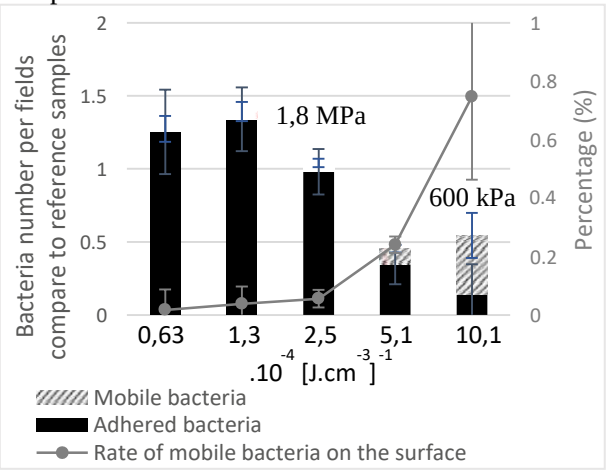

Fig. 1: Bacterial adhesion and mobility depending on plasma polymerization parameters.

DISCUSSION & CONCLUSIONS: These results, obtained under short- or long-term cultures, with or without hydrodynamic flow, demonstrate that soft hydrogel surfaces can prevent the efficient adhesion of pioneer bacteria and the further development of biofilm if hydrodynamic strains are applied. New investigations are currently performed to determine whether structure, density and thickness of the mature biofilm may be especially affected on soft hydrogel surfaces.

ACKNOWLEDGEMENTS: The authors acknowledge BpiFrance, European Commission, Fond Européen de Développement Régional (FEDER), Région Alsace (France) and Centre National de la Recherche Scientifique (CNRS) for their financial support.

Active Surfaces Engineered by Immobilizing Protein-polymer Nanoreactors for Selectively Detecting Sugar Alcohols

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INTRODUCTION: Sugar alcohols (*e.g.* D-sorbitol and D-mannitol) are essential metabolic intermediates involved in biological processes. The detection and concentration of sugar alcohols in aqueous solutions is necessary for a variety of applications, in biochemical, medical or even industrial domains. An elegant way to detect specific molecules with high sensitivity is by designing nanoreactors, which encapsulate active molecules that sense their presence inside polymer supramolecular assemblies (polymersomes, micelles, dendrimers, and capsules) with sizes in the nanometer range.

METHODS: First, such selective nanoreactors were engineered in solution by simultaneous encapsulation of specific enzymes (ribitol dehydrogenase, RDH) in copolymer polymersomes (poly(2-methyloxazoline)-*block*-poly(dimethylsiloxane)-*block*-poly(2-methyloxazoline, PMOXA-*b*-PDMS-*b*-PMOXA), and insertion of membrane proteins (*E. Coli.* glycerol facilitator, GlpF) for selective conduct of sugar alcohols. Ribitol was selected as a model sugar alcohol. Second, to obtain "active surfaces" for detecting sugar alcohols, the nanoreactors optimized in solution were then immobilized on a solid support: aldehyde groups exposed at the compartment external surface reacted *via* an aldehyde-amino reaction with glass surfaces chemically modified with amino groups.

RESULTS: Despite the artificial surroundings, and the thickness of the copolymer membrane, functionality of reconstituted GlpF was preserved, and allowed selective conduct of sugar alcohols to the inner cavity of the polymersome, where encapsulated RDH enzymes served as biosensing entities. After immobilizing the polymer nanoreactors onto modified glass surfaces, the nanoreactors preserved their architecture and activity, and represent active biosensing surfaces for selective detection of sugar alcohols, with high sensitivity. As the enzyme reaction produces NADH, which is fluorescent (with an emission wavelength at 445 nm), it was used as a probe to evaluate the activity after immobilization of polymer nanoreactors with inserted GlpF on a solid support by confocal laser scanning microscopy (CLSM) and UV/Vis spectroscopy. CLSM micrographs of the active surface showed a bright fluorescence of the surface (Fig. 1 left). The cross scratch part of the surface, where no nanoreactors were present after rinsing with water, showed no fluorescence. In addition, UV/Vis spectroscopy was used to evaluate the active surface performance: a linear biosensing reply was obtained for milli-molar range of ribitol, in agreement with the performance of our polymer nanoreactores in solution (Fig. 1 right).

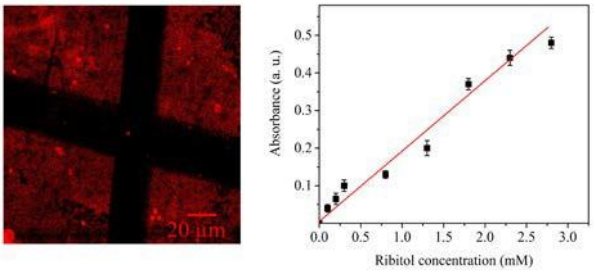

Fig. 1: CLSM micrographs of the active surface with immobilized nanoreactors with inserted GlpF (left) and absorbance intensity of NADH produced by the enzymatic reaction inside nanoreactors with GlpF immobilized on the interior wall of a glass cuvette, in the presence of 0.0 -3.0 mM ribitol (right) (±SD, n=3).

DISCUSSION & CONCLUSIONS: We have developed "active surfaces" for selective biosensing of sugar alcohols with time and space precision based on immobilization of nanoreactors with specific proteins inserted in their membranes, and sugar alcohol sensitive enzymes encapsulated in their cavities. Their immobilization on solid supports provides efficient "active surfaces" due to the rapid change in their fluorescence intensity in the presence of sugar alcohols.

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Nano-Structured Plastic Surfaces for Fluorescence-Based Biological Assays

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INTRODUCTION: Processing of plastic components has become a major market due to the high processing capabilities, producing complex products at large volume and low cost. Integrating nano-structure on the surface of such components has shown increased performances in a number of applications *e.g.* for antimicrobial surfaces [1] or control of the adhesion of biological cells [2]. This work focuses on nano-structuring surfaces in order to improve the homogeneity and sensitivity of biomolecular spotting for diagnostic applications.

METHODS:

Fabrication of nanostructured plastic parts

The overall process-flow for the fabrication of nanostructured plastic parts consists in the fabrication of a nanostructured steel mold insert and the replication of the structures into plastic

For the surface structuring of steel inserts, nanoparticles are first self-assembled on the surface of the steel and used as a template for the fabrication of an etch mask. The structures are then electrochemically etched into the steel. The lateral size of the structures is controlled by the nanoparticles while the vertical dimensions are controlled by the etching step. Structures with a diameter ranging from 300nm to 1micrometer can been produced. The nano-structured steel inserts were used as molds for replication into polycarbonate (Makrolon 2207) by hot embossing and injection molding.

Contact angle measurements: Wettability was characterized by measuring the dynamic contact angles of water on the different structures on the samples. A flat polycarbonate surface was used as reference.

Model fluorescent immunoassay: A model immunoassay was carried out by spotting mouse immunoglobulin on the chosen surfaces. Bovine serum albumin was used to prevent non-specific adsorption. After rinsing, the samples were placed in a solution of the complementary antibody conjugated with a fluorescent marker (Cy5). The fluorescent spots were imaged using a confocal microscope (Zeiss).

RESULTS & DISCUSSION: The nano-structures replicated in plastic are shown in Fig. 1. An advancing contact angle (ACA) of almost 100° is observed on the reference surface with a wetting hysteresis (WH) of 30°. Nano-structurating leads to an increase in ACA and also a significant increase in WH. The roughest sample shows an ACA of 135° and a WH of 85°. This increase in WH suggests the water drops are in the Wenzel mode, which corresponds to an increase in the adhesion of the drop on the surface [3].

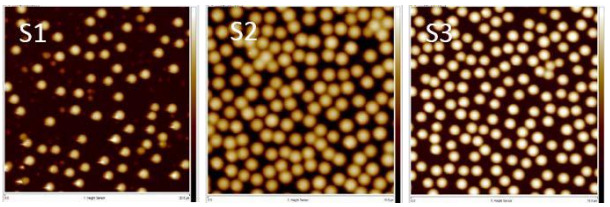

Fig. 1: nanostructures characterized by AFM.

After the immunoassay, homogenous fluorescence spots were observed on the polycarbonate samples, avoiding the so-called “coffee ring” effect. The quantification of the fluorescence signal revealed that one of the structure (S3) leads to an increase in fluorescence of 30%. The origins of this effect are still under investigation. It may be due to the increase in specific surface or the scattering of the light by the nano-structuration.

CONCLUSIONS:

Nano-structured surfaces aimed to improve the sensitivity and homogeneity of fluorescence-based bio-assays were produced in plastic from nanostructured steel inserts. The wettability of the surfaces was influenced by the surface structures and an increase in the adhesion of water drops was observed. One of the structures was also found to significantly increase the sensitivity of an immunoassay.

ACKNOWLEDGEMENTS: The transnational Interreg IVa project Piment is funded by the European Regional Development Fund, the OFES (Swiss Federal Office for Education and Science), the cantons of Neuchâtel and Vaud, and the Franche-Comté and the Rhône-Alpes regions. The authors thank them for their support.

Tuning InAs nanowire geometry for HEK293 cell viability, adhesion, and morphology: perspectives for nanowire-based biosensors

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INTRODUCTION: Arrays of nanowires (NWs) are currently being established as vehicles for molecule delivery and electrical- and fluorescence-based platforms in the development of biosensors [1]. It is conceivable that NW-based biosensors can be optimized through increased understanding of how the nanotopography influences the interfaced mammalian cells. However, in order to systematically evaluate the effects of nanotography on cell behavior, the NW array geometry has to be well-controlled. Here, we use state-of-the-art homogenous InAs NWs to perform such a systematic investigation of how the broad range of NW densities used by the community influences mammalian cells [2,3].

METHODS: Ordered InAs NWs of different densities (spacings 2-10 µm, densities 1-29 NWs/100 µm^2) were grown by an Au-assisted vapor-liquid-solid growth mechanism using molecular beam epitaxy and Au-droplets were positioned using electron beam litography. The different densities and flat controls were contained within single multidensity chips, so that observations could be performed in parallel.
HEK293 cells were interfaced dropwise with the InAs NW arrays and allowed to settle and spread for 24-48 h before staining and imaging.

RESULTS: The overall cell morphology was found to depend on NW density, as seen by the scanning electron microscopy (SEM) and live-cell images of cells in figure 1A. When investigating intracellular structures of immunostained cells, it was furthermore found that the cell cytoskeleton was interacting closely with the NWs (figure 1B) and that focal adhesion (FA) formation was upregulated on NWs compared with flat controls in a NW density-dependent manner (figure 1C). Finally, the cell division rate was shown to depend on NW density and to be generally increased on NWs compared to flat controls (figure 1D).

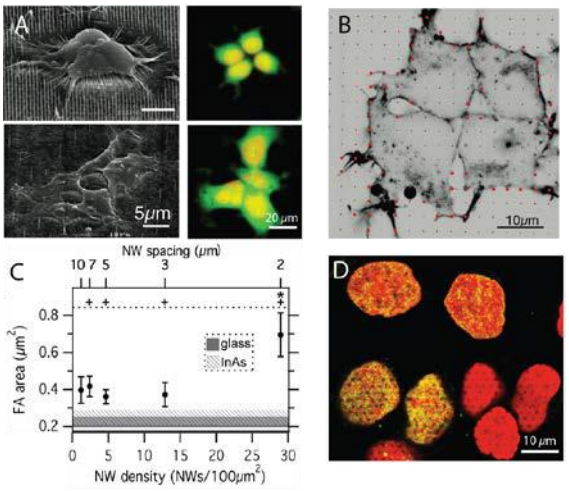

Fig. 1: Systematic evaluation of cell behavior vs. NW density. A) SEM images (left) and corresponding live-cell images (right, cytosol in green and nucleus in red) for two different NW densities. B) Actin (black) filaments of immunostained cells on NWs (pattern also in black). NWs in contact with the actin have been marked with red dots. C) FA density on different NW densities vs. the indicated flat controls. Markings indicate significant differences vs. glass (+), flat InAs (*) or 29 NWs/100 µm^2 (°). D) Overlay of all cell nuclei (red) with stained newly synthesized DNA (green, S-phase cells).

DISCUSSION & CONCLUSIONS: Using state-of-the-art multidensity chips, we show that different NW densities affect several key cellular parameters, such as cell morphology, FA formation and cell division. NW density is thus instrumental to take into consideration when optimizing NW-based biosensors for cellular applications.

ACKNOWLEDGEMENTS: For financial support, the authors thank the Danish Innovation Fond (ANaCell project).

IMAGING OF GLIOBLASTOMA CELLS USING SURFACE ENHANCED RAMAN SPECTROSCOPY WITH ANTI EGFR-CONJUGATED GOLD NANOPARTICLES

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INTRODUCTION:

Glioblastoma Multiforme (GBM) is the most lethal brain tumor that typically results in death in the first 15 months after diagnosis. Surgery is still the mainstay of care, but, because of the infiltrative pattern, a complete surgical resection of GBM cells remains an unmet challenge. Current intraoperative techniques do not allow for visualization of residual cancer cells beyond frank tumor margins. Surface enhanced Raman scattering (SERS) has proven to be a highly sensitive and selective technique in cancer therapy. In the present work, SERS active gold nanoparticles (GNP) are developed and characterized to specifically bind GBM cells. EGFR protein, which is overexpressed in 30-70% of primary GBM will be used as target. Only the GNP showing a high specificity and sensitivity will be used for further studies in xenograft mouse model.

METHODS: Commercial gold nanospheres are coupled with Raman reporter (a molecule that has intrinsic Raman signal) and covered with a protective polyethylene glycol (PEG) layer. GNP are conjugated with anti-EGFR antibodies. A full characterization of GNP dimension and chemical composition is performed by UV–vis absorbance spectra, dynamic light scattering (DLS) technique and transmission electron microscope (TEM) image. Their selectivity for EGFR is analyzed using three human GBM cell lines - BS153, U87MG, and LN319 - known to have differential EGFR expression levels. Overexpression and quantification of the EGFR protein in these cell lines is evaluated by Immunofluorescence staining and Western Blot analysis.

RESULTS:

Clear red shifts were observed after coating GNP with a large number of Raman molecules and stabilization with a layer of PEG. Surface modification was further confirmed by size and zeta potential data. The immunofluorescence

analysis confirmed a significant difference in the expression of EGFR among these three different cell lines with higher levels in BS153 compared to U87MG and LN319.

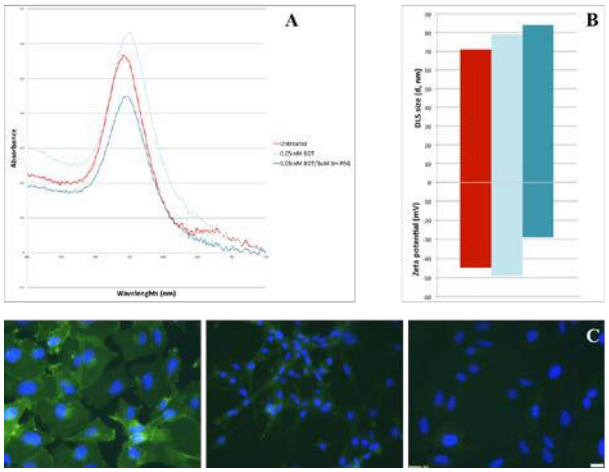

Fig. 1: A) Optical absorption, and (B) dynamic light scattering data obtained from the original (red), Raman marker (light blue), and thiol-PEG gold nanoparticles (blue).
C) Expression of EGFR in GBM human cell lines: BS153 (on the left), U87MG (in the middle), and LN319 (on the right). Nuclei are stained in blue, and EGFR in green. Scale bar 20µm.

DISCUSSION & CONCLUSIONS: Preliminary results suggest that GNPs directed against EGFR, visualized with Raman imaging spectroscopy, may be implemented intraoperatively to identify and to remove infiltrating tumor cells, with potential survival benefit for patients with GBM.

A Novel Method for Producing Cell Microarrays for the Controlled Release of Small Molecules

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INTRODUCTION: Traditional drug discovery involves the evaluation of lead compounds from a chemical library by using cell-based assay.[1] Therefore, the development of cell-based microarray chips to evaluate compounds has been progressively increasing over the past decade. [2-4] Although several cell-based microarray devices and procedures to evaluate chemical libraries have been reported [5-8], they all have room for improvement in terms of simplicity, speed, and cost. Here, we developed a simple method for producing multiple copies of microarray chip controlled release of small molecules for cell-based assay.

METHODS: In a test tube, 3 g of the powder of a biodegradable polymer DLG80-10 (poly[lactic-co-glycolic] acid, DL-lactic acid:glycolic acid=80:20, Mn=8,500, Mw=11,500, Mw/Mn=1.35) was added with or without 40 µM of calcein-AM, and was melted. Subsequently, one side of ~50 cm of PTFE tubing channel was dipped in melted polymer and the other side was connected to a channel on the heat-resistant cap for a glass bottle. The cap has 2 channels with valves through the cap and the valve of the channel connected tubing channel was closed and the other for air vent was opened. Then, the cap was connected on a 1 L of a glass bottle. After the bottle was put in the oven and incubated for 5 min, it was taken out of the incubator and put in cold water. Once the valve for air vent was closed immediately, the melted polymer in a test tube was pulled into tubing channel slowly. By repeating these steps, the polymer was filled in the PTFE tubing channel. Lastly, these tubings were stretched straight and then cooled at room temperature to be solidified the polymer.

Next, these tubing channels filled with solidified polymer with or without calcein-AM were arrayed with 3 × 3 channels using a mold. The channel with calcein-AM was only placed in the center. In a 50 ml of conical polypropylenes tube, 20 ml of slicone elastomer base solution (KE-1300T) and curing agent (CAT-1300) were mixed and centrifuged for 5min to remove air bubble, and then the mixture was poured in the metal mold and incubated at 60ºC for 2 h for curing. The cured block through arrayed tubings was sliced perpendicular to tubings to create multiple copies of microarray chip (Fig.1).

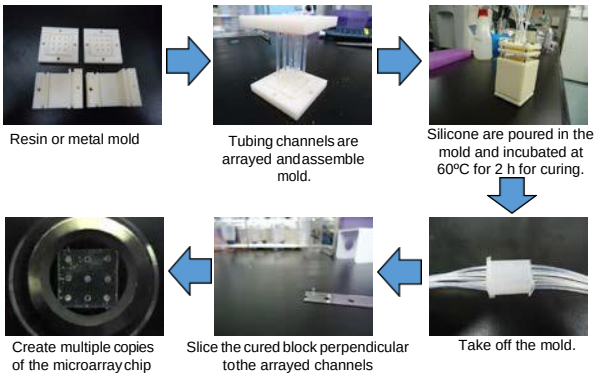

Fig. 1: Method for making multiple copies of small molecule microarrays

RESULTS & DISCUSSION: The chip was put over cells and the cells were incubated for 66 h, after 6 h of cellular seeding. We could not observe cells under the chip by epi-illlumination, because the degradation of DLG80-10 made the field of view opaque by 66 h of incubation. Calcein-AM including biodegradable polymer, DLG80-10, inside center channel is slowly released and introduced into cells under the polymer. We confirmed that cells can survive under the chip for more than 66 h. After 72 h of cell seeding, we successfully confirmed that calcein-AM locally introduced into cells on specific channels.

ACKNOWLEDGEMENTS: This work was partly supported by the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Functional Poly(3,4-ethylenedioxythiophenes) for Bioanalytics and Diagnostics

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INTRODUCTION: Conducting polymers, such as poly(3,4-ethylenedioxythiophene (EDOT)), have attracted significant attention as a new class of organic materials for biosensing because of their good electronic and ionic conductivities, feasibility to add functionality by chemical derivatization, smart responsiveness to external stimuli, tunability of bulk and surface properties, non-cytotoxicity, mass productivity, and workability. Low impedance interfaces originate in good electrical conductance of polythiophene backbone in a doped state and facilitated ionic currents that flow into and out of the bulk phase of conducting polymers. Zwtterions are effective for making non-fouling surfaces in biological environments [1]. We developed functional poly(EDOTs) bearing zwitterionic moieties for specific biosensing. Further, we developed oligosaccharide-tethered EDOT as a biomimetic ligand for influenza virus.

METHODS: EDOTs functionalized with zwitterionic moieties such as phosphorylcholine (PC), sulfobetaine (SB), and carboxybetaine (CB) (Fig. 1) were synthesized by thiol-ene click reaction between thiolated EDOTs and zwitterionic methacrylates as previously reported [2]. EDOTs functionalized with α-2,6' or α-2,3' Sial-Lac-Glc was synthesized by glycoblotting between the oxylamine-tethered EDOT and the reducing end of oligosaccharides [3]. These EDOT derivatives were electrochemically copolymerized with EDOT on a glassy carbon electrode in the presence of (poly)anions as a dopant. Differential pulse voltammetry (DPV) was performed using a working electrode modified with functional poly(EDOTs).

RESULTS: An EDOT derivative bearing PC (EDOTPC) was electrocopolymerized with EDOT at varying compositions to tune the conductivities and biorecognition ability for C-reactive protein (CRP), a marker for inflammation and infection, of the resultant conducting polymer films [4]. Circulating CRP binds to damaged cells and tissues via PC in a calcium-dependent manner and activates the classical complement pathway. The differential pulse voltammetry (DPV) measurements showed sensitivity and selectivity of poly(EDOT-co-EDOTPC)-based electrodes with the dynamic range that covers physiologically relevant circulating CRP levels in the acute-phase (Fig. 2). Nonspecific signals were suppressed by non-fouling nature of PC zwitterion. Moreover, an EDOT derivative bearing Sial-Lac-Glc (EDOTSLG) was electrocopolymerized with EDOT at varying compositions on an extended-gate of field-effect transistor (FET) for detecting influenza virus. The gate potential of poly(EDOT-co-EDOTSLG)-modified FET responded to virus in a dose-dependent manner.

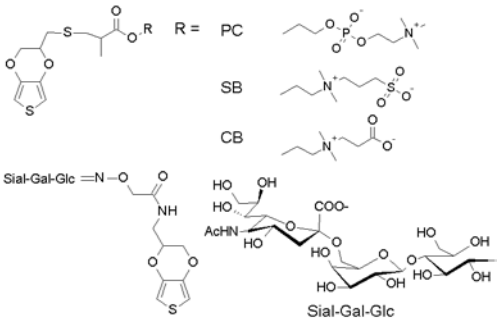

Fig. 1: Chemical structure of EDOT functionalized with zwitterions or oligosaccharides.

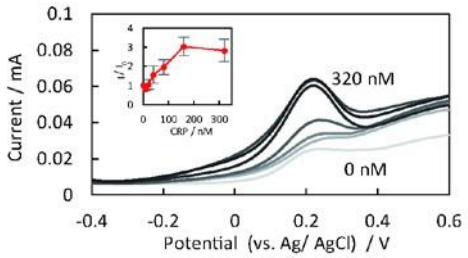

Fig. 2: DPV signal for CRP recognition by poly(EDOT$_{75}$-co-EDOTPC$_{25}$).

DISCUSSION & CONCLUSIONS: Our study reveals the potential of totally organic, non-fouling, and target-responsive conducting materials for applications in fast screening of biosamples in realistic conditions.

ACKNOWLEDGEMENTS: The author (T.G.) is grateful for financial support from the Grant-in-Aid for Scientific Research on Innovative Areas (#26107705) from MEXT of Japan and the Futaba Electronics Foundation.

Subsurface structuring as a new means to control protein adsorption

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INTRODUCTION: The affinity adsorption of proteins to a surface is at the heart of many bio-analytical tools. The general understanding is that protein adsorption, e.g. protein resistance or the conformation and activity of adsorbed proteins can be controlled by modification of interactions with the surface. We have discovered a new means of controlling protein adsorption using longer range interactions that originate from the hydrated subsurface.

METHODS: Porous plasma-polymer films were produced exhibiting different hydrophilic-hydrophobic character. A high degree of stability in aqueous environment was realized by enhancing the crosslinking during film growth. Using neutron reflectometry we have investigated the hydration of altered films. Using the label-free transmission interferometric adsorption sensor (TInAS [1]) we have studied how the adsorbed mass of bovine serum albumin (BSA) is modified by the hydrophilic-hydrophobic balance of the nominally 50 nm thick films, as well as by structural changes introduced into the subsurface of the plasma polymer by a change of plasma conditions on the terminal 2-8 nm surface vicinity.

RESULTS: By variation of input power we can readily adjust the properties of the plasma films [2]. The kinetics of contact angle measurements suggests that there is a subsurface hydration taking place on timescales of few hours to days.

The hydration of the subsurface was further investigated using neutron reflectometry [3] and it could be shown that water is hydrating the subsurface regions of different plasma polymers to different amounts. The result of scattering length density (SLD) fits to neutron reflectometry data for two selected film types clearly shows the presence of subsurface hydration water.

To evaluate the effects of subsurface structuring and hydration on protein-surface interactions, we have investigated the adsorption of (BSA) from deionized water onto TInAS sensors with plasma polymer coatings [4]. As shown in Figure 1, there is a significant difference of adsorbed mass depending on the surface hydrophilic balance, as one might expect.

In addition, there is a significant reduction of adsorbed mass on the "vgrad" film made of a 4 nm thick hydrophobic-to-hydrophilic vertical gradient, although it has the same chemical termination and water contact angle as the ppHMDSO reference. The significant difference in adsorption behaviour is related to the different subsurface structure alone.

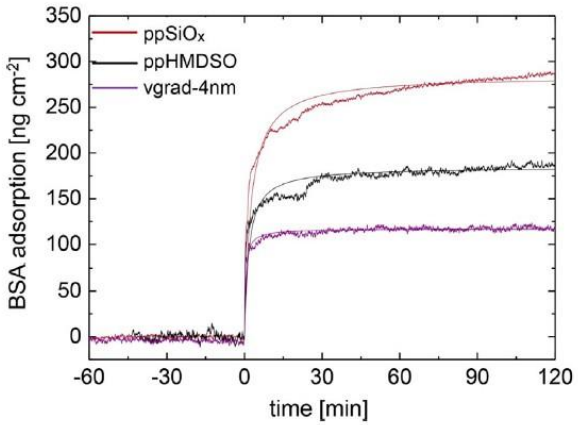

Fig. 1: Adsorbed mass of BSA on different polymer coatings measured with the TInAS; both the ppHDMSO and the vgrad films exhibit the same contact angle, yet different protein adsorption due to different subsurface structure. ppSiOx represents a more hydrophilic reference.

DISCUSSION & CONCLUSIONS: The unexpected effect of the subsurface structure may be due to water structuring or long-range e.g. dipolar interactions. Further investigations are planned to identify the interaction mechanism and its influence on protein conformation and activity. Our results suggest that there are new means to control protein adsorption to surfaces via subsurface chemical structuring.

ACKNOWLEDGEMENTS: This work was supported by the Swiss National Funds (SNF). We thank Prof. N.D. Spencer for hosting this thesis project.

Altering the physics and chemistry of biological interfaces for diagnostics: Micro–Fluorescent *in situ* Hybridization (µFISH) and SPATIALYSE

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INTRODUCTION: Technique to study, work and locally probe adherent cells & tissues at micrometer distances from cell surfaces in "open space" would represent a major advance for the biology of biointerfaces. Here, we use a non-contact, scanning technology, which spatially confines nanoliter volumes of chemicals for interacting with cells at the µm-length scale. This technology called the vertically-oriented microfluidic probe shapes liquid on biological surfaces hydrodynamically and is compatible with standard biological supports. By altering the shape of this confined liquid termed – hydrodynamic flow confinement – the physics and chemistry of the interface is dynamically altered for spatiotemporal studies. We apply this technology **to realize two new methods for the interrogation of nucleic acids content on cell layers and expression studies in diagnostics.**

RESULTS:

First, we present a micrometer-scale implementation of fluorescence *in situ* hybridization, a key cytogenetic technique in research and diagnostics. Conventional *in situ* hybridization (ISH) is an important class of cytogenetic techniques, allowing high-resolution detection, quantification, and localization of nucleic acid (NA) targets. Here, by localizing FISH probes at the tip of this microfabricated scanning probe, we selectively expose approximately 1000 MCF-7 cells of a monolayer to perform probe incubation — the rate-limiting step in conventional FISH. Our µFISH implementation is compatible with the standard workflow of conventional FISH, allows re-budgeting of the sample for various tests, and results in a 15-fold reduction in probe consumption [1]. The continuous flow of probes on these selected cells resulted in a 70-fold reduction of the hybridization time compared with the standard protocol (3 min vs. 6 h) and efficient rinsing, thereby shortening the FISH assay time.

Secondly, we will present a novel method — Spatialyse — to selectively lyse adherent cells for nucleic acid analyses (Fig. 1a) and deliver this lysate in minimum volume for downstream molecular analysis. This method can be used for spatio-temporal studies of heterogeneous cells while in culture in contrast to current methods,

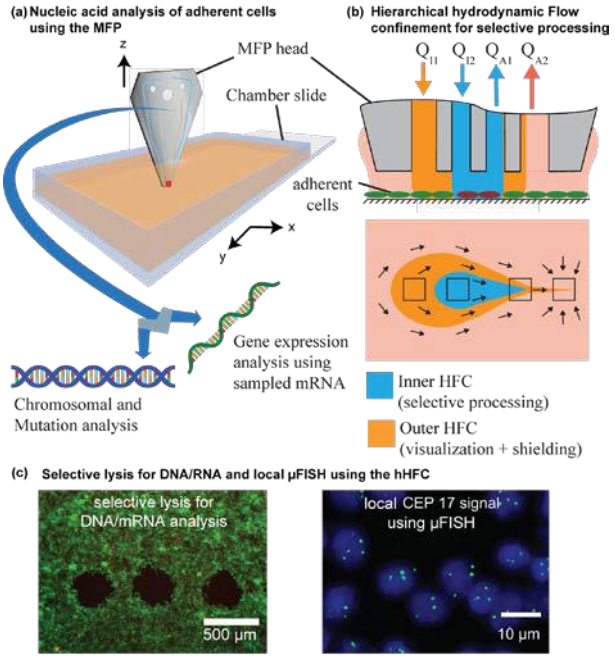

Fig. 1: Illustration of the probe on a biological surface to stain and lyse cells selectively.

thus providing insight into cellular signaling [2]. To demonstrate spatially accurate cell lysis and retrieval, we performed local lysis of multiple footprints (1, 5, 10 and 15; ~400 cells/footprint) using NaOH as processing liquid and quantified the DNA in the lysate.

OUTLOOK:

Compatibility between the chemical systems used combined with the flow confinements ability to shape multiple liquids (& altering the sheer profiles) can be leveraged to perform simultaneous DNA and mRNA retrieval and newer FISH implementations. These suite of methods can be applied to study cell-cell, cell-matrix interactions spatio-temporally, which when combined with application on tumor tissue sections, provides better understanding of growth, progression and drug response of a tumor.

ACKNOWLEDGEMENTS: This work was supported by an ERC Grant, under the 7th Framework (Project No. 311122, BioProbe).

A PATH FOR SUPERSELECTIVE GLYCAN RECOGNITION AND BIOMARKER DETECTION FOR PROSTATE CANCER

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INTRODUCTION: Prostate cancer (PCa) is one of the most common cancers diagnosed in men worldwide. [1] The current blood based ELISA test, relies on detecting elevated levels of prostate specific antigen (PSA) via an epitope region recognised by anti-PSA. This current test has led to many false positive and negative results. [1] Approximately 50 different glycoforms of PSA exist, and only a few of these are indicative of PCa. [2] Elevated levels of PSA may be due to age, medication or life style. Glycans are emerging as invaluable markers for disease detection. These glycans are found on the cell surface of protein, and allow a multifunctional path for proteins. They are known to differentiate in cancer, making them useful biomarkers. [2] Creating a system to differentiate amongst these subtle differences is crucial in developing a super-selective platform for accurate prostate cancer detection. The use of synthetic antibodies is a great alternative for platforms such as glucose sensing. Here a synthetic ligand, boronic acid, well-known to bind covalently and reversibly to saccharides is used. [3] The challenges in creating a platform with high specificity for saccharide chains indicative of disease is in the complexation and isolation of pure target-ligand complexes. Here, we demonstrate a fine tuned approach, which can discriminate between same length saccharide chains and glycan fragments.

METHODS: The imprinted surface for glycan detection has been developed via a series of highly devised strategies which perfectly match the pattern of hydroxyl groups on the glycan target surface. The synthesis of the target-ligand complexes are prepared via a reflux reaction and precipitation of the free ligand, to obtain a pure product. Using free radical polymerization, as previously described by us, [4] these complexed targets are cross-linked onto a gold surface which is formerly treated with a self-assembled monolayer, attributing the acrylamide cross-linking site. The target is removed using an acidic washing buffer, and a binding response is monitored using surface plasmon resonance (SPR).

RESULTS: Isolation of ligand-target complexes, consisting of boronic acid derivatives and saccharides, Cellobiose, Maltose, Raffinose, Meletizose and Stachyose hydrate, were isolated *in vacuo*. The isolated ligand-complexes were used to form molecular imprinted surfaces (MIPs), which allow us to select for target oligosaccharide. Fig. 1 shows the binding response at equilibrium for a non-imprinted surface (NIP) (a) as well as a MIP (b). The response on the NIP shows a high selectivity for Palatinose hydrate, compared to the other two disaccharides. However, once we create a MIP incorporating Cellobiose, we are successfully able to enhance the selectivity for the target over the other two disaccharides. We have successfully created MIPS for Meletizose, Cellobiose and Stachyose hydrate.

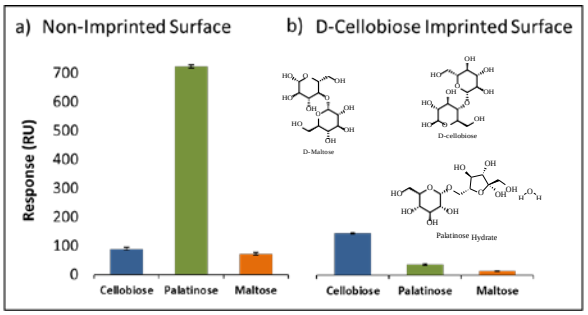

Fig 1. (a) The SPR response for disaccharide binding on a boronic acid monolayer. (b) A MIP for Cellobiose, showing selective SPR response for Cellobiose over other disaccharides.

DISCUSSION & CONCLUSIONS: By manipulating the arrangements of the boronic acids on the surface, we have created a platform which can be used to target specific saccharides of our choice. The power of the methodology is now being demonstrated by the preparation of platforms to identify a series of complex carbohydrates (multi-antennary, sialylated, fucosylated and mannosylated) and identify global differences in glycosylation of PSA from men with and without PCa. This will allow us to detect cancerous PSA over non cancers, creating a highly accurate diagnostic tool.

Nanozyme-based approach for H_2O_2 sensing

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[1] *ETH Zurich, Switzerland.* [2] *Karolinska Institutet, Stockholm, Sweden.*

INTRODUCTION: Cerium oxide nanomaterials have received a lot of attention recently due to their antioxidant *enzyme-like* (nanozyme) properties [1]. Here, uncoated europium doped cerium oxide (Eu:CeO_2) nanoparticles with well-defined size (d_{XRD}: 4.4 – 16 nm) were prepared by flame aerosol technology and characterized in regards to H_2O_2 sensor response in physiologically-relevant solutions. Temporal stability was compared to a commercially available fluorescent dye in a peroxidase coupled reaction.

METHODS: CeO_2 nanoparticles containing 5 at% europium were prepared by flame spray pyrolysis. The particle size was controlled by tuning the flow rate of the liquid precursor solution (x) and the flow rate of the O_2 dispersion gas (y) [2]. Crystallinity and crystal size was characterized by X-ray diffraction.

Particles were dispersed in deionized water (diH_2O) or a phosphate based buffer (pH 7.4) by vigorous vortexing and sonication. For all sensing measurements 75 µL of 0.25 mg/mL Eu:CeO_2 were added to 75 µL blank or H_2O_2 and kept at room temperature (RT) for 30 min prior to measuring the signal intensity by phosphorescence ($\lambda_{Ex/Em}$: 330/590). Applied H_2O_2 concentration ranged from 0.05 to 1000 µM, a blank sample without substrate was included for each measurement. The sensor response (SR) was derived as follows from the sample intensity (I_S) and blank intensity (I_0):

$$SR = 1 - I_S/I_0 \quad (1)$$

For stability testing a H_2O_2 sensitive probe, Amplex Ultra Red (AUR) was employed in a peroxidase (HRP) coupled reaction. 50 µL AUR/HRP were added to 50 µL H_2O_2 and the signal measured directly by fluorescence ($\lambda_{Ex/Em}$: 490/585). Eu:CeO_2 particles and AUR/HRP were kept for 8 h at RT, unprotected from light and the signal intensity for 2 µM H_2O_2 monitored over time.

RESULTS: While largest Eu:CeO_2 particles (d_{XRD}: 16 nm) showed highest signal intensity in the absence of substrate, smallest nanoceria (d_{XRD}: 4.4 nm) showed highest sensor response (**Fig. 1a**) and were therefore selected for subsequent H_2O_2 sensing. A clear substrate concentration signal dependency could be observed (**Fig. 1b**). A limit of quantitation of 0.4 µM for phosphate buffered saline and 1.16 µM for 100 mM potassium phosphate was observed with linearity up to 5 µM. When the concentration and sensor response reciprocals were plotted, as this is typically done for enzymatic reactions, a broader linear range could be observed.

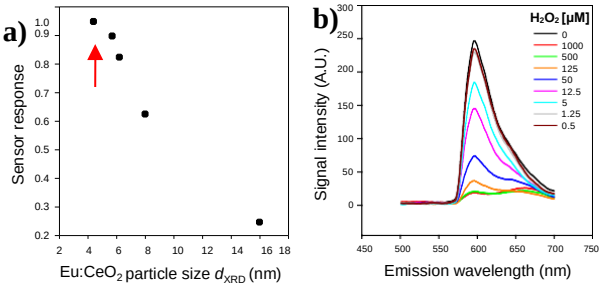

Fig. 1: ***(a)*** *Sensor response for 1 mM H_2O_2 measured in diH_2O given as a function of particle size (d_{XRD}).* ***(b)*** *Eu:CeO_2 emission scan for increasing H_2O_2 levels assayed in diH_2O.*

In contrast to AUR/HRP, nanoceria particles were able to maintain their H_2O_2 detection properties after 8 h demonstrating superior stability and reduced susceptibility to photobleaching.

DISCUSSION & CONCLUSIONS: Fine-tuning the particle size revealed higher substrate sensitivity for smallest particles. Eu:CeO_2 demonstrated signal linearity in both diH_2O and phosphate-based solutions enabling H_2O_2 quantitation in biological systems using standard fluorimetric approaches (e.g. plate reader). Finally, improved stability over currently available H_2O_2 detection systems highlight the potential of the robust and inexpensive Eu:CeO_2.

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Interaction of hematopoietic and leukemic cells with hyaluronans

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INTRODUCTION: The interaction of leukemic and haematopoietic cells with their microenvironment is of upmost importance for extravasation and homing. Cell rolling on vessel walls has intensively been investigated and shear flow activated catch bond interactions were identified as key mechanism for selectin mediated extravasation of leukocytes [1]. Hematopoietic cells are able interact with their extracellular environment via the CD44-hyaluronan (HA) pathway. It is known to be essential for the proliferation, homing and engraftment of hematopoietic stem and progenitor cells [2]. We were able to show that CD44 interacts via a catch-bond mechanism with HA and that the mechanism is already established in hematopoietic stem cells. In addition we show the relevance of this interaction in hematopoietic diseases, such as leukaemia.

METHODS: To quantitatively assess the interaction, a microfluidic experiment has been developed that allows studying the interaction of cells with interfaces under well-defined flow conditions [3]. To test the shear dependent rolling behaviour of hematopoietic stem cells, an increasing shear was applied and the rolling response quantified.

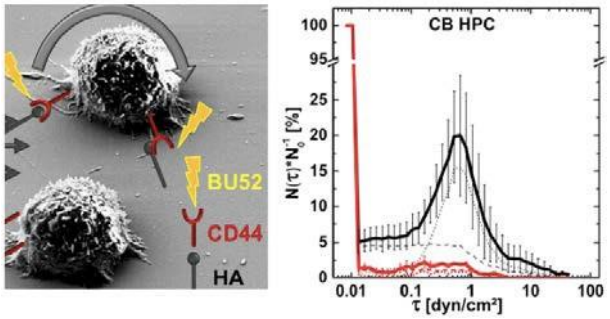


Fig. 1: Rolling of leukemic and hematopoietic cells and shear induced activation of the rolling process on hyaluronans (reprinted from [5]).

RESULTS: We found that similar to the selectin mediated rolling, also the CD44 interaction with HA requires a minimum shear stress to become activated and enable cells to roll on HA surfaces [4]. Similar critical shear values were found for rolling on mesenchymal stroma cells, which are present in the bone marrow niche creating the microenvironment required for haematopoietic stem cell renewal [5]. Interestingly, not only hematopoietic stem cells but also acute leukemic blasts show a shear flow induced rolling. The proportion of rolling cells was correlated with the pathogenesis of the disease [5].

DISCUSSION & CONCLUSIONS: A microfluidic experiment revealed that CD44 mediated rolling of hematopoietic and leukemic cells on synthetic hyaluronan is shear force activated and thus a catch-bond mechanism. The ability of cells for this interaction is already established in hematopoietic stem cells and conserved in leukemic blasts. Screening the adhesion pattern of a variety of leukemic blasts revealed its potential to serve as diagnostic tool for risk stratification.

ACKNOWLEDGEMENTS: The authors acknowledge support from the Sander Stiftung (D10051281), the German Research Foundation (SFB 873), and the Cluster of Excellence RESOLV (EXC 1069).

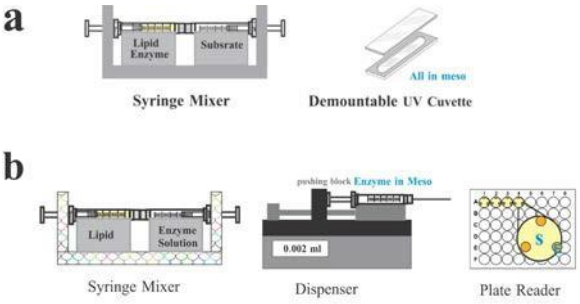
Lyotropic Liquid Crystalline Cubic Phases as Versatile Host Matrices for Membrane-Bound Enzymes

Wenjie Sun,[1] Jijo Vallooran,[1] Wye-Khay Fong,[1,2] Raffaele Mezzenga[1,*]

Food and Soft Materials Science, Department of Health Science and Technology, ETH Zurich
Monash Institute of Pharmaceutical Sciences, Monash University, Australia

INTRODUCTION: In general, enzyme activity decreases when immobilized, no matter whether it is physically entrapped or covalently bonded. Here we present that the lipidic bilayers in the 3D structures of cubic mesophases provide an ideal environment for the reconstitution of the membrane bound enzyme, D-fructose dehydrogenase (FDH). The preserved activity, long-term stability and reusability demonstrate that these hybrid nanomaterials are ideal matrices for biosensing and biocatalytic fuel cell applications.

METHODS: Two sample preparation and detection methods were applied in this study:



Scheme 1. The illustrations of the sample preparation procedures for the enzyme activity study in bulk mesophase (a) and the in meso membrane enzyme stability study (b).

RESULTS: The *in meso* immobilized FDH, was found to follow the Michaelis-Menten model (Equation 1) as per the FDH in solution (Figure 1), while a huge deviation from normal Michaelis-Menten kinetics was previously observed for the hydrophilic enzyme HRP.[2]

$$v = \frac{V_{mmm}}{K_m + [s]}[s] \quad (1)$$

Moreover, the enzymatic kinetics parameter, K_m in bulk mesophase (1.7 mM) is only slightly larger than K_m in water (1.3 mM), indicating a greatly preserved activity in the mesophase.

The membrane bound enzyme, FDH, stayed stable *in meso* for 5 days (100% activity), whilst the hydrophilic enzyme HRP retained only 21% activity after 5 days. However, the opposite was observed in water, FDH lost over half of its activity within 10 days while HRP retained more than 90%.

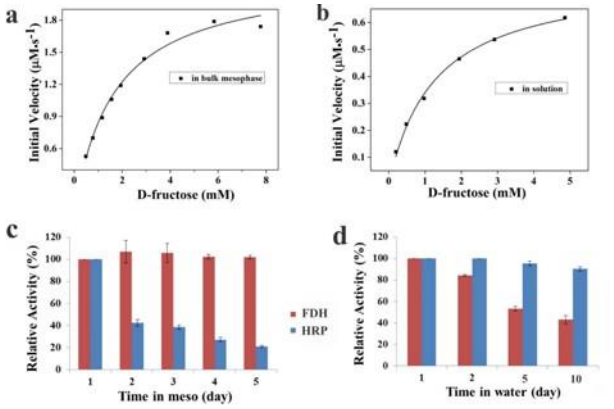


Figure 1. The FDH saturation curves between substrate concentration and reaction rate in bulk Pn3m (monolinolein: 30% water) (a) and in solution (b). The stability of FDH and HRP incorporated in meso and stored in solution (c, d).

Table 1. K_m of FDH as obtained from Michaelis-Menten model in solution and bulk lipidic cubic phase, compared to the K_m of HRP adapted from [2]

Kinetic parameters	*In meso*	In solution
$K_{m\ FDH}$ (mM)	1.7	1.3
$K_{m\ HRP}$ (mM)	7.5	1.4

DISCUSSION & CONCLUSIONS: This study demonstrates the preservation of the enzymatic activity for the membrane bound enzyme, FDH, when immobilized in a bulk lipidic cubic phase, where the enzyme kinetics follows the Michaelis-Menten model. This improved enzyme performance can be attributed to both the improved reconstitution of the membrane bound enzyme, FDH, within the lipidic cubic mesophase and the faster diffusion of the hydrophobic converted product through the mesophase. The improved reconstitution also resulted in the greatly extended stability of the immobilized membrane-bound enzyme, demonstrated by remaining the 100% activity *in meso* after 5 days compared to the reduction of activity to 50% in solution. [1]

ACKNOWLEDGEMENTS: The China Scholarship Council and ETH Zurich are acknowledged for the financial support.

Self-assembled lipid systems for diagnostics

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INTRODUCTION: Lipidic cubic phases are optically isotropic, transparent lyotropic liquid crystals containing highly confined water nanochannels in-between percolating lipid bilayers following defined space groups. They provide an excellent matrix for the entrapment of proteins and enzymes in its active states due to their biocompatibility, the presence of both hydrophilic and hydrophobic environments and their thermodynamic stability in excess water [1]. Here lipidic cubic phases are further developed as a bio-sensing platform by introducing a new convenient and general assay principle, based on the monitoring of birefringence within the ensued mesophases.

METHODS: *In-meso* immunosorbent assay method based on birefringence was used in this study:

Scheme 1. Illustration of the birefringent ELISA.

RESULTS: The *in meso* peroxidase enzymatic reaction generates a unique optical signal, birefringence due to the crystallization of the converted product within the mesophase domains. This is due to the unique environment of the water channels in bicontinuous cubic phase where most of the water being bound to the polar heads of the lipid. Birefringence signal originated from converted product is used for the real-time monitoring of enzymatic reactions in lipidic mesophases.

By engineering the *in-meso* enzymatic reaction, the proposed concept is suitable for bi-enzymatic cascade reactions within the lipid mesophases and followed the birefringence development to detect glucose and cholesterol in a wide range of concentrations. Additionally, cubic phases are used for peroxidase enzymatic reaction with antibody-mediated molecular recognition to detect pathogenic microorganisms and viruses, again, based on the sole detection of birefringence. In the case of analytes with intrinsic birefringence such as the hemozoin crystallites produced by *Plasmodium falciparum* parasites, the same methodology can be used for naked-eye detection and diagnosis of *Malaria* infection.

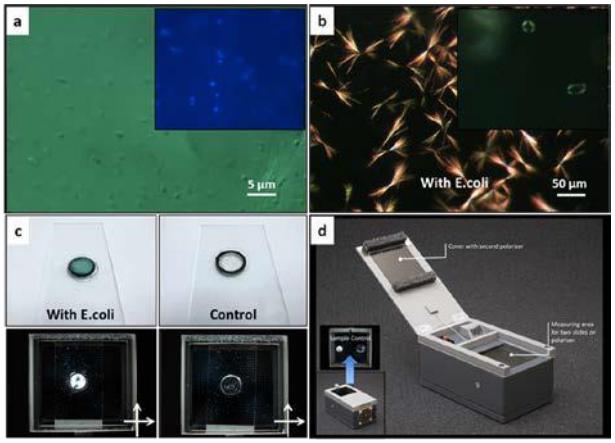

Figure 1. Diagnosis of E.coli in lipid based cubic phase (a) Light microscopy image of immobilized bacteria in the cubic phase. Inset shows the fluorescence microscopy image with DAPI. (b) Birefringent pattern observed in cubic phase after the immunoassay. (c) Sample photographs and their visualization in the cross-polarized filter device after the immunoassay. (d) Photograph of the portable birefringent ELISA device.

DISCUSSION & CONCLUSIONS: The bicontinuous cubic phases serve as the ideal platform due to their isotropic and transparent nature combined to the high nanoconfinement of the lipid and water domains. The methodology proposed here exploiting both exogenously and endogenously-generated *in-meso* birefringence is general enough to be adapted to the detection of a vast class of analytes in a broad range of biotechnological fields and may pave the way to new detection strategies in the pharmaceutical, food, medical and bio-threat diagnostics.

ACKNOWLEDGEMENTS: ETH Zurich is acknowledged for the pioneer fellowship and for financial support.

Plasma-based stable micropattern coating for biosensing purposes

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INTRODUCTION: Biosensor optical devices, such as surface plasmon resonance (SPR) or transmission interferometric adsorption sensor (TInAS), [1] are used to detect some biomarkers in biological fluids. These label free detection devices are based on interactions between biomarkers from the analyzed fluids and biomolecules, such as proteins, lipids or DNA, immobilized at the surface of the sensor chip. Surface preparation of the biosensor-chips is thus crucial in order to ensure relevant, repetitive and reproducible results while analysing biological fluids. In the present study, we investigate a stable plasma-based micropatterned coating. By this way, two different functionalities can be provided simultaneously by the coating: avoidance of the unspecific adsorption of proteins and specific recognition of one target protein.

METHODS: In this study, two different plasma polymer films (PPFs) are prepared, i.e. amino-containing and carboxylic acid-containing PPFs, thanks to an electrical discharge in a mixture of C_2H_4/NH_3 and C_2H_4/CO_2, respectively. Using the high control over both deposition processes that has recently been achieved in our laboratory [3,4], multilayer and micropatterned films were prepared in order to obtain a bifunctional coating that can enhance biomolecule sensitivity. Coatings were characterized by SEM, XPS and WCA measurements. Immobilization of an engineered fluorescent protein (Y1-GFPuv) [4] onto amino groups using tyrosinase was performed in order to highlight the chemical micropatterning generated during plasma deposition: the fluorescent pattern was then observed thanks to a microarray scanner (Tecan, Switzerland).

RESULTS: A first layer of amine-containing plasma polymer nanofilms (~5 nm) were deposited onto the substrate, and then, a carboxylic-acid containing plasma polymer nanofilm was deposited through a high precision mesh used as a mask in this study. As the thickness of the coating is regularly different all over the substrate surface, it was possible to observe the presence of the patterning thanks to SEM, varying contrast and brightness conditions. But, from this analysis, it is not possible to have information on the chemistry at the surface. XPS analyses were thus carried out to determine the atomic percentage in the plasma coating: most of the amino-containing plasma polymer film was covered with the carboxylic acid-containing polymer film, but still some nitrogen atoms were observed ([N]/[C]=5%).

Adsorption of GFP onto the amine groups, thanks to electrostatic attraction, was performed in order to highlight the micropattern structure. Treated coatings were then analysed thanks to a microarray scanner (Fig. 1) where the fluorescence (green) shows the regular spatial positioning of the amine groups.

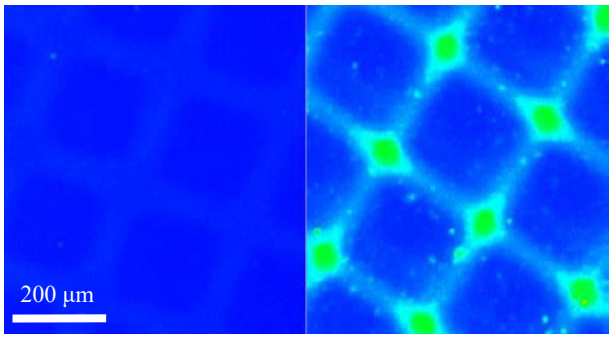

Fig. 1: Images taken by microarray scanner showing no fluorescence for the micropatterned sample immersed in buffer (left), and fluorescence for the one treated with GFP, revealing the positions of the amine groups (right).

DISCUSSION & CONCLUSIONS: A stable micropatterned plasma polymer nanofilm was investigated, and the micropatterning was visualized thanks to fluorescent technique, showing a regular spatial deposition of the amine and carboxylic acid groups all over the surface of the substrate. This patterning technique represents a promising basis for further sensor developments on the basis of functionalized plasma polymer films.

ACKNOWLEDGEMENTS: The authors gratefully acknowledge Swiss National Science Foundation (SNSF, Bern) that funded this study under the grant no. IZ73Z0_152661 (SCOPES).

Fluorescent nanocellulose based biosensor for the analysis of heavy metal contents in human serum

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INTRODUCTION: Composite materials engineered to elicit specific bioactive responses have gained increased attention for various biomedical applications. Recently, nanofibrillated plant-derived celluloses (NFC) were introduced as promising carriers for the controlled immobilization and retention of bioactive molecules [1], e.g. able to detect heavy metal contents such as copper in body fluids. Copper is an essential element for the human body and, unless tightly regulated, a toxicant. A dramatic form of copper toxicity is found in Wilson Disease, characterized by increased free copper in blood. Unfortunately, detection of copper levels in blood is still difficult and time consuming [2]. Therefore we developed a fluorescent nanocellulose based biosensor for the analysis of copper ion contents in complex probes such as human serum. Furthermore, we demonstrate the NFC based biosensors to be a promising tool for biomedical diagnostic applications as they combine high sensitivity and specificity with a generally facile application [3].

METHODS: The α-subunit of the fluorescent protein C-phycocyanin (CPC), isolated from *Spirulina platensis*, sensitive for heavy metal ions such as Cu(II), was genetically engineered to optimize the yield of the production process based on previous publications [4]. Spontaneous- and covalent immobilization of the purified sensor protein to a TEMPO oxidized nanofibrillated cellulose (TO-NFC)-matrix (Figure 1) was performed and characterized as previously described [1].

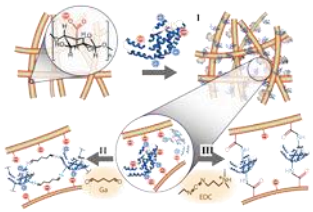

*Fig. 1: Immobilization of the fluorescent biosensor protein α-subunit of C-phycocyanin to a TO-NFC matrix (spherical icons) via electrostatic interaction **(I)**, covalently crosslinked using either imine-**(II)** or peptide bond forming reactions **(III)**.*

The fluorescence of the suspensions and the thin films of CPC-TO-NFC obtained by precipiation was measured by recording the change of the specific emission at 575-680 nm using spectrophotometric-

and/or laser array-scanning analysis (excitation: 532-609 nm). Cu(II)Cl_2 was diluted in either MilliQ™ water as a reference to calibrate the biosensors or in filtrate of commercial human serum.

RESULTS: Adsorption isotherm modelling of CPC interacting with a TO-NFC matrix [1] revealed electrostatic interactions as the major driving force for physisorption with a maximum capacity of 35.2 nmol CPC/mg TO-NFC. Covalent conjugation resulted in a stable biosensor system resistant to desorption in suspension but as a trade-off, in a reduced sensitivity for Cu(II) ions (Figure 2).

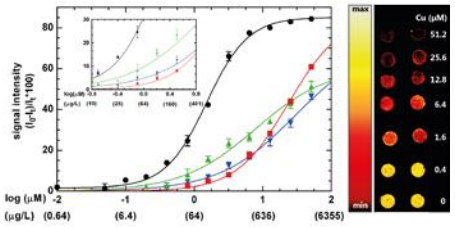

*Fig. 2: The sensitivity of CPC suspensions (1.47 nmol/mL) for Cu(II) ions in log(µM) (left). Fractions: Soluble (black), physisorbed **(I)** (red), or covalently coupled **(II)** (blue) & **(III)** (green). A biosensor array of fluorescent TO-NFC composite thin films incubated in human serum filtrate spiked with different concentrations of Cu(II) (right).*

The biosensor thin films facilitated sensor arrays (Figure 2, right) for the detection of Cu(II) ions in complex probes such as human blood serum filtrate with a current detection limit as low as 20 µM.

DISCUSSION & CONCLUSIONS: Current results confirmed NFC based substrates as a suitable carrier for bioactive molecules, such as fluorescent biosensor proteins[1]**.** Currently the developed microarrays are further optimized to meet the sensitivity relevant for the diagnosis of Wilson Disease (≤ 2 µM) [2].

ACKNOWLEDGEMENTS: The authors thank M. Kämpf and L. Widmer for their contribution.

INFLUENCE OF LPS INFECTION ON STRETCHED ENDOTHELIAL CELLS IN A LUNG-ON-CHIP

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INTRODUCTION: The organs-on-chip technology, which aims at accurately mimicking in-vitro the human microenvironment and organs functions, is expected to provide deeper insights in complex cellular phenomena. We recently developed a lung-on-a-chip to study the behaviour of the air-blood barrier under physiological cyclic stress induced by respiratory movements[1]. The mortality rate of patients suffering from acute lung injury together with bacterial infection is between 30-40%[2]. The outcome is even worse, if the patients have to be mechanically ventilated. In this study, we aim at investigating the effects of the mechanical strain on pulmonary endothelial cells exposed to bacterial particles in order to understand better the role of ventilation in patients suffering from septic lungs.

METHODS: The lung-on-a-chip consists of a thin and elastic membrane, which is sandwiched between two cell compartments. Human lung microvascular endothelial cells (HMVEC-L, Lonza) were seeded on the apical side of the membrane at a density of 50'000 cells/cm^2. The cells were allowed to grow for 48h. The cells were then exposed for 24h to 8% linear cyclic mechanical stretch to model the physiological lung distention during respiration. The cells were then exposed to lipopolysaccharide (LPS, 1µg/ml, Sigma-Aldrich) for 24h. During exposition to LPS the cells were kept either under static conditions or were cyclically stretched (8% cyclic stress). After exposition, the cells were stained with calcein AM and ethidium homodimer 1 (Invitrogen) to assess cell viability. Medium was exchanged daily. ELISAs for Interleukins 8 and 6 were performed following according to the manufacturer's protocol.

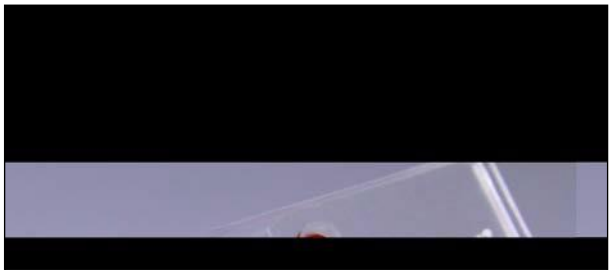

Fig. 1: Picture of a lung-on-a-chip

RESULTS: Neither the cyclic stretch, nor the LPS treatment, nor the combination thereof did influence the cell viability of the microvascular endothelial cells (data not shown). After 48h of 8% linear cyclic stretch there was a significant increase in IL-8 secretion compared to static control (1006 pg/ml vs. 648.7 pg/ml, p=0.026), whereas no difference was observed in Il-6 secretion. The exposure to 1µg/ml LPS did increase the secretion of both cytokines by a factor of more than 7. A slight increase in both IL6 and IL-8, but no significance, was observed between experiments carried out under static or dynamic conditions (8% linear cyclic stretch).

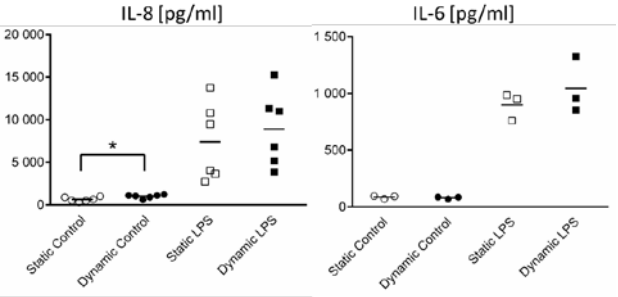

Fig.2: Effect of the mechanical cyclic stretch on interleukin (IL) 8 and 6 secretion upon exposure to LPS.

DISCUSSION & CONCLUSIONS: In the lung-on-a-chip, an 8% linear cyclic stretch influences the secretion of basal IL8 but not IL6 secretion in human lung microvascular endothelial cells. However, only a slight increase in cytokine expression was observed upon LPS exposure and cyclic stretch. These preliminary experiments suggest that a physiologic stress does not significantly aggravate the inflammation from the endothelium that is typical in acute lung injury. Additional experiments are on-going to support these data.

ACKNOWLEDGEMENTS: Simone Ebener for kindly providing LPS.

Cartilage decellularized extracellular matrix as photocrosslinkable hydrogel for mechanical gradients in near native environments

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INTRODUCTION: In this study we present a layerable hydrogel based on decellularized extracellular matrix (dECM) intended for in vitro models of cartilage tissue formation. Decellularized extracellular matrices (dECM) can provide cellular environments that are close to the native tissue since most of the natural ECM components are still present. As a photocrosslinkable hydrogel dECM provides a good control of mechanical properties and enables the formation of gradients. These gradients can mimic the zonal organization of articular cartilage. It was already demonstrated that mechanical gradients can direct differentiation of MSCs to chondrocytes under dynamic mechanical loading [1]. The study of cartilage tissue formation in mechanical gradients as in vitro models might ultimately lead to improved scaffold design and new cartilage repair strategies.

METHODS: Minced pieces (~1x1 mm) of bovine articular cartilage were decellularized by repeated freeze-thaw cycles, osmotic shock, enzymatic digestion and detergent. Between each freeze and thaw cycle the osmolarity of the solution was changed between hypoosmotic and hyperosmotic to introduce osmotic shock. This was followed by enzymatic digestion with trypsin and a detergent treatment with Triton X-100 to remove cell membranes and debris. The pieces were then washed with dH_2O until foam formation was stopped. The dECM pieces were then digested in acetic acid supplemented with pepsin at RT until a homogenous solution was obtained. Methacrylic anhydride (Sigma-Aldrich) was added dropwise to a solution of dECM under constant stirring while working on ice and the pH maintained at 8. A hydrogel gradient was then obtained by layering different wt.%tages according to a partial UV-crosslinking procedure described elsewhere [2]. The DNA content was determined by the NucleoSpin Tissue XS kit (Machery-Nagel). Protein content was analysed by an SDS-PAGE with a high molecular weight standard and Collagen I and II as reference. Decellularization efficiency was analysed by hematoxylin and eosin (HE) staining of histology sections. Rheological properties were obtained by a rheometer (Bohlin Instruments) and compression tests were performed by an Instron E3000.

RESULTS: The decellularization protocol efficiently removed cells which was confirmed by HE staining. Reduction of DNA content was observed from 55 ng/mg tissue to only 5 ng/mg tissue. Photocrosslinking of methacrylated dECM under UV-A light (365 nm) was successful resulting in two different stiffnesses by variation of wt.%.

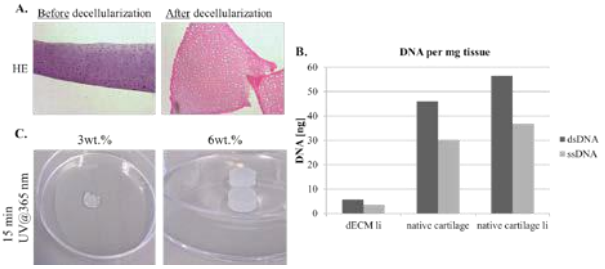

Fig. 1: Decellularization of bovine cartilage and photocrosslinking, A. Tissue before and after decellularization; B.DNA content C. 3wt. % vs. 6wt. % methacrylated dECM hydrogel.

DISCUSSION & CONCLUSIONS: This study presents a photocrosslinkable hydrogel that is based on dECM from bovine articular cartilage. It can be layered to create defined mechanical gradients and would be an interesting system to study cartilage tissue formation in vitro. Future work would include analysing the cell differentiation in different stiffness gradients.

ACKNOWLEDGEMENTS: The authors like to thank the Swiss National Science Foundation for generous support. Thanks to Quentin Vermeire for his work on the decellularization and gel characterization.

Physiologically functional perfused microvessels composed of primary human endothelial cells and pericytes

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INTRODUCTION: The lung microvasculature plays an important but often unclear role in the progression of multiple diseases. To elucidate the morphology and function of lung microvessels, we developed an in vitro model of 3D perfusable and long-lived microvasculature, using primary human cells[1].

METHODS: Pericytes from the distal airway were isolated using fluorescence activated cell sorting based on Lineage-EpCAM-CD73+CD90+ markers. Primary human endothelial cells from the umbilical vein or lung microvessels were used in the experimental setup. Both cell types were suspended in a fibrin solution and filled in the central chamber of a microfluidic device, separated from the adjacent flow channels by pillars. The outer chambers were filled with pericytes only to guide vessel orientation. After fibrin clotting, the flow channels were filled with EGM2 cell culture medium to enable signal diffusion across chambers and establish a pressure drop across the chambers.

RESULTS: Endothelial cells formed microvascular networks within seven days in the presence of pericytes. Microvessel diameters ranged from 20 to 200 µm and spanned the 2.4 mm chamber, opening up on towards the flow channels on either side. Continuous expression of junctional markers VE-Cadherin and CD31 and deposition of basement membrane components Collagen IV and Laminin (Fig.1) on the ablumenal side confirmed vessel patency. The vessels were accessible from the outside, as confirmed by perfusing fluorescently labeled dextran. If pericytes were directly co-seeded with endothelial cells, instead of only in the side chambers, the vessel morphology was less tortuous, and permeability decreased (Table 1). Furthermore, the microvesssels were functionally responsive to the vasoactive agent phenylephrine: pericyte-lined microvessels constricted by 3% in diameter over the course of two minutes. This constriction was not observed in the absence of pericytes. Comparisons between microvessels supported by normal and non-small cell lung cancer derived pericytes showed that both are able to support *in vitro* microvascular assembly. Interestingly, we found that tumor pericytes led to significantly higher vessel permeability compared to normal pericytes.

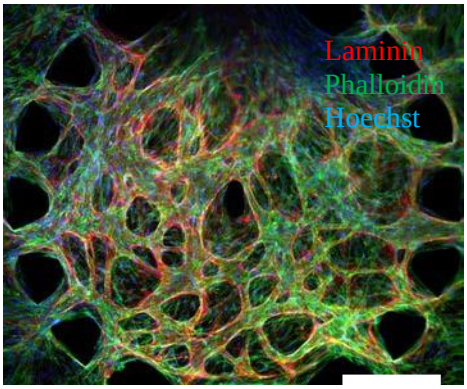

Fig. 1: Immunostained in vitro microvascular network after 7 days in culture. Scale: 500 µm.

Table 1. Microvascular permeability in direct or separated endothelial-pericyte co-culture.

	direct	separated
Permeability ($\cdot 10^{-6}$ cm/s)	2.0±1.0	17.8±7.0

DISCUSSION & CONCLUSIONS: Primary human endothelial cells and pericytes self-arranged to perfusable microvessels. They showed close similarity to *in vivo* microvessels in terms of morphology, marker expression and functional response. Our results on tumor-derived pericytes further suggest that tumor-derived pericytes retain abnormal functionality *in vitro*, showing that the present device is valuable in uncovering pathological processes in microvessels.

An in-situ crosslinkable, adhesive scaffold for cartilage repair: *in vitro* and *in vivo* characterization

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INTRODUCTION: In this study we present a novel biomimetic hydrogel for cartilage repair. It is based on a modified hyaluronic acid (HA-TG) that can be crosslinked with the activated transglutaminase (TG) factor XIII. The hydrogel is injectable with a crosslinking time of less than 2 minutes and strongly adheres to cartilage tissue. When seeded with human epiphyseal chondroprogenitor cells (ECPs), this hydrogel is able to promote cell proliferation and cartilaginous matrix deposition. HA-TG demonstrated to be stable and to support cell maturation in vivo in a subcutaneous mouse model.

METHODS: Lysine-modified hyaluronic acid (HA-lys) and glutamine-modified hyaluronic acid (HA-glu) were synthetized according to a recently developed protocol and mixed with 15 mio/ml ECPs. Gels were investigated both alone and in a bovine ex-vivo explant model. The scaffolds were cultured for 3 weeks in chondrogenic media and then implanted subcutaneously in nude mice. Gene expression and mechanical testing were evaluated at the beginning of the culture and after 3 weeks of in vitro culture, while histological analysis and push-out testing were conducted at the time of implantation and after additional 6 weeks.

RESULTS: After 3 weeks of culturing, the cells in the hydrogels expressed up to 100,000 fold increase in collagen type 2 and up to 10 fold aggrecan expression compared to cells in 2D (*Figure 1*). This resulted in a dramatic increase in mechanical properties in the softer gels: from the initial 2, 6 and 9 kPa to 250, 25 and 10 kPa (1, 2 and 3% HA-XIII gels respectively) at 3 weeks.
Adhesive properties of the gels were investigated by push-out test and showed initial bond strength 4 times higher than fibrin glue. After 3 weeks of culture of the hydrogel/ECPS within bovine cartilage explants, the bond strength increased to 6 kPa likely due to matrix production and remodeling. HA-TG gels were stable for 6 weeks in vivo, prevented vascular ingrowth and supported the construct maturation reaching 160 kPa in bond strength (*Figure 2*).

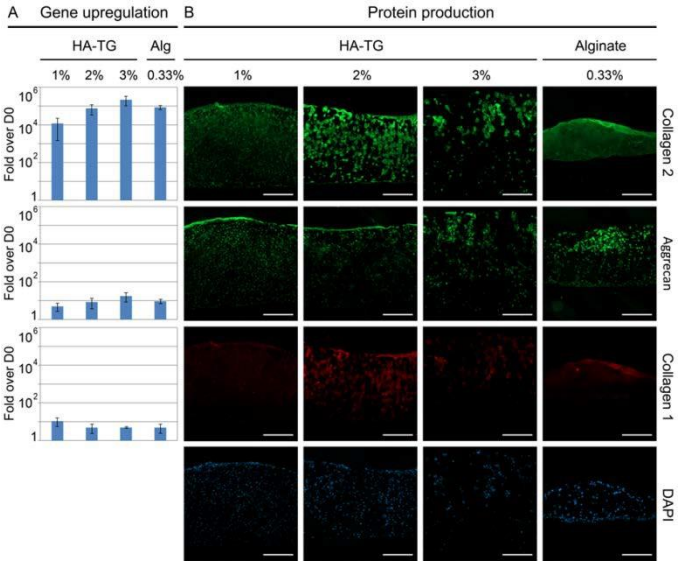

Figure 1: Gene expression (A) and protein production (B) of HA-TG gels after 3 week of in vitro culture. Scale bar: 500µm.

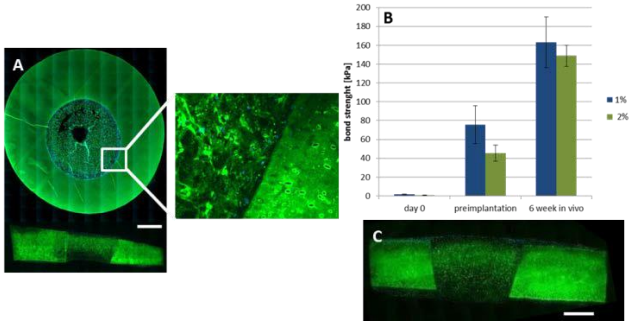

Figure 2: Collagen 2 staining a hydrogel/explant construct after 3 weeks in vitro (A). Bond strength of HA-TG gels to bovine cartilage explants after crosslinking, after 3 weeks of in vitro culture and after 6 more weeks in vivo (B). Collagen 2 staining of a 2% hydrogel/explant construct after 3 weeks in vitro and 6 in vivo (C). Scale bar: 2mm.

DISCUSSION & CONCLUSIONS: Due to its adhesive properties, injectable potential and in vivo stability, this novel scaffold represents a promising alternative to the current cartilage repair techniques.

ACKNOWLEDGEMENTS: This project was funded by the ETH Foundation (Grant ETH-50 13-1).

Surface Functionalization Strategy for Bone Biomimetic Osteocyte Models

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INTRODUCTION: Osteocytes, the most abundant cells in bone, are responsible for sensing the mechanical strains that bone is subjected to. *In vivo*, osteocytes are tightly surrounded by the rigid bone matrix, which is composed predominantly of collagen and hydroxyapatite (HA). How mechanical signals are perceived by these cells is strongly influenced by the three dimensional (3D) shape of the cavity and channel system they reside in [1]. Nevertheless, *in vitro* osteocytes are conventionally cultured on 2D collagen-coated substrates, devoid of HA, which has been shown to be important for the characteristic gene expression by these cells *in vitro* [2].

We aim to develop a bone biomimetic surface functionalization strategy applicable to 3D substrates that we generate by micro-3D printing with the Nanoscribe system based on two photon polymerization. Therefore, we implemented solution-based strategies that are suitable for coating complex geometries. As a base material, we used OrmoComp (micro resist technology GmbH), an inorganic–organic hybrid polymer amenable to micro-3D printing, which is biocompatible and, due to its transparency, well suited for fluorescence microscopy.

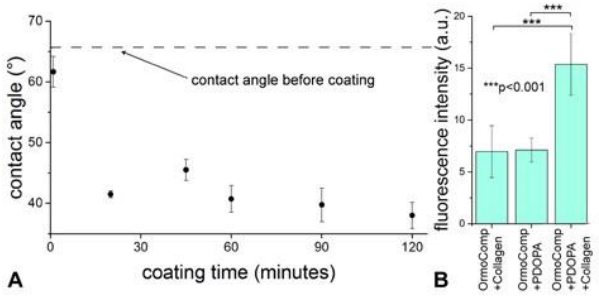

Fig. 1: A: Contact angle of PDOPA coated surfaces. B: Relative quantification of immobilized collagen by immunofluorescence

METHODS: Planar OrmoComp substrates were fabricated by spin-coating on glass slides, followed by post-processing steps as recommended by the manufacturer. Polydopamine (PDOPA) coatings were prepared by dip-coating according to [3] for the indicated times. Collagen coating using a solution of collagen I (Sigma-Aldrich) in PBS was carried out for 24 hours in a humidified incubator at 37°C. Collagen was detected by indirect immunofluorescence and confocal microscopy. HA coatings were prepared by dip-coating in 10x Simulated Body Fluid [4] for 6 hours at room temperature.

RESULTS: By dip-coating, a homogeneous, thin layer of PDOPA was formed on the OrmoComp surface. Longer coating times resulted in a larger increase in surface hydrophilicity (Fig. 1A), and a plateau was reached at a contact angle of around 38°. Pre-coating with PDOPA for 90 minutes lead to significantly more collagen immobilization on the surface compared to direct adsorption to the OrmoComp surface (Fig. 1B). Collagen-coated OrmoComp surfaces were coated with a crystalline HA layer (Fig. 2A). The coating consisted of spherical crystalline mineral aggregates of 6-10 µm in diameter. The thickness of the layer was determined to be 22 µm by cross-sectional Scanning Electron Microscopy (SEM), in accordance with profilometry measurements.

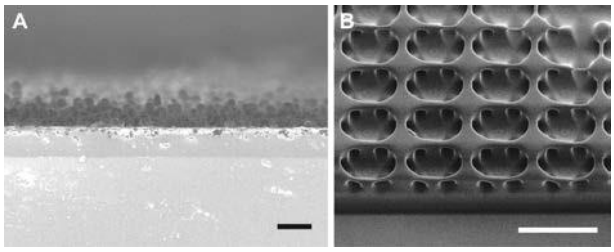

Fig. 2: A: Cross-sectional SEM of HA layer on collagen-coated OrmoComp substrate. B: Micro 3D-printed cavity and channel structures. Scale bars: 30 µm

DISCUSSION & CONCLUSIONS: Using dip-coating processes, a bone biomimetic coating combining collagen and HA on OrmoComp was achieved. However, the high surface roughness should be further improved. Using direct laser writing, we are able to fabricate freeform 3D structures out of OrmoComp with sub-micron resolution, for example channel and cavity networks resembling cellular microenvironment in bone (Fig. 2B). We propose that in combination, these fabrication approaches can provide a promising platform to mimic the composition and geometry of the bone microenvironment for functional studies of live osteocytes *in vitro*.

Lung-on-chip to study idiopathic pulmonary fibrosis

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INTRODUCTION: Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive and fatal disorder of unknown aetiology characterized by defects in lung epithelium regeneration, fibroblast activation and extensive lung remodelling (1).Preclinical IPF models mostly rely on animal testing, which have so far failed to reproduce the disease and have been of little help in testing anti-fibrotic compounds (2). The development of advanced *in-vitro* models that enable to mimic key aspects of the fibrotic process in the lung is thus urgently needed. These so-called "organs-on-chip" are widely seen as having the potential to revolutionize the way drug discovery is carried out.

Recently we reported about a lung-on-chip that reproduces the alveolar microenvironment properties such as the thin alveolar barrier and the three-dimensional cyclic strain applied to alveolar wall (3). Here, we apply this technology to study the pathophysiology of IPF.

METHODS: Either normal human lung fibroblasts (NHLF) or A549 lung epithelial cells were cultured in the lung-on-chip devices in growth media (10% FBS, DMEM). The media was then switched to 1% FBS / DMEM and the cells were cultured for 48h in presence of 5ng/ml TGFβ (NHLF cells). RNA was harvested using NucleoSpin® RNA XS kit (Macherey nagel), cDNA were prepared with SuperScript® III First-Strand Synthesis System and then qPCR analysis was performed. A wound-healing assay was carried out upon confluence of A549 cells. The epithelial layer cultured on an ultra-thin flexible and porous membrane was gently scratched using a pipette tip. The cells were then cultured with100ng/ml HGF a potent proliferation factor and the wound closure recorded.

RESULTS: We measured gene expression of TGFβ-induced markers of myofibroblast differentiation, such as alpha-smooth muscle actin (αSMA), Collagens (Col1A1, Col3A1), Tenascin (TNC) and Connective tissue growth factor (CTGF). Physiological cyclic stretching applied to NHLF for 48h led to reduced expression of αSMA, collagens and CTGF (Fig. 1). We have also observed that cyclic stretching reduced HGF-stimulated regeneration of a wound in A549 monolayer (Fig. 2).

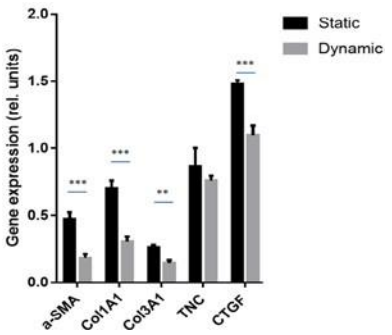

Fig. 1: Effect of physiologic cyclic stretch on myofibroblast marker expression in normal human lung fibroblasts stimulated by TGFβ for 48h.

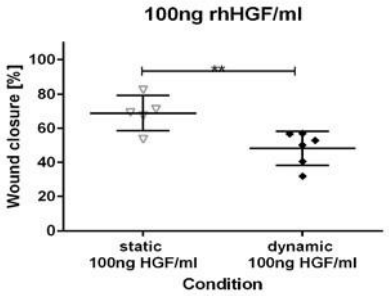

Fig. 2: Impact of physiologic cyclic stretch on wound closure in HGF supplemented medium after 24h.

DISCUSSION & CONCLUSIONS: Mechanical stretching plays an important role in the pathogenesis of IPF, however data uncovering its effects on different cell types is limited and often controversial. Here we confirm previous data that cyclic stretch reduces expression of αSMA and collagens in normal human lung fibroblasts. The cyclic stress also significantly reduces CTGF in NHLF as well as the healing rate of a wounded A549 cell monolayer. The lung-on-chip enables the investigation of several key aspects that take place during the fibrotic process in the lung. The effects of the physiologic mechanical cyclic stress can be evaluated using various read-outs, such as wound-healing or qPCR analysis.

1. 0

Aniline tetramer-*co*-polycaprolactone fibres as novel materials for biodegradable conductive scaffolds

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INTRODUCTION: Conductive substrates based on conjugated polymers have been proposed to enhance cell proliferation of C2C12 or MC3T3 and/or differentiation from precursor cells into mature myotubes or osteoblasts, respectively. Furthermore, conjugated systems have been reported to alter protein adsorption profiles, depending on their oxidative state [1]. However, restricted processability and biodegradability of conjugated polymers limit their use for biomedical application. In this respect, the synthesis of conjugated oligomers, presenting improved solubility, is expected to overcome the current limitations. This study focuses on the development of a biodegradable, electrically conductive scaffold based on aniline oligomers to meet the current demand for functional scaffolds [2].

METHODS: In a two-step synthesis, a block-*co*-polymer of an aniline tetramer (AT) and polycaprolactone (PCL) was obtained [3]. AT-PCL polymers were blended with pure PCL at different ratios and processed into fibrous mats by electrospinning. Electrical conductivity was calculated based on I-V curves. Fibronectin adsorption was quantified spectrofluorometrically, whereas the strength of fibronectin binding to the substrate was assessed by atomic force microscopy. Doped and undoped AT-PCL scaffolds were then evaluated in cell cultures of neonatal rat ventricular myocytes (NRVM), skeletal myoblasts (C2C12) and osteogenic precursor cells (MC3T3).

RESULTS: AT-PCL was successfully processed into homogenous fibrous mats by electrospinning (fibre diameter: 1.1 ± 0.4 µm). Electrical conductivity was shown to be highly dependent on the dopant, ranging from $6 \cdot 10^{-9}$ $S \cdot cm^{-1}$ (camphorsulfonic acid doped) to $9.9 \cdot 10^{-10}$ $S \cdot cm^{-1}$ (phytic acid doped). Fibronectin adsorption was enhanced on phytic acid doped substrates, compared to undoped AT-PCL membranes. Binding strength of fibronectin was reduced on doped substrates, indicating a more folded protein conformation with decreased surface interaction. C2C12 and MC3T3 adhered and proliferated on all substrates and formed homogeneous layers on the fibres. After 14 days in culture, C2C12 fused into multinucleated myotubes. NRVMs on the other hand formed aggregates and did not spread into homogeneous monolayers.

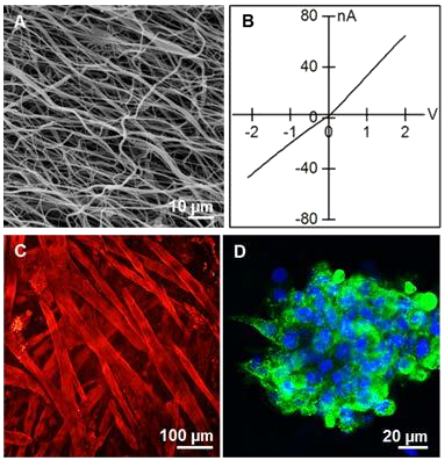

Fig 1: A) SEM image of electrospun AT-PCL fibres. B) IV curve of AT-PCL, doped with camphorsulfonic acid. C) myotubes on the generated scaffolds and D) NRVM, positively stained for α-actinin on AT-PCL scaffold.

DISCUSSION & CONCLUSIONS: Despite their very low electrical conductivity, an altered oxidative state of AT-PCL has an effect on protein adsorption and hypothetically protein conformation. Preliminary *in vitro* evaluation confirms cell adhesion and growth of various cell types on AT-PCL fibres. Future experiments will quantify myogenesis (tube formation of C2C12) and osteogenic differentiation of MC3T3, respectively on doped and undoped substrates.

ACKNOWLEDGEMENTS: A.G.G has been funded by the Swiss National Science Foundation SNF, Grant No. P2BEP3_152091 and P300PB_161072.

A LUNG-ON-A-CHIP ARRAY FOR LONG-TERM CELL CULTURE

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INTRODUCTION: Organs-on-chip are widely believed to have the potential to reduce or replace animal testing in the future. To achieve this goal a number of challenges must be solved. Alongside the accurate reproduction of the in-vivo parameters, cells have to be cultured on chip for several days or even weeks (long-term cell culture) to meet regulatory requirements. Here we present a novel lung-on-a-chip array that reproduces the cyclic in-vivo strain of the respiration and enables long-term cell culture.

METHODS: The lung-on-a-chip array consists of two reversibly bonded parts: a fluidic and a pneumatic part. They are made of PDMS and fabricated using soft lithography techniques. The fluidic part comprises two structured plates, sandwiching a 3.5µm thin, porous and flexible membrane. The pneumatic part includes a network of microchannels in which pressurized air actuates two valves by the mean of a 40µm thin PDMS membrane. This membrane also mimics the in-vivo diaphragm, whose function is to induce respiration. The cyclic movements of this microdiaphragm (Fig.1A) create a 3D cyclic strain on the cells cultured on the ECM-protein coated porous membrane [1]. Lung epithelial cells (16HBE14o-) were used and cultured on chip for several days in dynamic and static conditions. The apical and basal supernatants (SN) were collected every 22h to perform an ELISA assay.

RESULTS: The lung-on-a-chip array has two operation modes: I) breathing and II) medium exchange mode (Fig.1A). During breathing the valves are closed and the microdiaphragm is actuated, recreating the 3D cyclic strain on the porous membrane. In the medium exchange mode, the breathing is stopped, the inlet well filled with medium and the outlet well emptied. The medium is then exchanged passively in the basal chamber by hydrostatic and capillary forces, once the valves are open. The collected SN is used to analyse the IL-8 concentration in both the basal and apical cell culture chamber for static and dynamic conditions using an ELISA assay, see figure 1 B.

DISCUSSION & CONCLUSIONS: The new lung-on-a-chip allows to easily exchange the cell culture medium without the need of an active pump. The design modelled using numerical simulation minimizes the deflection of the 3.5µm thin membrane during medium exchange, which induces a minimal mechanical strain on the cells of less than 0.5% linear. Lung epithelial cells were cultured on chip for up to six days. The ELISA results showed, that the sampled SN can be successfully used to screen for cytokine levels. This lung-on-a-chip array now enables long-term cell culture experiments at the air-liquid interface and allows for more elaborate experiments. For example inhalation assays using drugs or particles and/or adding immune cells from either side of the barrier following for instance lung inflammation.

ACKNOWLEDGEMENTS: This work is supported by the Swiss Commission for Technology and Innovation (CTI).

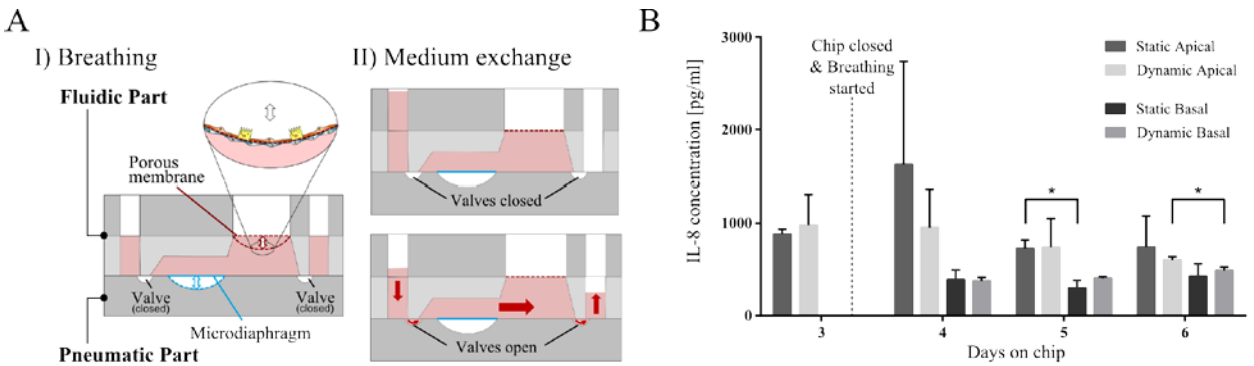

Figure 1. A shows a schematic cross-section of one (out of six) cell culture chambers of the lung-on-a-chip array and its two operation modes. In B one can see the concentration of IL-8 in the apical or basal side for static and dynamic cell culture.

BEHAVIOR OF MOUSE EMBRYONIC STEM CELLS ON PATTERNED SUBSTRATES

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INTRODUCTION: Embryonic stem cells (ESCs) are characterized by two special features: the capacity to self-renew and the ability to differentiate into every cell type of an organism (pluripotency). *In vitro*, all stem cell types reside in a specialized microenvironment – the so called stem cell niche – where they are exposed to different types of stimuli that affect cell behavior. These stimuli can be of biochemical nature, like growth factors and nutrients, but also of physical nature, like stiffness or topography.[1] Concerning physical cues, most studies have concentrated on different adult stem cells. Our work aims to investigate the effect of physical cues on the behavior of ESCs. In more detail, we want to gain knowledge on the effect of certain substrate types on mouse ESC (mESC) behavior. Therefore we use geometrically defined two-dimensional (2D) and three-dimensional (3D) cell culture setups as well as substrates with varying roughness levels. The studies were conducted on a single cellular level to exclude effects from neighboring cells.

METHODS: 2D patterned substrates with varying adhesive areas were fabricated via microcontact-printing. All 3D patterns were produced by direct laser writing and coated with fibronectin. Chemically identical substrates with different topographies were produced by UV-initiated free radical copolymerization of 2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate (HEMA-EDMA) in the presence of a porogenic mixture.

RESULTS & DISCUSSION: In the first project we investigated the impact of adhesion area on mESC behavior in geometrically defined 2D and 3D growth substrates. Cells were cultivated on quadratic fibronectin-coated 2D-microislands with sizes ranging from 15 to 35 µm side length. We observed that under these conditions the proliferation rate of mESCs positively correlates with adhesion area. Next we produced squared 3D-microwells with walls 20 µm in height and a varying ground area ranging from 15 to 35 µm side length. Surprisingly, smaller microwells resulted in higher mESC proliferation rates, indicating that dimensionality of the environment can influence stem cell behavior.

The second project investigates the impact of topography on mESC behavior. We could show that synthetic surfaces with a hierarchical micro-nano roughness (MN-surfaces) promote self-renewal of mESCs. In contrast, cultivation of mESCs on either smooth or nano-rough polymer surfaces resulted in their fast differentiation. Moreover, long-term cultivation of mESCs on MN-surfaces lead to a homogeneous population of undifferentiated stem cell colonies. These cells still expressed pluripotency markers like Oct4 and were able to differentiate into all three germ layers. Immunostainings of singe cells revealed the absence of focal adhesion markers on all studied polymer substrates. However, only the MN-surfaces elicited the formation of actin-positive cell protrusions anchoring the cells to the polymer agglomerates. This might indicate an alternative adhesion mechanism involved in the maintenance of mESC pluripotency.[2]

CONCLUSIONS: With our studies, we demonstrate that single mESCs - like adult stem cell types - strongly respond to changes of the physical properties in their microenvironment. Such physical factors can thus be harnessed in future to gain more control over ESC self-renewal and differentiation.

ACKNOWLEDGEMENTS: Dr. B. Richter and Dr. M. Thiel are acknowledged for the production of DLW-based substrates and master structures. We thank Prof. Dr. M. Wegener for providing access to the DLW setup. We also thank Prof. D. Wedlich and Prof. Dr. R. Kemler for the Oct4-eGFP mouse embryonic stem cell line.

IN VITRO PERIODONTAL LIGAMENT MODEL TO ASSESS SYNTHETIC SELF ASSEMBLING PEPTIDES FOR TISSUE REGENERATION

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INTRODUCTION: The periodontal ligament represents a very complex tissue structure between the tooth cementum and the alveolar bone. The matrix of the periodontal ligament is build up by periodontal ligament fibroblasts (PDLF). In case of sustainable bacterial colonization inside the periodontal pocket, the ligament is degraded and requires regenerative methods to replace the missing tissue.
Enabling the migration of PDLF seems to be the key success factor for a guided regeneration of the periodontal tissue. To achieve this, a degradable, suitable matrix has to be placed into the periodontal gap [1].
Self-assembled peptide nanofibers moved into the focus of several research groups in UK, China, US or the Netherlands, as they exhibit adequate characteristics of a matrix for tissue regeneration processes [2]. In order to evaluate the feasibility of SAPs for periodontal regeneration, a 3D test model was established.

METHODS: Bovine and human teeth were cleaned and cut to present pure dentin surface. Adhesion and proliferation rates of PDLF were determined. Visualization of the cells on the dentin was realized by SEM. For assessment of migration speed and distance, a collagen I based cell donor compartment was placed on the dentin inside a special designed chamber, simulating a periodontal pocket and visualised by MTT reduction and subsequent colour change. Migration into the peptides was assessed by confocal microscopy. Moreover, assessment of interaction of self-assembling peptides (SAP) as therapeutically acting materials to the tooth root surface and degradation kinetics in presence of microorganisms and neutrophilic elastase were determined by protein quantification.

RESULTS: Cells adhere and proliferate on pure dentin surface. Cell attachment was visualized by SEM (Fig.1A). Cell migration out of the donor compartment could be determined by MTT staining of viable cells (Fig. 1B). The interaction of the SAP with the root matrix could be performed with fluorescing labelled peptide (Fig. 1C). SAPs are able to fill dentin channels and provide a strong interaction with the given natural surface. Apparently, the PDLFs are able to migrate through the SAP hydrogels (Fig. 1D). Degradation of the hydrogel is due to dissolution of the structure, but is not increased by the action of neutrophilic elastase and reaches equilibrium after 24h.

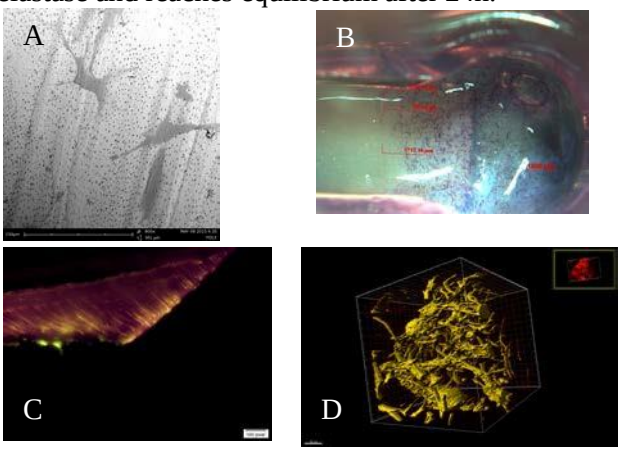

Fig. 1: PDLFs adhesion on pure dentin (A), in 3D periodontal model on SAP hydrogel (B), diffusion of fluorescent SAP into dentin channels (C) and distribution in SAP hydrogel (D)

DISCUSSION & CONCLUSIONS: Effective treatment of periodontitis has emerged to one of the most important issues in dentistry, the need for uncomplicated and straight forward therapies is obvious. Within the study we were able to establish an *in vitro* model of the periodontal ligament and first results supported the proof of concept of this approach. Further investigations and subsequent integration of newly developed materials are ongoing and will allow deeper insight in periodontal tissue regeneration.

ACKNOWLEDGEMENTS: We gratefully thank the CTI for project funding and the support of Credentis

Effect of exogenous hyaluronan on human Mesenchymal Stem Cell chondrogenesis

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INTRODUCTION: The presence of hyaluronan (HA) in synovial fluid is known to have an impact on the elastoviscosity of joint synovial fluid and consequently on the friction coefficient of the opposing articular surfaces[1]. This important biological function will also potentially change at cellular level the amount of activated TGF-β with an impact on human mesenchymal stem cell (hMSCs) response *in vivo*. In addition, HA may also provide further protection to the developing cartilage engineered implant by improving lubrication. Therefore, a medium containing HA may better recapitulate the rheological and biological features of the synovial fluid present in the patients' intra-articular environment. Thus, the aim of the present study is to improve the chemical composition of a standard chondrogenic media by assessing the influence of Sodium Hyaluronate supplemented cell culture media on the chondrogenesis of hMSCs.

METHODS: Cylindrical (8 mm x 4 mm) porous polyurethane (PU) scaffolds were prepared. hMSCs isolated from bone marrow aspirates were suspended at passage 3 in a fibrinogen-thrombin-solution, and seeded evenly throughout the PU scaffolds. Scaffolds were cultured in control standard chondrogenic medium without sodium hyaluronate (HA-) or supplemented with 0.2% sodium hyaluronate (HA+) with a molecular weight of 1.800 kDa (*Stanford Chemicals*) in order to simulate synovial fluid concentration under normal conditions (2.3 mg/ml)[2,3]. After 14 and 28 days of culture, total RNA was extracted from the constructs with TRI reagent and TaqMan reverse transcription and Real-time PCR were performed. A panel of human genes associated with chondrogenic markers (Collagen II, Collagen X and Aggrecan) was investigated. Relative quantification of target mRNA was performed according to the comparative Ct method and RNA was normalized to 18S rRNA. After 28 days of incubation, the constructs were digested with proteinase K and total DNA content and sulphated glycosaminoglycans (GAG) were measured spectrofluorometrically. Total GAG content of the culture media was also measured to assess the release of matrix molecules from the constructs into the media.

RESULTS: Collagen II, a relevant marker for chondrogenesis, was upregulated after 14 days in HA+ group. Additionally Aggrecan was expressed only in HA+ group while was undetectable for HA- group at both time points. Collagen X was equally expressed in both groups over the two time point (Fig 1A, and B). Furthermore, levels of sulphated GAG retained in the scaffolds and released into the culture media were higher when the chondrogenic media was supplemented with 0.2% HA (Fig 1C and D).

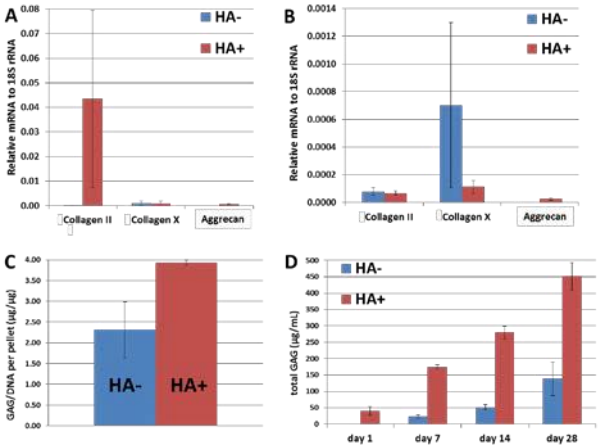

Fig. 1: Effect of HA on: gene expression after 14 days (A) and 28 days (B); total GAG/DNA ratio retained into the scaffolds after 28 days (C); total GAG released into the medium at different time points (D).

DISCUSSION & CONCLUSIONS: These preliminary results suggest that the addition of HA to the standard chondrogenic media, improves the intrinsic capacity of hMSCs to differentiate *in vitro* towards a chondrogenic phenotype. Therefore enhanced chondrogenic differentiation of hMSCs may be also achieved through the development of an appropriate culture medium. Further experiments are ongoing in our laboratory to better investigate the signalling mechanisms.

ACKNOWLEDGEMENTS: VivoLoad Project funded by AO Research Institute, Davos, CH.

Towards a new xeno-free microcarrier-based cell culture system for the scalable expansion of human ASCs

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INTRODUCTION: Human adipose tissue-derived stem cells (hASCs) are very promising candidates for cell-based therapies. However, for therapeutic use, these cells need to be isolated from a biopsy and efficiently expanded as they cannot be harvested in sufficient quantities from the body[1]. We believe that efficient expansion of clinical grade hASCs can be achieved in a microcarrier-based cultivation system. Microcarriers (MCs)[2] offer the advantage of providing a large surface area for cell growth. In this study we optimized our new xeno-free and biodegradable microcarrier-based system for the scalable expansion of hASC in serum-free cell culture conditions.

METHODS: hASCs were isolated from discarded human adipose tissue and cultured in xeno- and serum-free conditions. Several types of MCs manufactured by Micro-Sphere SA[3] were examined to test their ability to promote attachment on their surface and growth of hASCs in serum-free culture conditions. hASCs cells were analyzed for the expression of typical markers (FACS analysis) and by quantitative RT-PCR to monitor the expression of some marker genes. The growth of the cells on MCs was monitored by immunohistochemical methods and by Scanning Electron Microscopy.

RESULTS: We found a suitable MC for the expansion of hASC in defined cell culture conditions and we optimized cell seeding strategies. hASCs retained their differentiation potential and their cell surface markers, indicating that the MC did not alter phenotypic properties of the cells.

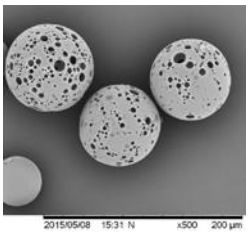

Fig.1: Scanning electron microscope (SEM) pictures of the biodegradable microcarrier BR61A

DISCUSSION & CONCLUSIONS: This study will allow us to implement our approach by introducing a bioreactor-based manufacturing system for the preparation of cell-based therapeutics. Micro-sphere SA is also exploring new manufacturing technologies for MCs production with different material composition with the aim to improve industrial scalability and the process reproducibility.

ACKNOWLEDGEMENTS: The authors gratefully acknowledge the financial support of this project by the Cardiocentro Ticino Foundation and micro-sphere SA.

An Implantable Device for Screening Hematopoietic Stem Cell Niche Factors

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INTRODUCTION: For many cancers involving blood cells (e.g. leukemia or lymphoma), the only available therapy necessitates bone marrow transplants. A limiting factor in this approach is the availability of hematopoietic stem cells (HSCs). Expanding HSCs *ex vivo* presents a major medical challenge. When plated *in vitro*, HSCs rapidly lose their regenerative capacity, primarily due to lack of the molecular and cellular components normally present in the HSC niche [1]. The generation of more tractable *in vivo* models may provide tools for identifying these critical factors. One approach is to combine a blank biomaterial with different growth factors and cell types known to be present in the niche *in vivo*. To date, there remains a great need for methods to achieve heightened throughput in screening such constructs *in vivo*. Here we present a novel device, which allows the screening of multiple unique conditions in a single mouse, and have applied it for use in discovery of critical HSC niche factors.

METHODS: Human mesenchymal stem cells (hMSCs) were encapsulated in functionalized biomimetic polyethylene glycol (PEG) hydrogels [2] supplemented with or without bone morphogenetic protein-2 (BMP-2). These PEG gels were polymerized directly in individual wells of novel multiplexing polydimethylsiloxane (PDMS) devices. Then, the screening devices containing PEG hydrogels were subcutaneously implanted in immunocompromised mice (4x per animal) (Fig 1A). After 8 weeks, the devices were explanted and analyzed for bone and bone marrow formation by microCT, histology, and FACS.

RESULTS: In our screening devices, wells of at least 2mm diameter were shown to be required for niche formation allowing 8 conditions to be screened per implantable site, resulting in 32 different conditions per mouse. Bone formation was observed in all wells containing gels with hMSCs and BMP-2 (Fig 1B), but not in those lacking the growth factor (circle), as assessed by microCT. H&E stained sections complemented these findings. Gels containing hMSCs and BMP-2 were shown to be enclosed by a bone shell filled with marrow containing trabecular bone structures (Fig 1C). Finally, FACS characterization of the murine hematopoietic cell population revealed that constructs implanted with hMSCs and BMP-2 were enriched with long term HSCs, whereas gels lacking hMSCs were not (Fig 1D).

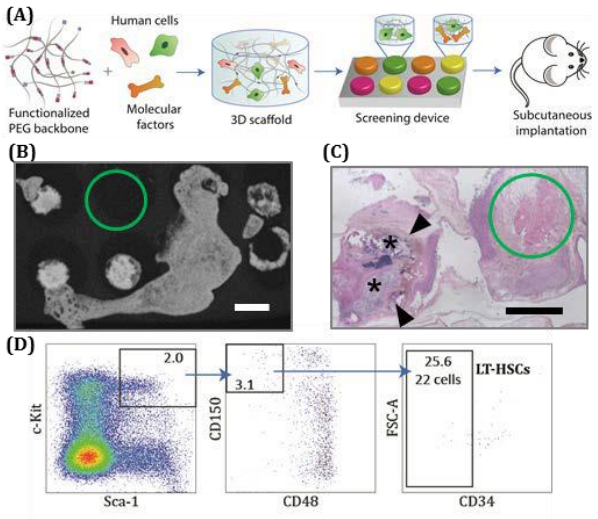

Fig. 1: In vivo HSC niche screening device. (A) PEG precursors, different growth factors and cell types were employed to create microenvironments of variable composition in subcutaneously implanted screening devices. (B) MicroCT and (C) H&E staining depict ectopic ossicle formation (arrows) and bone marrow-like cavities (*) in gels loaded with hMSCs and BMP-2, or fibrous tissue formation in absence of BMP-2 (circle). (D) FACS analysis of ossicles shows the presence of long term HSCs. Scale bars: 2mm.

DISCUSSION & CONCLUSIONS: A PDMS device for increased screening throughput was successfully used to determine ideal HSC niche conditions in a subcutaneous implantation model. Multiple cell types and soluble factors were varied to find optimal conditions for bone marrow niche formation *in vivo*. Results indicated a functional ectopic niche in BMP-2 and hMSCs-laden gels. This device represents a powerful new tool for heightened *in vivo* screening of tissue engineering constructs with a broad range of applications.

ACKNOWLEDGEMENTS: This work was funded by the Swiss National Science Foundation grant 153316.

EXPLOITING PEPTIDE SELF-ASSEMBLING HYDROGELS FOR 3D CELL CULTURE OF A VARIETY OF CELL TYPES OF INTEREST FOR REGENERATIVE MEDICINE

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INTRODUCTION: We have developed a platform for the design of hydrogels with tailored properties and functionalities exploiting the self-assembly of short (8-10 amino acids) β-sheet forming peptides. The design of these peptides is based on the alternation of hydrophilic and hydrophobic residues. By altering the primary peptide structure, the formulation and the concentration the properties of these scaffolds (e.g.: modulus and functionality) can be easily controlled. We have shown that various cell types can be cultured successfully within the peptide scaffolds [1-3].

METHODS: All cells were maintained in specific media required for their growth in a 5 % CO_2 humidified incubator at 37°C. Cells were either cultured on or suspended in gels (PeptiGelDesign) at varying concentrations and cultured in ThinCerts™ (Greiner). The viability of encapsulated cells was determined using LIVE/DEAD kit (ThermoScientific), according to the manufacturers' instructions. Subsequently, the distribution of red (dead) and green (live) cells was visualized using a fluorescent microscope. Immunohistochemistry was carried out to detect various cell specific markers.

RESULTS: A range of cell types, including: human mesenchymal stem cells, cardiomyocytes, dorsal root ganglion explants, adipose derived mesenchymal stem cells, cardiac progenitor cells, nucleus pulposus cells, chondrocytes, hepatocytes, oesophageal stromal fibroblasts and oesophageal epithelial cells were cultured either on the surface (2D) or encapsulated within (3D) peptide self-assembling hydrogels. Excellent viability was observed after LIVE/DEAD staining for up to 14 days. Cell specific markers were also detectable; for example, oesophageal epithelial cells cultured on the surface of the hydrogels exhibited ZO-1 staining equivalent to that observed in control tissue (Figure 1). Physiological morphology was observed in all cell types cultured on or in the hydrogels.

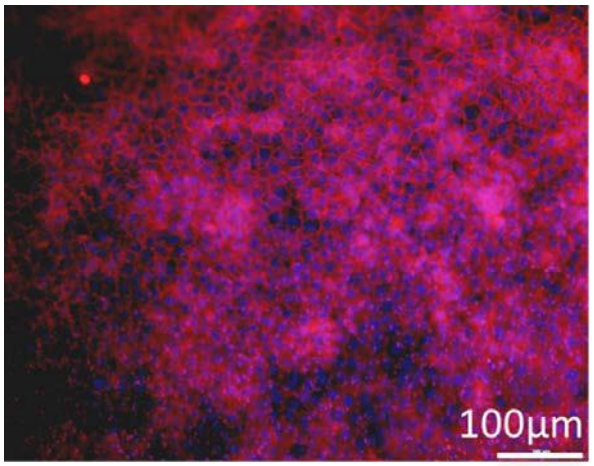

Fig. 1: Primary rat oesophageal epithelial cells cultured on the surface of SAPH for 3 days. Cell nuclei (blue) and ZO-1 (red).

DISCUSSION & CONCLUSIONS: Self-assembling peptide hydrogels are suitable for the culture of a wide variety of cell types, including stem cells and primary cells. Physiological morphology was observed when using these hydrogels without the need for RGD functionalisation. The cells cultured are from all parts of the body and are of particular interest for regenerative medicine applications. Self-assembling peptide hydrogels could be crucial in regenerative medicine applications for growing cells in an environment more closely resembling physiological ones.

ACKNOWLEDGEMENTS: The authors acknowledge an EPSRC Fellowship (EP/K016210/1) for funding.

Sandwich culture of human mesenchymal stem cells with ephrinB2 oriented-immobilized polyacrylamide hydrogels

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INTRODUCTION: The objective of this study is to investigate the effect of hydrogels properties and culture condition on the behavior of human mesenchymal stem cells (MSC) sandwich cultured between poly(acrylamide) (PAAm) hydrogels with or without ephrinB2 immobilization. It has been demonstrated that ephrinB2 of an Eph signal ligand could stimulate the osteoblastic differentiation of MSC through a direct binding between ephrinB2 and EphB4 via the cell-cell contact [1]. In this study, the immobilization of ephrinB2 in an orientation-regulated manner was designed to achieve the efficient ligand-receptor binding [2].

METHODS: PAAm hydrogels with varied elasticity were prepared by the radical polymerization of acrylamide (AAm), and *N,N'*-methylenebisacrylamide (BIS). The storage modulus of hydrogels was measured by a rheometer (Rheostress I, Thermo Haake, Inc.) to assess the elasticity. Rat tail collagen type I was immobilized onto the surface of the hydrogel for MSC attachment. Then, a chimeric protein of ephrinB2 and Fc domain was orientated-immobilized onto protein A-conjugated PAAm hydrogels through the bio-specific interaction between the Fc domain and protein A. As a control, the ephrinB2 chimeric protein was chemically conjugated on the collagen type I-immobilized hydrogel. MSC were sandwiched between the resulting hydrogels and maintained in culture for 5 days [3]. Runx2 expression of MSC was measured by quantitative polymerase chain reaction to evaluate their osteoblastic differentiation.

RESULTS: Upon sandwich cultured between the bottom conventional culture dish and the upper ephrinB2 oriented-immobilized PAAm hydrogels with varied elasticity, the oriented-immobilized ephrinB2 significantly enhanced the level of Runx2 expression for the PAAm hydrogel with an elasticity of 3.6 kPa (Figure 1). This phenomenon was well corresponded with the significantly down-regulated RhoA activity in the same sandwich culture condition (data not shown). Moreover, the level of Runx2 expression was significantly enhanced by sandwich culture with two ephrinB2 oriented-immobilized PAAm hydrogels (Figure 2).

DISCUSSION & CONCLUSIONS: The present study suggests that cell signaling pathways for osteoblastic differentiation in MSC could be activated by the oriented-immobilized ephrinB2 even in the sandwiched condition.

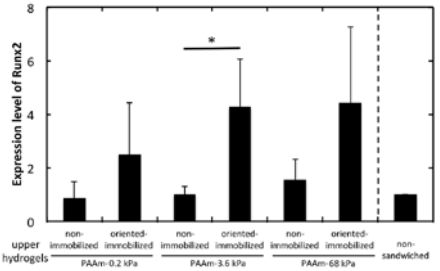

*Fig. 1: Runx2 expression of MSC sandwich cultured between the bottom conventional culture dish and upper PAAm hydrogels with or without ephrinB2 immobilization or cultured on the bottom conventional culture dish. *p<0.05; significance between the groups of upper hydrogels with and without ephrinB2 immobilization at the corresponding hydrogel.*

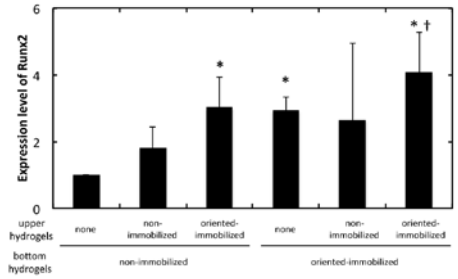

*Fig. 2: Runx2 expression of MSC sandwich cultured between the upper and the bottom ephrinB2 non- and oriented-immobilized PAAm hydrogels, or cultured on the ephrinB2 non- and oriented-immobilized PAAm hydrogel. *p<0.05; significance against the Runx2 expression of MSC cultured on the ephrinB2 non-immobilized PAAm hydrogel. †p<0.05; significance against the Runx2 expression of MSC sandwich cultured between two ephrinB2 non-immobilized PAAm hydrogels.*

ACKNOWLEDGEMENTS: MSC were kindly provided by Prof. Junya Toguchida, Kyoto University.

Development of an *in-vitro* cornea-eyelid friction model to understand the role of glycerol and PRG4 (lubricin) in preventing dry eyes)

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INTRODUCTION: Malfunction of the lacrimal gland due to trauma, old age or Sjögren's syndrome causes the problem of dry eyes, which has a serious impact on the patient's quality of life. Often the patients are prescribed one of the numerous tear substitutes. Comparing the different substitutes objectively *in vivo* is difficult thus an *in vitro* model is necessary for this purpose where relevant surfaces are used, mimics the blinking process and dry eyes. Recently, presence of PRG4(Schmidt et al., 2013) has been shown on the corneal surface in absence of which damage ensues, furthermore the role of glycerol is known in corneal wear protection(Barros et al., 2015) against glass. The aim of this study is to illustrate the role of these two molecules in alleviating dry eye symptoms and to explore the synergism if any.

METHODS: In the first part of the talk the development of the eyelid-cornea friction model mimicking blinking will be discussed (Figure a, b and c) where the cornea slid against the eyelid at 10 mm/s for 1 cm with a 6 second idle time between each blink. Initially the coefficient of friction (COF) was measured in dry condition for 15 cycles followed by bringing a small drop of lubricating fluid at the cornea-eyelid interface. The drop in COF is taken as the relief in dry eye symptom and the time for which the COF remained low would be the duration for which the relief will stay.

Relief an relief duration were then used to study the dose response of glycerol and PRG4 individually and together to understand the synergism.

RESULTS: Figure 1 (e) shows the results of relief from various fluids. Glycerol, PRG4 individually and together provide better relief then just buffer but no synergism was observed between PRG4 and glycerol in fact the combination was worse than individual.

DISCUSSION & CONCLUSIONS:

Both Quartz crystal microbalance and FT Raman is used to understand the reason for this antagonism between glycerol and PRG4.

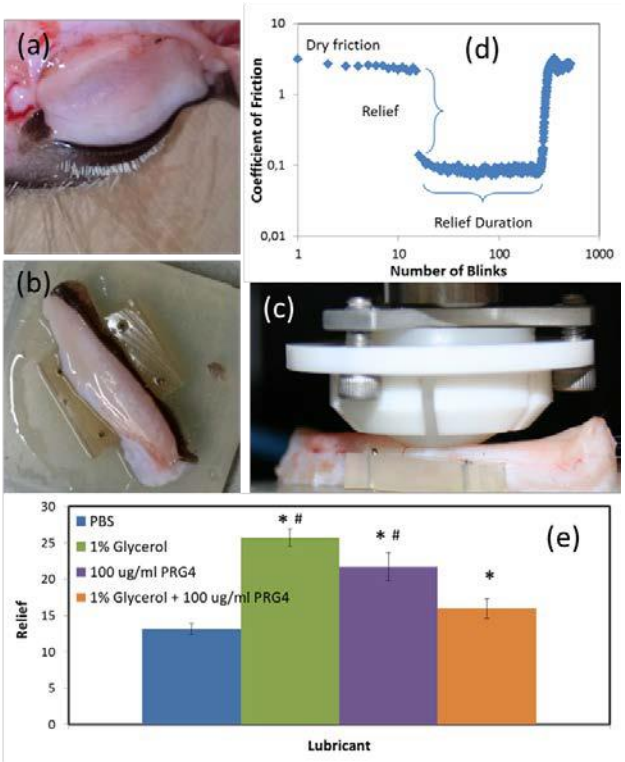

*Fig. 1: (a) As received porcine eyelids, (b) Mounted eyelid to be placed in the tribometer, (c) Sliding of porcine cornea against the eyelid and mimicking the blinking motion, (d) The COF Vs time curve showing the relief and relief duration for water and (e)the relief due to different lubricants. Where *and # shows statistical significance (P<0.05) with respect to PBS and 1%Glycerol+100 µg/ml PRG4.*

STUDYING CELL MEMBRANE-NANOWIRE INTERACTIONS USING FLUORESCENT NANOWIRES

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INTRODUCTION: Epitaxially grown nanowires (NWs) are increasingly used as tools for biology applications, such as sensors, manipulators, injectors, and model particles for toxicology studies [1-4]. However, the interactions that take place at the interface between NWs and the cell membrane are not well understood. To study these interactions it is crucial to visualize the NWs in relation to the cell membrane. For this reason, we are using inherently fluorescent gallium-indium-phosphide (GaInP) NWs in our investigations of the cell membrane-NW interactions, which consist of studies of living cells on NWs, as well as physicochemical studies of model membranes on NWs.

METHODS: The NWs are synthesized using gold catalyzed metal organic vapor phase epitaxy (MOVPE), which allows for good control over the NW size, shape and composition [1,5]. We demonstrated the synthesis of highly fluorescent and photo stable GaInP NWs with emission around 780 nm [1]. In our experiments, we seed and culture cells directly on the nanowire substrate in order to study cell-NW interactions using confocal microscopy.

RESULTS: A SEM- and a confocal image of an array of GaInP NWs can be seen in Fig. 1a and Fig.1b respectively.

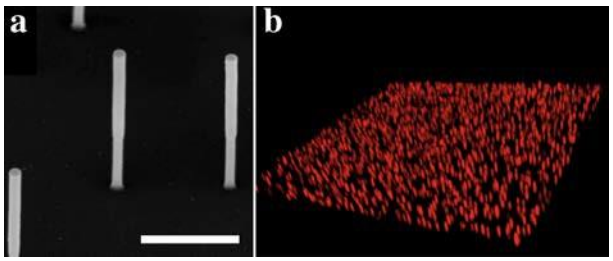

Fig. 1: a) SEM image of GaInP NWs (scale bar: 1 µm) and b) confocal 3D reconstruction image of fluorescence from GaInP NWs.

A confocal image of a cell that has been cultured on top of an array of vertical GaInP nanowires can be seen in Fig. 2. In this case, the cell membrane, which has been labeled with a green fluorescent membrane dye appears to conform to the geometry of this type of NW (20 nm in diameter and 3 µm long).

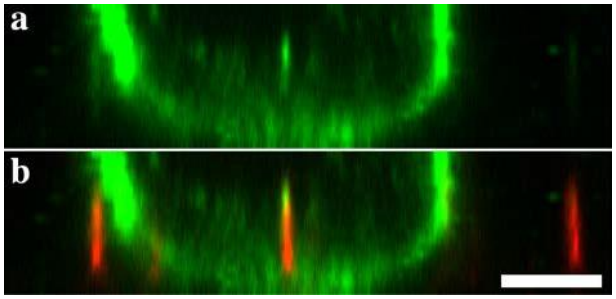

Fig. 2: Confocal images (orthogonal view) of a cell, cultured on top of GaInP NWs. The cell membrane can be seen in green (CellMask) and the GaInP NWs can be seen in red. a) only the cell membrane and b) both channels. Scale bar: 4µm.

Furthermore, we have previously shown that artificial cell membranes can form on NW substrates and they conform to the NW geometry [6]. This allows us to impose highly defined curvatures on model membranes in order to study cell membrane-NW dynamics in a system with reduced complexity

DISCUSSION & CONCLUSIONS: Using fluorescent GaInP NWs we were able to observe that the cell membrane conform to the nanowire geometry when indented by 20 nm in diameter NWs. Consequently, GaInP NWs allows for the localization of NWs in relation to the cell membrane with high precision, which is inherently crucial when studying interactions at the cell membrane-NW interface.

ACKNOWLEDGEMENTS: This work is financed by NanoLund, the Swedish Research Council (VR), and the Carl Trygger foundation.

Characterization of Biomedical Nanoparticles Mixtures as *N*-dimensional Optical Parameter Vectors for On-line Control

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INTRODUCTION: The multiparametric analysis of simultaneous optical data for systems of nano- and /or micro- particles (ensembles, colloids, dispersions) by presentation of system characteristics as *N*-dimensional vectors of optical parameters (*ND*-vectors) can help to elucidate changes in the state of the particles in systems [1-4]. Previously [2], mixtures of anthracene with cyclodextrin nanoparticles were characterized by *4D*-vectors. It was supposed that the position of mixture *ND*-vector on the line connecting the separate components *ND*-vectors points could be justification that there is no interaction between particles in mixture. In this work, the application of *ND*-vector approach is shown on the example of biomedical dispersions mixtures.

METHODS: The analysis includes: a) simultaneous measurements of dispersions by compatible nondestructive optical methods such as refractometry, absorbance, fluorescence, light scattering (integral and differential, static and dynamic, unpolarized and polarized), and b) solution of inverse optical problem by different methods and technologies of data interpretation. Taking into account optical theory and results of experiments helped to elaborate the *ND*-vector characteristics [1–4] for studied particles system state.

RESULTS: Mixture of influenza virus and serum albumin protein nanoparticles can be considered as a model dispersion of vaccine production technological process. It is important to know the degree of virus dispersions purification from the protein impurities. The influenza virus particle was approximated: a) as a homogeneous sphere with the mean diameter - d = 100 nm; b) at the determination of virus particles concentration the bilayer sphere approximation was used. In order to design the optimal scheme for dispersions on-line control the *3D*-vectors **P** $\{P_i, P_k, P_m\}$ of the second class optical parameters (dimensionless and independent from the concentration of particles [1–4]) from the integral light scattering were elaborated. The following dispersions were used: N1 - influenza virus (strain A1 - H1N1); N2- bovine serum albumin protein; N3 - mixtures of influenza virus and serum albumin dispersions in the ratio 1:1. Measurements of the dispersions were made at the same conditions.

DISCUSSION & CONCLUSIONS: It can be concluded that the positions of the *3D*-vectors **P** $\{P_i, P_k, P_m\}$ for constituent dispersions N1 and N2 are suitable for differentiation of these dispersions not only by the value but also by the sign. The preparation of vaccine will be better if the position of mixture (N3) *3D*-vector points will be closer to the pure influenza virus dispersion (N1) *3D*-vector point. It is also possible to suppose from mixture *3D*-vector position under the line connected the constituent dispersions *3D*-vectors points, that there is interaction between particles in mixture (especially if to consider that both influenza virus and serum albumin dispersions are inclined to aggregation and its particle size distributions are polymodal [3]). At the *ND*-vector approach, the polymodality of particle system is not the obstacle. The number of informative parameters is not limited: it is possible to use different polarization and wavelength of incident light, different light scattering angles, etc. [4]. *ND*-vector can reflect most of the changes in the state of particles in dispersion as a whole ensemble. This approach can serve as the on-line control platform for the study and management of the processes with particles mixtures.

ACKNOWLEDGEMENTS: Author would like to thank Prof. Dr. V. J. Klenin (SSU), Dr. N.V. Katushkina (SPbPU), Prof. Dr. O.L. Vlasova (SPbPU), Prof. Dr. H. Hofmann (EPFL), Dr. M. Hofmann-Amtenbrink (MSCH) and Prof. Dr. M. H. Textor (ETHZ) for the help in research and useful discussions.

Neutron Reflectometry: A Powerful Tool to Characterize Biofilms at Interfaces

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INTRODUCTION: X-ray and neutron scattering techniques have shown a great potential in providing detailed picture of complex materials such as biomolecule arrays and nanoparticles at liquid/air or liquid/solid interfaces. While high intensity X-rays often cause radiation damages in the biomaterial film samples, neutrons can be used as a destruction-free probe to uncover the thin films inner structure. Moreover, through tuning of the reflectivity contrast of the liquid phase (use of deuterated liquids) inner film structures can be revealed during a neutron reflectometry (NR) experiment. Paul Scherrer Institute provides a peer-reviewed access to a complete suite of neutron scattering instruments (including small angle scattering, SANS and NR) for the scientific community. Examples from biointerfaces research such as complexation of phospholipid and cholesterol films as well as investigation of the gastric stability of thermo-responsive biopolymers are demonstrated.

METHODS: The principal setup of PSI neutron reflectometer AMOR allows measurements with polarized or non-polarized neutrons in white beam time-of-flight mode (3Å<λ<12Å). The scattering plane of the sample is oriented vertically in order to allow measurements at open liquid surfaces, too. The inclination angle and thereby the accessible q-range is adjusted by tilting a deflection mirror and/or the sample. An area detector or two single detector tubes can be operated alternatively with new fast detector read-out electronics.

RESULTS: The interactions between a model phospholipid 1,2-dipalmitoyl-glycero-3-phosphocholine (DPPC) and a biosurfactant Quillaja Bark Saponin (QBS) obtained from the bark of Quillaja saponaria Molina were studied using simple models of biological membranes. In contrast to the synthetic surfactants of similar surface activity, QBS does not collapse DPPC mono- and bilayers, but penetrates them. The mechanical performance of materials at oil/ water interfaces after consumption is a key factor affecting hydrophobic drug release. Secondly, we methylated the surface of nanocrystalline cellulose (NCC) to produce thermosensitive biopolymers. These methylated NCC (metNCC) were used to investigate interfacial thermogelation at air/water and medium chain triglyceride (MCT)/water interfaces at body temperature. In contrast to bulk fluid dynamics, elastic layers were formed at room temperature, and elasticity increased significantly at body temperature, which was measured by interfacial shear and dilatational rheology and neutron reflectometry in situ.

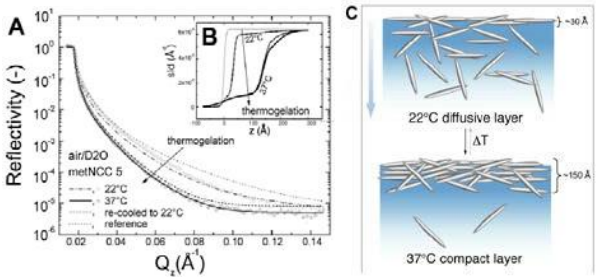

Fig. 1: *(A) Neutron reflectivity of equilibrated Langmuir films of metNCC 5 at 22 and 37 °C at air/deuterium interfaces with its corresponding fits using Parratt formalism. (B) Scattering length density (sld) as a function of layer depth. (C) Illustration of the morphological change of metNCC before and after its thermoresponse.*

DISCUSSION & CONCLUSIONS: The neutron reflectometer AMOR at PSI offers extremely flexible opportunities to measure all kinds of biofilms and/or nanoparticle arrays to study phenomena at liquid/solid or, , due to the horizontal sample geometry, liquid/air interfaces (adsorption, wetting and de-wetting, diffusion etc). Examples of current measurements of thin biofilms in time-of-flight mode are shown.

ACKNOWLEDGEMENTS: This work is based on experiments performed at the Swiss spallation neutron source SINQ, Paul Scherrer Institute, Villigen PSI, Switzerland.

Temperature dependent spectral ellipsometry reveals hydration of thin organic coatings

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INTRODUCTION: Besides physico-chemical properties like surface energy, surface charge or topography, the hydration of interfaces is essential in preventing biofouling.[1] This effect is particularly pronounced in ethylene glycol containing hydrogel coatings. MC-simulations[2] and ab-initio calculations[1] predict that both, the specific ethylene glycol chemistry and the structure of the thin films are prerequisites for water stabilization. For instance, the resistance of self-assembled hexa(ethylene glycol)-monolayers against protein adsorption and the low adhesion strength of zoospores of the green algae *Ulva linz*[3] demonstrate that a stable hydration barrier leads to inert coatings. Besides ethylene glycols, polysaccharides are biopolymers with hydrogel properties capable to store water.[4]

METHODS: To investigate hydrated interfaces and their hydration state, thermogravimetric analysis, QCMD, X-ray and neutron scattering, and temperature programmed desorption are frequently used. Here we present an optical approach which is able to work under ambient conditions. Spectral ellipsometry is applied in a variable temperature cell with controlled atmosphere. This setup allows to monitor changes in optical constants as a function of temperature and thereby giving information on the change in water content and thus the hydration state. As model surfaces to establish this method, highly hydrated hydrogels like ethylene glycol and coatings based on the biopolymers alginic and hyaluronic acid were chosen. As complementary methods, thermogravimetric analysis (TGA) and infra-red spectroscopy were applied to compare the bulk material properties to the thin film coatings.

RESULTS: As two characteristic effects, the temperature induced dehydration and decomposition of the materials were observed. In the bulk studies one can distinguish between materials which show two separated effects and the ones with a combined behaviour. Spectral ellipsometry measurements revealed the separate dehydration and decomposition where TGA measurements could not for some bulk materials. The changes in surfaces chemistry at the different temperatures were monitored with IR spectroscopy, which prove to be particularly sensitive to the decomposition of the films.

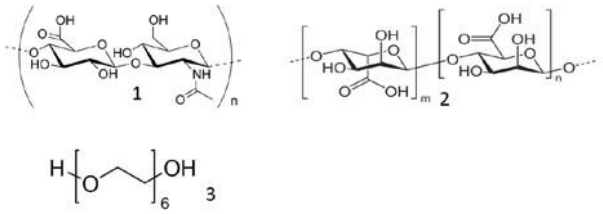

Fig. 1: Structure of the monomers of the studied model hydrogels. 1: Hyaluronic acid; 2: Alginic acid; 3: Hexa(ethylene glycol)

DISCUSSION & CONCLUSIONS: Temperature dependent spectral ellipsometry allows to investigate the hydration state of hydrogels. Though the amount of material in thin film coatings is several orders lower than used for conventional TGA, the sensitivity is high enough to detect the changes in optical properties. For several model surfaces we found different dehydration and decomposition patterns.

ACKNOWLEDGEMENTS: This work is supported by the Cluster of Excellence RESOLV (EXC 1069) funded by the Deutsche Forschungsgemeinschaft and the ONR.

Exploring protein-nanoparticle interactions in reconstituted artificial coronas by combining experiment and computation

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INTRODUCTION: Nanoparticles are novel materials being used increasingly in a range of fields, including biomedicine [1] and with great industrial market potential -estimated at 10^{12} $ (trillion) [2]. When nanoparticles enter a living organism (best example: the human body), they often interact with proteins to form a "corona" (*Fig. 1*), which may change the properties and function of the nanoparticle; it is also hypothesized that formation of the corona is related to toxic effects of the materials. However, exactly which proteins bind to nanoparticles and how this protein-inorganic interaction occurs remain largely unknown, and therefore any prediction of corona formation remains challenging. Consequently, reliable knowledge about nanoparticle behaviour *in vivo* is lacking, thus hindering predictions of their toxicity [1]. Moreover, there are no general rules how to characterize and define nanoparticles. Such lack of standards may lead to legal issues [1]. This project aims to tackle these problems by investigating protein nanoparticle interactions on a molecular level.

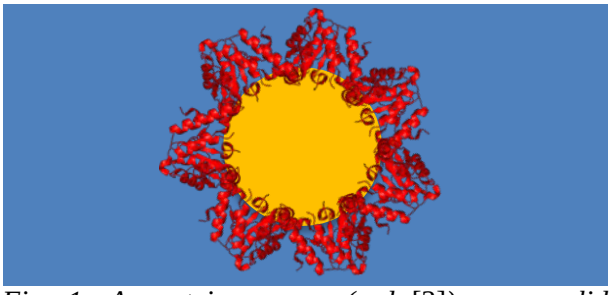

Fig. 1: A protein corona (red [3]*) on a solid nanoparticle (gold). The whole protein-nanoparticle complex is assumed to be in solution.*

METHODS: Here we aim to define the interactions at the interface of a subset of proteins with model standard nanoparticles of defined size and surface chemistry. We plan a re-iterative approach that combines experiment and computation to explore such "reconstituted artificial coronas". Our predictions will be experimentally tested with recombinant protein mutants designed to probe potential interaction sites.

PERSPECTIVES: The detailed exploration of these model bio-inorganic interfaces at the molecular level will help determine how size, material curvature and surface properties affect protein interactions. These studies pave the path towards computational modelling and prediction of corona formation, with the long-term aim of applying such detailed molecular understanding to predictive toxicology and nanomedicine.

REFERENCES: [1] C. Hirsch, M. Roesslein, H.F. Krug et al (2011) *Nanomedicine*, **6**(5):837–847. [2] Nano.gov, FAQs (2016, April 17) Retrieved from http://www.nano.gov/nanotech101/nanotechnology-facts. [3] Protein model (2016, April 17) Retrieved from http://www.rcsb.org/pdb/explore/jmol.do?structureId=1JBK

ACKNOWLEDGEMENTS AND PARTNERS: This PhD Project is sponsored generously by the NanoScreen Consortium, as part of the Competence Centre for Materials Science and Technology (www.ccmx.ch). The Nanoscreen Consortium includes the Eidgenössische Materialprüfungs- und Forschungsanstalt (Empa), École polytechnique fédérale de Lausanne (EPFL), CETICS Healthcare Technologies, Midatech Pharma and the Swiss federal office of public health.

INJECTABLE HYDROGELS FOR INNOVATIVE BIOMEDICAL APPLICATIONS

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INTRODUCTION: Hydrogels have been extensively investigated for biomedical applications and tissue engineering, because of their biocompatibility and the resemblance with living tissues. Since their first introduction in 1960[1], they have been exploited as drug delivery systems, biosensors and scaffolds for cell culture[2]. On top of performing as extracellular matrix and to be able to trap a very high content of water[3], the introduction of addressable groups into the skeleton could allow the generation of "smart" materials. Herein, we report the synthesis and characterization of polyamidoamines-based hydrogels with an equilibrium water content (EWC) up to 96%. *In vivo* and *ex-vivo* properties of these hydrogels were evaluated, in particular as adhesive sealants for gastrointestinal fistulas. The current approach to heal fistulas involves the use of painful and often not resolutive surgery, while our hydrogels can be injected through non-invasive endoscopic procedures and ideally seal the fistula without further complications.

METHODS: Hydrogel synthesis was accomplished via a one-pot Michael-like addition of N,N-methylenebisacrylamide with a diamine as a crosslinker. Amino-group containing moieties can also be inserted into the hydrogel and they give to the material special stimuli-responsive or adhesive properties.
The reagents are mixed in a small vial with 1,5 mL of water and the mixture is shaked for two hours and then left to rest. After several minutes up to few hours, depending on the composition, the hydrogel is formed. Hydrogel formation is tested with the upside down method and rheological measurements are in progress. Biocompatibility has been assessed monitoring for 48h cell viability inside the hydrogel. Ex-vivo test were conducted on porcine stomach and intestine to evaluate adhesion and in-vivo gelation.

RESULTS: Various hydrogels with different morphological, swelling and mechanical properties were obtained by changing the reaction conditions and the relative concentrations of every component of the polymeric network. The presence of amine and carboxylic groups allowed us to tune the swelling properties of the scaffolds according to the variation of pH to modulate.
Swelling test conducted at different pH, showed a maximum up to 96% at pH=5.
Initial injection tests in the submucosa layer of a porcine stomach and in the intestine, have shown that our hydrogels have good adhesion properties and a fast gelation time (less than three minutes).

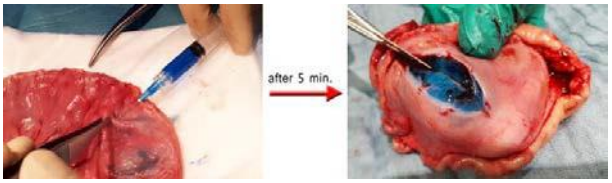

Fig. 1: Injection of methylene blue-stained hydrogel in a porcine stomach (left) and his adhesion after five minutes from the injection (right)

DISCUSSION & CONCLUSIONS: Hydrogel formation time and swelling has been tested at different pH (2, 5, 7.4, 9) to simulate the real conditions inside the stomach and in the different tissues. Interestingly, the hydrogels are liquid and easily injectable while in the sol phase. Initial injection tests, in the submucosa layer of a porcine stomach and in the intestine, have shown that our hydrogels after injection have perfect adhesion properties and a fast gelation time (less than five minutes). Due to the versatility in the synthesis and the large number of precursors we can use a modulation of the adhesion and of the mechanical properties is possible. Also the hydrogels can become degradable and we are now testing the degradation time in different conditions.

ACKNOWLEDGEMENTS: We thank the European Union Horizon 2020 research and innovative programme under the Marie Sklodowska-Curie grant agreement No 642192.

Ex-Vivo Cultivation Of Human Cardiac Biopsy-Derived Stem Cells In Physico-Chemically Defined Nutrient Media

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INTRODUCTION: Physico-chemically defined culture media are fully synthetic nutrient mixtures free of complex additives such as serum or hydrolysates. Furthermore, such media are made of non-animal derived ingredients. The advantage of using physico-chemically defined animal-component free media is the maximum control over cell metabolic functions, and a higher reproducibility in the synthesis of the cell therapy product while avoiding immunogenic risks for the clinical administration. The objective of the present study was to evaluate whether specific recombinant human animal-component free substrates could support human Cardiac Stem Cell (hCSC) adhesion and proliferation in serum free culture conditions.

METHODS: A panel of physico-chemically defined media was evaluated. Commercially-available Essential 6 proprietary medium (Life Technologies) was used as a reference. Unfractionated and MACS-enriched hCSCs were cultivated at sequentially reduced Fetal Bovine Serum concentration (10%, 5%, 3%, 0% FBS i.e. serum free) onto animal-component free recombinant human Vitronectin, Fibronectin, and pharma-grade hyaluronic acid-coated plates. A study on cell seeding density was also performed. Cell adhesion, proliferation and morphology were observed for 4 weeks. Experimental results were evaluated quantitatively by Population Doubling Time (PDT) assay.

RESULTS:

Cells grew successfully in serum free condition in proprietary Essential 6 medium containing a mixture of growth factors. However, in favorable physico-chemically defined media, such as Ham's F12, further reduction of FBS concentration below 5% was not tolerated. Quantitative measurement of PDT for cells grown in physico-chemically defined HAM'S F12 medium at the concentration of 10% and 5% FBS showed comparable values (28 hours ± 1.89). To further reduce animal-derived components in our cultures, we evaluated the use of recombinant human or pharma-grade adhesion substrates. We used the natural composition of the cardiac extracellular matrix as a model system.

Cells did not adhere successfully on Vitronectin (0.5 µg/ cm^2). By contrast, the use of Fibronectin (1 µg/ cm^2) as a coating culture surface allowed us to reduce sequentially to 3% FBS and further to serum-free condition. On a preliminary observation, cell adhesion was slightly lower on pharma-grade Hyaluronic acid (30 µg/ cm^2) compared to Fibronectin substrate. Currently, our cells are cultivated in serum-free condition.

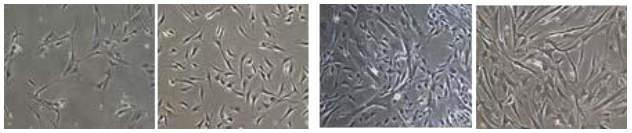

Fig. 1: Effect of Fibronectin on human Cardiac Stem Cell adhesion and morphology in serum-free: Ham's F12 physico-chemically defined medium, uncoated and coated wells (left) vs. Essential 6™ proprietary medium, uncoated and coated wells (right).

DISCUSSION & CONCLUSIONS:

Our experimental design included the use of animal-component free recombinant human, and pharma-grade adhesion substrates. The data that we collected so far support the hypothesis that hCSCs can be cultivated *in vitro* in physico-chemically defined nutrient media in serum-free culture condition by preserving their safety profile for future therapeutic use.

ACKNOWLEDGEMENTS: The authors acknowledge Cardiocentro Ticino Foundation and the Foundation for Cardiological Research and education (FCRE) for funding this project.

Advanced bioengineering approach for promoting nerve regeneration

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INTRODUCTION: Peripheral nerve reconstruction is critical and challenging due to lack of effective ways to create a complex, multifactorial and dynamic microenvironment required for axonal path finding[l]. Here, we report on bioengineered nerve conduits (NCs) endowed with important biological functions, namely structural, cellular and molecular cues for supporting complex requirements of axonal path finding and target re-innervation.

METHODS: The required materials were developed and thoroughly characterized *in vitro* prior to animal testing [2]. Rat Schwann cells were isolated, purified, and desired phenotype S100+ was confirmed by immunostaining. Efficient (99% transduction efficiency), tropism modified, recombinant adenovirus was generated using homologous genetic recombination process for mono or bicistronic expression of GDNF and NGF (Ad5-CMV-GDNF-IRES-NGF/F-RGD. Purified virus was used for genetic modification of Schwann cells (GMSCs), which were further analysed *in vitro* and *in vivo* for viability and transgene expression. Nanostructured NCs were made of collagen (CNC) or silk fibroin (SFNC) using a newly developed layer-by-layer fabrication method and enriched by using unmodified SCs or GMSCs, or GDNF and NGF proteins. Thus, the developed NCs encompassed 8 different compositions. Adult rats (Sprague Dawley, 250-300g) were randomly allocated into 9 groups (6 and 12 weeks; n=12 per group) for treating 10 mm sciatic nerve gap by using the variants of bioengineered NCs. Animal response for nerve regeneration was evaluated by measuring the anatomical, behavioural and electrophysiological recovery. For examining the potential induction of an immune response against the recombinant adenovirus, serum samples were collected and prepared for the virus neutralization assay.

RESULTS: Resulting recombinant virus, within GMSCs, proved to be safe and efficacious both *in vitro* and *in vivo* in nerve crush injury model. SEM analysis of nanostructured collagen NC (CNC) and silk fibroin NC (SFNC) confirmed the alignment and stable adherence of nanofibers on the luminal surface. Further enrichment of NCs with biological functions such as SCs, GMSCs, and GDNF/NGF proteins produced 9 different NC types varying in cellular and trophic support. All biofunctionalized NCs augmented significantly axonal outgrowth of sensory or/and motor neurons *in vitro*, and determined differentially the extent of axonal elongation as well as growth direction in comparison with non-biofunctionalized NCs.

The anatomical, behavioural and electrophysiological outcomes differed significantly between the treatment groups. The virus neutralization assay indicated absence of undesired immune response against the recombinant adenovirus used for genetic modification of the Schwann cells. Importantly, bioengineered collagen NCs carrying most important biological functions, in contrast to silk fibroin NCs, showed potential to overcome the present hurdles of nerve regeneration and matched well with autograft performance.

DISCUSSION & CONCLUSIONS: This study not only revealed the importance of new bioengineering approach for creating dynamic and effective microenvironment for axonal path finding, but also demonstrated the impact of biomaterial composition (advanced therapeutic medicinal product for complex tissue organization) in mounting effective nerve tissue regeneration.

ACKNOWLEDGEMENTS: We would like to acknowledge the enabling financial support by SNSF and CTI.

Biomimetic and adhesive alginate sulfate hydrogels provide a chondrogenic and anti-inflammatory microenvironment for articular chondrocytes

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INTRODUCTION: Damage of articular cartilage from aging or trauma is often coupled with inflammation that further accelerates cartilage matrix degradation. Autologous cell-based therapies for cartilage repair require implantation of a high number of chondrocytes which demands their 2D expansion and causes loss of phenotype. Therefore, scaffolds for cartilage engineering should both provide biological cues that induce cartilage matrix production and act as a protective microenvironment against the inflammatory effects from the defect site. Here, we show that biomimetic alginate sulfate hydrogels induce proliferation and preservation of native phenotype of primary chondrocytes and suppress IL-1β-induced inflammation. Furthermore, for clinical translation of the approach, we introduced tyramine modification on alginate sulfate to enable enzymatic crosslinking of the material as well as adhesion to the cartilage tissue.

METHODS: Sulfation of alginate was carried out as previously described[1]. Human or bovine articular chondrocytes were isolated and encapsulated in alginate (Alg), alginate sulfate (S-Alg, ds: 0.32 sulfates/monomer) or mixtures of alginate/alginate sulfate (ds: 0.9) hydrogels and cultured up to 3 weeks. For inflammatory induction, the cells were serum starved overnight and treated with 1 ng/ml IL-1β for 24h or 48h. The gels were collected at given time points and assessed for cell viability and morphology, gene and protein expression and immunohistochemistry. The polymers were modified with tyramine groups using DMTMM as previously reported[2]. For the crosslinking, alginate sulfate tyramine (ASTA) was mixed with tyrosinase (20 kU/ml) and pipetted in cartilage explant rings. For assessment of adhesiveness, push-out tests were performed.

RESULTS: Alginate sulfate hydrogels promote proliferation of primary chondrocytes in a dose-responsive manner to the degree of sulfation. 3D growth of chondrocytes in alginate sulfate hydrogels lead to great amounts of cartilage-specific matrix formation after 3 weeks of culture (Fig. 1). Moreover, expression of de-differentiation markers such as collagen 1 is suppressed in alginate sulfate hydrogels compared to unmodified alginate via activation FGF signaling.

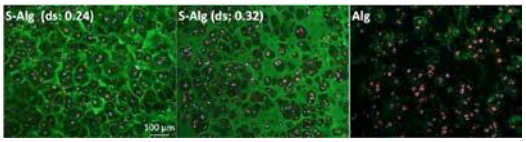

Fig. 1: Immunostaining images showing Collagen2 (green), actin cytoskeleton (red), nuclei (blue).

Sulfated alginate hydrogels prevent IL-1β-induced expression and activation of inflammatory markers (COX-2 and NF-κB) compared to unmodified alginate via suppressing an upstream activator (p38-MAPK) (Fig. 2).

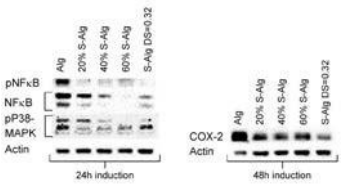

Fig. 2: Western blots showing the expression of inflammatory markers in IL-1β induced cells.

Tyramine modification of alginate sulfate is achieved with tunable degree of substitution. In the push-out tests, enzymatically crosslinked ASTA hydrogels show stronger binding and adhesiveness to native cartilage compared to ionically crosslinked gels.

DISCUSSION & CONCLUSIONS: Here we show that alginate sulfate hydrogels promote the growth and cartilage matrix production of primary chondrocytes and prevent expression of IL-1β-induced inflammatory markers. We further demonstrate tyramine-modified ASTA hydrogels with enzymatic crosslinking and adhesive properties to native cartilage. On-going studies investigate the chondrogenic re-differentiation of human chondrocytes within ASTA hydrogels and the stability of ASTA hydrogels and cartilage matrix formation *in vivo* with subcutaneous implantation in mice.

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Sulfated hyaluronan alters fibronectin matrix assembly and promotes osteogenic lineage commitment of human bone marrow stromal cells

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INTRODUCTION: In the bone marrow niche, human bone marrow stromal cells (hBMSC) are surrounded by an extracellular matrix (ECM) rich in fibronectin (FN) and sulfated glycosaminoglycans (GAG); whereas at the site of bone formation the osteoblasts are surrounded by a mineralized collagen matrix. Synthetically sulfated hyaluronan derivatives were seen to support osteogenic lineage commitment of hBMSC [1].
FN is secreted by hBMSC, assembles into fibrils and interconnects ECM components and cells with each other. FN has three binding regions for the natural sGAG heparin (Hep) which controls growth factor binding to FN.
In this study we describe the interaction of the synthetically sulfated hyaluronan derivative sHA1 with FN and its impact on FN matrix assembly and osteogenic differentiation of hBMSC. Hep and hyaluronan (HA) were used as controls [1].

METHODS: hBMSC were cultured in DMEM with 10% FCS supplemented with sHA1 (42,000 g/mol, sulfation degree 1.3), low molecular weight HA (84,000 g/mol) or Hep (Sigma-Aldrich, sulfation degree 2.0, 18,000 g/mol) (200 µg/mL each). Side-on modification with Atto565-NH_2 was used for fluorescent labeling of HA and sHA1. Immunofluorescence staining [1], FRET-analysis [2] and DOC (desoxycholate)-solubility assay [3] were performed to characterize hBMSC-derived FN matrix. The impact of GAG derivatives on osteogenesis was evaluated by determining the activity of tissue non-specific alkaline phosphatase (TNAP) [1].

RESULTS: Immunofluorescence staining indicated a colocalization of Atto565-sHA1 with hBMSC-derived FN fibrils similar to FITC-Hep. The electrostatics driven GAG interaction with FN was dependent on sulfation as ATTO565-HA did not show any colocalization with FN. FRET-analysis gave evidence that sHA1-induced FN stretching was similar to Hep-induced effects [2] whereas HA did not alter FN fibril conformation.
Beside close similarities of synthetic sHA1 and natural Hep concerning their interaction with FN, they differentially affected hBMSC. sHA1 induced *fn* gene expression and FN formation which mostly appeared in detergent soluble protein fraction (Fig. 1) indicating the presence of less assembled FN fibrils.

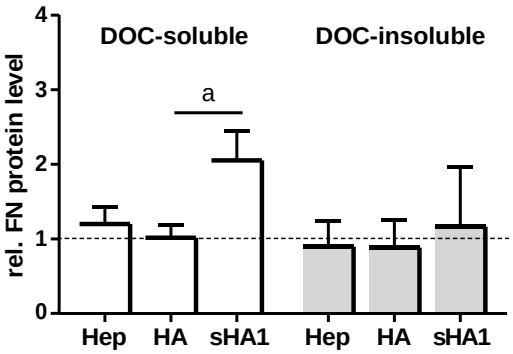

Fig. 1: Impact of GAG derivatives on FN matrix assembly status in hBMSC at day 8.

sHA1 significantly increased TNAP activity in hBMSC by about 3.5-fold while HA and Hep did not.

DISCUSSION & CONCLUSIONS: The present study shows one putative mechanism how sHA1 affects ECM assembly and remodeling. The interaction of sHA1 with FN led to altered composition and stability of the ECM. This assumption is also supported by the reported effects on matrixmetalloproteinases and their inhibitors [4] and might be partial reasonable for the pro-osteogenic effect of sHA1.

ACKNOWLEDGEMENTS: The study was supported by DFG TRR67, projects B1 and A3.

Ag nanoencapsulation for antimicrobial implant coatings

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INTRODUCTION: Medical progress and an ageing world population have led to an increasing use of foreign materials inside the human body. Consequently also the number of infections related to these implants has grown significantly. Antimicrobial coatings that prevent the formation of infectious biofilms on the surface of the implants could make an important contribution to overcome that issue. Silver is known for its good antimicrobial and biocompatible properties and could therefore play an important role in the fight against implant infections, especially if they are caused by antibiotic resistant bacteria.[1]

This project investigates $Ag@SiO_2$ nanorattles as antimicrobial agent for implant coatings. These nanoparticles are characterized by a void between a silica shell and a Ag nanoparticle as cargo.[2] The shell protects the Ag cores from aggregation and prolongs the release of the antimicrobial active Ag^+ ions. Moreover it provides reactive sites to functionalize the nanocontainers in order to attach them covalently to implant surfaces or to incorporate them into polymer materials.

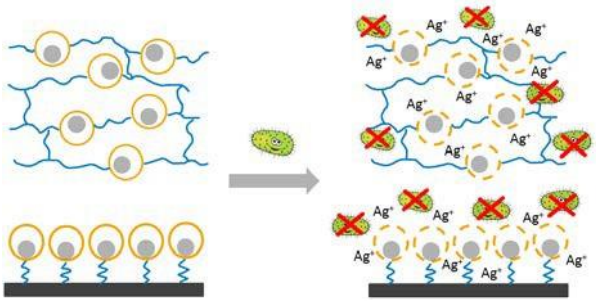

Fig. 1: Antimicrobial Ag^+ release from $Ag@SiO_2$ nanorattle containing coatings or polymers.

METHODS: $Ag@SiO_2$ nanorattles, 25 nm: A reverse microemulsion was used to coat Ag nanoparticles that were formed by reduction of $AgNO_3$ with hydrazine monohydrate with a silica shell co-condensed from TEOS and APTES. During the purification process the inner part of the shell was removed by washing with hot water.[3]

$Ag@SiO_2$ nanorattles, 80 nm: Ag nanoparticles synthesized by a variation of the polyol process were coated with a silica shell by a modified Stöber method. The inner part of the silica shell was removed in a surface protected etching step by refluxing in presence of polyvinylpyrrolidone.

RESULTS: We have developed two different synthetic routes to yield $Ag@SiO_2$ nanorattles of different sizes. The microemulsion method[3] gives access to nanorattles with a diameter of about 25 nm (Figure 1, top) that were evaluated for their Ag^+ release properties, antimicrobial potential and for their impact on cells of the immune system (in collaboration with C. Bourquin, Fribourg).[4]

The second synthetic approach based on a modified Stöber method results in very homogenously filled $Ag@SiO_2$ nanorattles of about 80 nm (Figure 1, bottom). Cytotoxicity tests showed a good biocompatibility determining a nontoxic concentration of ≤0.113 mg/ml. Their antimicrobial efficiency is currently under investigation. First tests against *E.coli* suggest a therapeutic window from 0.003 mg/ml.

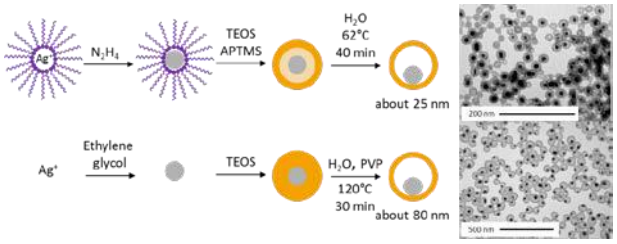

Fig. 2: Synthesis and TEM images of $Ag@SiO_2$ nanorattles: microemulsion method (top) and Stöber conditions followed by surface protected etching (bottom).

DISCUSSION & CONCLUSIONS: Both type of silver-containing silica nanorattles thus fulfill several requirements for the development of novel antibacterial nanocoatings on biomaterial surfaces. Further investigations will compare their Ag^+ release kinetics and develop strategies to link them to implant model surfaces of titanium, gold or polymers.

ACKNOWLEDGEMENTS: The authors would like to thank the University of Fribourg and the SNSF for the generous funding of the project. It is part of the National Center of Competence in Research "Bio-inspired Materials".

MOBILITY DEPENDENT CELLULAR BEHAVIOUR ON SUPPORTED LIPID BILAYERS

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INTRODUCTION: The physical properties of biomaterials (e.g. stiffness & topography) are of great importance to the field of biomedical engineering[1] and have a profound effect on cellular behaviour, including differentiation. This behaviour can also be affected by the mobility of a surface[2]. Supported lipid bilayers (SLBs) provide an opportunity to study the effect of this property in more depth. Easily produced and readily functionalised, the fluidity, and thus the mobility (defined by the diffusion coefficient, *D*), can be tuned by lipid composition, due to their phase transition temperature, T_m, which defines the point at which they transform from a gel to a fluid state. In this work we have used lipids with T_m above and below cell culture temperature, functionalised with the cell binding peptide RGD, to study how cells respond to different mobility.

METHODS: Small unilamellar vesicles (diameter <90 nm), containing 2% biotinylated lipid (b-cap-PE) were formed from DOPC (T_m = -19°C), with up to 40% cholesterol or DPPC (T_m = 41°C) and incubated, above their T_m, on glass surfaces cleaned with RCA solution and activated with oxygen plasma. These were then functionalised with neutravidin (NA) and biotin-RGD. Characterisation was performed via AFM and QCM. *D* was measured via FCS upon the inclusion of 0.01% - 0.5% fluorescent lipid (TopFluor-PE). C2C12 mouse myoblast cells were used to determine cellular behaviour, with morphology and adhesion measured. Results were compared to an immobile RGD-Glass control, produced by linking RGD to a silanised glass surface, via NA and a NHS-biotin crosslinker.

RESULTS: The presence of a single bilayer on a glass surface was confirmed via AFM in both imaging and force mapping modes. FCS resulted in a *D* of 3.64, 1.76 and 0.13 $\mu m^2/s$ for DOPC, DOPC-CHOL and DPPC respectively. We have observed that the changing of mobility has a statistically significant effect on the morphological characteristics of the cells, with both area and perimeter increasing as mobility of the surface reduces. Furthermore, cells were qualitatively observed to have a more organised cytoskeleton and different focal adhesion properties on less mobile surfaces.

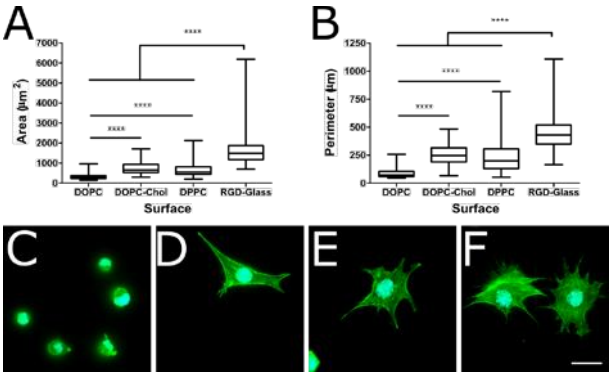

Fig. 1: Mobility dependent morphology. (A) & (B) show area and perimeter of cells respectively. (C), (D), (E)& (F) are representative images of DOPC, DOPC-CHOL, DPPC & RGD-Glass respectively. (Scale Bar = 25μm).

DISCUSSION & CONCLUSIONS: We believe that the detection of mobility in SLBs is linked to the ability of cells to exert force on a surface. The lack of cytoskeletal organisation and the rounded shape on the more mobile substrate imply that cells are not able to exert the strong contractile forces associated with the formation of well-defined stress fibres on the immobile control. Indeed, reducing ligand mobility by tuning the lipid composition leads to an increase in cell spreading and cytoskeletal organisation. These findings point to a critical role of mobility in the regulation of cellular mechanics and, eventually, cell fate through a mechanotransductive cascade. Hence, we believe this system may be used to explore the potential of surface mobility as a tool to control cell behaviour and tune their differentiation.

ACKNOWLEDGEMENTS: Funding support from the EPSRC and the ERC is acknowledged.

Determination of normal and shear residual stresses in plasma-sprayed hydroxyapatite coatings by X-ray $\sin^2\psi$-Method

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INTRODUCTION: Because of the similarity of chemical composition of Hydroxyapatite (HA) to the bone, it has been widely used on the metallic implants to provide long-term fixation of the implant to bone [1]. The residual stress acts a critical role on the durability of the implants. Therefore, investigation of the residual stress levels present within the coating would be useful in order to improve fatigue resistance and long term stability of the HA coating. In this study, an X-ray diffraction technique based on the X-ray $\sin^2\psi$-method was used to estimate the residual stress in the plasma sprayed HA coatings fabricated in vacuum and atmosphere. In this method, the lattice strain as a function of $\sin^2\psi$ will be calculated and then the normal/shear residual stresses will be directly determined from the measured data.

METHODS: X-ray diffraction is considered as a reliable method for studying of residual stress within HA coating [2]. This Method is able to measure the residual stress at different depth in the material. The selected diffraction peak should be a high angle with high diffraction intensity, i.e. not significantly overlapping with diffractions of the substrate material. In the calculation of residual stresses in this examination, the (522) plane was used for the stress measurements of the HA coating. The diffraction intensity of this peak is high enough for the stress measurement. The detailed diffraction conditions used for the stress investigations are listed in Table 1.

Table 1. X-ray diffraction conditions

Diffraction plane, hkl	**522**
Equipment	Bruker D8 Advance
Diffraction angle, deg	139.3
Characteristic X-ray	Cr-Kα
Kβ-Filter	Vanadium
Tube voltage, kV	30
Tube current, mA	50

RESULTS: The stress obtained directly from the slope of data taken at various ψ-angles. A biaxial stress state was assumed by fitting the peak profile with the Pearson VII function [3]:

$$I(x) = a_0/[1+4((x-a_1)/a_2)^2(2^{a3}-1)]^{a3} \quad (1)$$

Where a_0 = amplitude (I_0), a_1 = center (2θ), a_2 = width1 and a_3 = width 2 are.

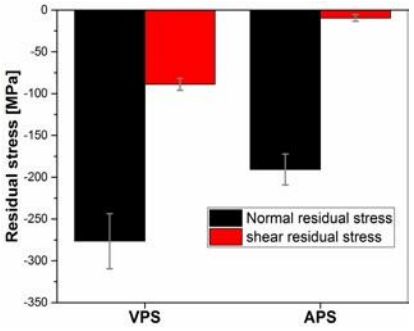

Fig. 1: Normal and shear residual stresses of HA plasma sprayed coating fabricated in vacuum (VPS) and atmosphere (APS).

DISCUSSION & CONCLUSIONS: This study focused on the estimation of the thermal residual stresses in the HA coatings fabricated in vacuum and atmosphere by means of X-ray $\sin^2\psi$-Method. This allows a direct comparison between the deposited coatings at different mediums. The residual stresses measured in the HA coating of both samples were in a compressive mode, but the value of compressive normal and shear residual stresses in the coating fabricated in vacuum were significantly higher relative to the atmospheric coating. Therefore, the VPS coating with higher residual stress are more failure resistant than the APS coating.

ACKNOWLEDGEMENTS: This work is part of the research program "Kooperatives Promotionskolleg Generierungsmechanismen von Mikrostrukturen (GenMik)", which is financed by the state of Ministerium für Wissenschaft, Forschung und Kunst Baden-Württemberg.

Adipose-derived stem cell seeded biominerizable nanocomposite for chest wall repair: suppression of inflammatory response in a murine model

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INTRODUCTION: Defects to the chest wall can occur after tumour resections or trauma caused by accidents, and appropriate chest wall reconstruction is therefore needed. Stability and integrity of the repaired chest wall should reach similarity to natural physiology. Addressing the treatment of critical size full-thickness chest wall defects, the ideal graft should be stable, fluid- and air-tight, biocompatible inducing no inflammatory reactions, biodegradable during the healing with non- toxic degradation products as well as rapidly integrating into the surrounding tissue. Here, we present the implantation of a biocompatible, biodegradable and easily vascularizable nanocomposite seeded with adipose-derived stem cells (ASCs) as a chest wall graft in a murine model. The cellular response towards this graft is compared to the cell-free graft.

METHODS: An electrospun poly(lactic-co-glycolide)/amorphous calcium phosphate (PLGA/aCaP) nanocomposite was seeded on both sides with murine ASCs and cultivated for two weeks before implantation as a chest wall graft. In addition, a cell-free analogous PLGA/aCaP scaffold was implanted on top of the cell-seeded scaffold towards the skin in order to be able to study not only direct cell-to-cell contact-based effects, but also to address paracrine effects caused by ASCs (control: cell-free scaffold alone). Histomorphometric analysis was performed at 4 and 8 weeks post-operation, respectively, to assess cell density of macrophages, lymphocytes and foreign body giant cells (Figure 1).

RESULTS: Inflammatory response towards the graft material was significantly reduced for macrophages, lymphocytes and foreign body giant cells in the presence of ASCs compared to cell-free scaffolds. Moreover, this anti-inflammatory action caused by ASCs was not only found on the side where direct cell-to-cell contact between seeded ASCs and local cell population was enabled and studied, but also on the scaffold side where predominantly diffusible factors secreted by ASCs were active (paracrine function).

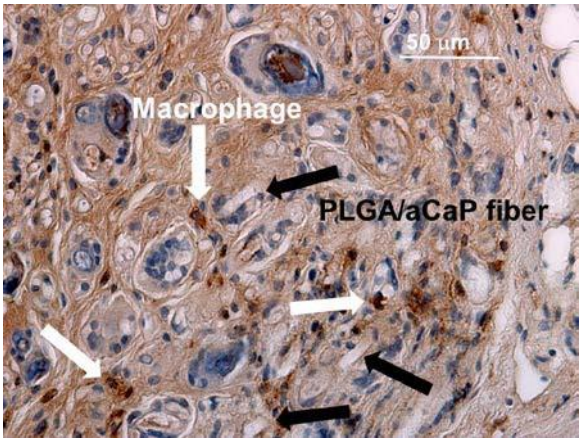

Fig. 1: F4/80 stained histological section at 8 weeks post-operation showing selected macrophages (white arrows) and electrospun PLGA/aCaP fibers of the degrading chest wall graft.

DISCUSSION & CONCLUSIONS: In clinics, the state of the art of repairing critical size chest wall defects is to use inert materials such as Goretex® which are not easily vascularizable and not biodegradable. Here, we present a biocompatible, biodegradable and well vascularizable nanocomposite for chest wall repair. In order to enhance integration of this graft material and accelerate wound healing, ASCs were seeded. A beneficial effect of these ASCs was that the inflammatory response towards the implant was significantly reduced. Therefore, such cell-seeded nanocomposites may be applied as chest wall grafts in clinics in the future.

ACKNOWLEDGEMENTS: We thank Gabriella Meier Bürgisser for her help with analysis of histological sections and Pia Fuchs for histological staining.

Bioactive, elastic and biodegradable double – layered emulsion electrospun DegraPol tube delivering PDGF-BB for tendon rupture repair: *in vitro* and *in vivo* assessment

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INTRODUCTION: Healing of tendon ruptures represents a major challenge in musculoskeletal injuries. Approaches of repair by conventional suture may lead to re-rupture. A new, highly elastic polyester urethane (DegraPol® (DP))[1,2] was explored as a delivery device for platelet derived growth factor – BB (PDGF-BB) to promote tendon healing.[3] Morphological, mechanical and delivery properties of the bioactive DP scaffolds were studied. Assessment of double-layered emulsion electrospun DP was performed *in vivo*, in a rabbit Achilles tendon rupture model.

METHODS: Water-in-oil emulsions were produced by dropwise addition of aqueous phase with biomolecules to the polymer solution, stirred, ultrasonicated and electrospun using in-house built electrospinning device. Changes in fiber morphology and diameter as a function of different electrospinning parameters were determined from SEM images. Mechanical properties of bioactive DP scaffolds were determined from stress/strain curves and compared to pure DP scaffolds. The delivery of rhPDGF-BB was analysed by ELISA. Bioactivity of the released PDGF was tested on rabbit tenocytes *in vitro* by analysing cell proliferation (EdU assay). Performance of bioactive scaffolds in a form of double-layered tube, with inner layer delivering PDGF-BB at the site of tendon rupture (Figure 1A) was assessed in an *in vivo* rabbit model where biomechanical and histomorphological analysis of the regenerating tendons was performed 3 weeks post – surgery. t – test and one-way ANOVA were used to test differences between groups (*$p < 0.05$, **$p < 0.01$, *** $p < 0.001$).

RESULTS: Emulsion electrospinning resulted in significantly smaller fiber diameters, compared to single electrospinning. PDGF-BB was released in a sustained manner within a period of 30 days. No differences were observed in Young's modulus, but bioactive scaffolds had decreased strain at break [%], however still high enough for proper surgeon handling. Tenocyte proliferation was higher on them and significantly different from pure DP scaffolds (Figure 1B)[3]. *In vivo* results revealed the positive impact of PDGF-BB by significant higher failure stress (MPa) and stiffness (N/mm) of the regenerated tendons, when compared to conditions with only pure DP electrospun tubes.

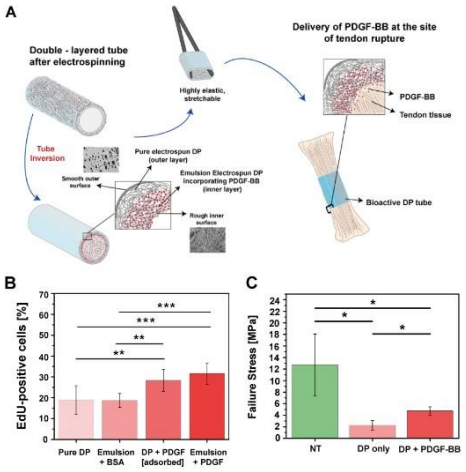

Fig. 1: A) Scheme of double-layered DP tube. B) B) Bioactivity of incorporated PDGF-BB in DP scaffolds. C) Failure stress (MPa) of tendons 3 – weeks post-surgery.

DISCUSSION & CONCLUSIONS: The data suggests that emulsion electrospun DP tubes deliver bioactive PDGF-BB, thus increasing tenocyte proliferation and leading to enhanced biomechanical properties of regenerating tendons *in vivo* 3 weeks post-surgery, presenting a promising application in aiding tendon regeneration after rupture.

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Interface engineering in colloids and composite materials by specific interactions of biocombinatorially selected peptide-polymer conjugates

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INTRODUCTION: A bioinspired strategy for bone tissue engineering uses highly porous, biodegradable polymer scaffolds prepared via additive manufacturing or 3D printing techniques. But pure polymers are mechanically too weak to be used in load-bearing bone replacement therapies.[1] Hence, composites with enhanced mechanical properties are needed. To overcome limitations of common composites the internal material interfaces are engineered to enhance the interactions between the inorganic filler particles and the organic matrix polymer.[2]

METHODS: Sequence-specific peptide sequences were selected via biocombinatorial phage display. Subsequently, peptide-PEG conjugates with high affinity for the filler particles were prepared by automated SPPS and used for nanoparticle stabilization in colloidal dispersion and interface engineering in composite materials. For this compatibilization approach composites made from polycaprolactone (PCL) and magnesium fluoride (MgF_2) particles were chosen. The adhesion and stabilization efficiency of the conjugate in solution were proved via SEM, TEM, DLS and ^{19}F-NMR. The composites were printed by bioextrusion (fused deposition modeling, FDM) into filamentous scaffolds with fully interconnected pores. The influence of the interface modifications on 3D processability, mechanical properties (tensile, compression and nanoindentation), degradation rates, ion release and biocompatibility in vitro was investigated. Mechanical approaches combined. Cell proliferation and ALP activity was tested on the 3D scaffolds.

RESULTS: Peptide-PEG conjugates showed excellent stabilization properties for MgF_2 sol nanoparticles. The stabilized nanoparticles were redispersible even after complete removal of solvent. The compatibilized composites with engineered interfaces showed enhanced processability in different 3D printing techniques and enhanced mechanical properties in tensile, compression and indentation setups. Degradation rates and ion release was raised with higher filler amounts.

DISCUSSION & CONCLUSIONS: The mechanical reinforcement seems to be a result of the increased interactions of filler particles in the PCL matrix, since the change of crystallinity could be excluded as the main reason. Remarkably, not only the elastic moduli were increased but the tensile toughness was raised at the same time. The compatibilization of interfaces in composite led to strengthened materials which proved to be biocompatible as well. The degradation and ion release profiles can be adjusted to respective applications by choosing the amounts of compatibilized particles. Our interface engineering approach provides a generic way to modulate mechanical properties of composites by increasing the interactions between components.

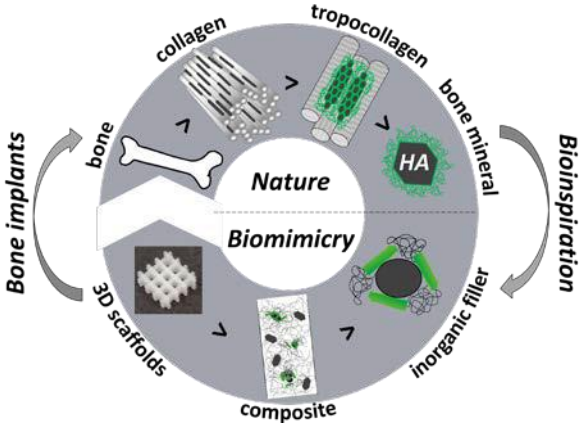

Fig. 1: Bioinspired concept of interface engineering in polymer-particle composites for 3D printed bone replacement materials: surface-specific peptide-polymer conjugates mimic the non-collagenous proteins in bone's hierarchical build-up and enhance interactions between filler particles and matrix polymer.

ACKNOWLEDGEMENTS: Financial support was granted by HU Berlin and the ERC under the European Union's 7th Framework Program (FP07-13)/ERC Starting grant "Specifically Interacting Polymer−SIP" (ERC 305064).

Molecular interactions in crystal packing of dipeptide gels

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INTRODUCTION: In general peptide hydrogels are a promising class of soft biomaterials for cell culture, regenerative medicine, or drug delivery applications having advantages in biocompatibility, biodegradability and injectability[1,2]. So far, many different longer peptide hydrogel systems like Max1 and P_{11}-2[2] are well studied but the literature about dipeptide hydrogels is limited. Dipeptide gels have certain advantages over longer peptide gels being less cost intensive, more versatile and easier to synthesize in high quantities. The most commonly studied one is the Fmoc-Phe-Phe dipeptide[3]. The major driving force of the self-assembly of such peptides is proposed to be π-π stacking. Other forces known to play a role are hydrophobic interactions, ionic interactions, hydrogen bonding and electrostatic interactions[4]. Nevertheless, a better understanding of the self-assembly process would allow a more rational design for specific applications.
In our work, we found out that a dipeptide based on two β-alanine groups ($Boc(\beta ala)_2N_2H_3$), hence excluding π-π stacking, was able to form a gel-like state. In order to explore this phenomenon, we started to intensively characterize this system.

METHODS: To obtain the dipeptide $Boc(\beta ala)_2N_2H_3$ a standard liquid phase synthesis was used (Fig.1). ^{1}H-NMR, as well as a melting point analysis was performed to verify the purity of the product. Gel formation protocols were developed with the solvents ethyl acetate, acetonitrile and dioxane. The obtained gels were visually analysed by SEM and AFM. Their structure was analysed via XRD and SAXS.

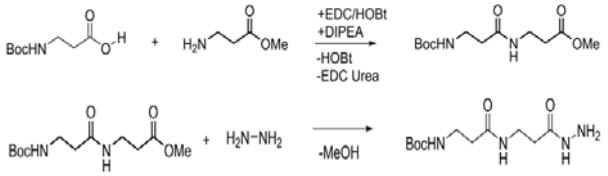

Fig. 1: Synthesis scheme of the dipeptide $Boc(\beta ala)_2N_2H_3$

RESULTS: The obtained yield of three dipeptide batches is 44 ± 9 %. In addition, the ^{1}H-NMR (400 MHz, DMSO-D_6) measurement gave the following values: δ = 8.98 (s, 1H, NH), δ = 7.86 - 7.85 (t, 1H, NH), δ = 6.7 (s, 1H, NH), δ = 4.1 (s, 2H, NH_2), 3.24 – 3.19 (q, 2H, βCH_2), δ = 3.12-3.07 (q, 2H, βCH_2), 2.2 – 2.15 (q, 2H, αCH_2), 1.36 (s, 9H, Boc). Furthermore, the average melting point of three dipeptide batches (Büchi melting point B-540) is 176.7 ± 0.4 °C.
With the three solvents ethyl acetate, acetonitrile and dioxane a gel could be formed by heating and sonication. The results with SEM (Fig.2) and AFM show that the gels contain long fibers. Both SAXS and XRD (Fig.2) show that the structures are crystalline.

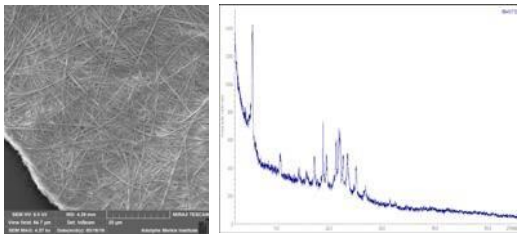

Fig. 2: SEM picture of a dried dipeptide gel and its XRD diffractogram (with ethyl acetate)

DISCUSSION & CONCLUSIONS: First of all, the results show that the obtained dipeptide is pure. Furthermore, with different solvents a gel-like structure was observed. Those structures are composed of crystalline fibers as the SEM and XRD data shows.
To understand the system in more detail rheometric - as well as thermal analysis are ongoing. Furthermore, we aim to solve the solid state structure of the dipeptide either by XRD or by x-ray single crystal diffraction. With that information at hand, we will understand the packing of the molecules and we will be able to perform modelling regarding their self-assembly process. In addition, slight structural modifications in the dipeptide are planned to specifically target the different driving forces of assembly.

ACKNOWLEDGEMENTS: The authors thank the Swiss National Science Foundation for generous support, as well as Anne Schuwey and Dr. Veronique Trappe for help in the synthesis and analysis of the dipeptide.

ACTIVE LONG-TERM IMPLANTABLE MICRO-PACKAGES

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CSEM SA, Switzerland.

INTRODUCTION: Miniaturization of Active implantable medical devices (AIMDs) for sensing and actuation, shows a lot of potential for improvement of quality of life of many chronically ill patients. Packaging is one of the main constraint for the miniaturization of AIMDs. The packaging technologies currently used for AIMDs, are bulky and has not changed for decades. Miniaturization and new features like Micro electro Mechanical systems integration, transparency for RF and optical signal transmission are required for enabling new medical applications. Here we are presenting a novel long-term implantable, miniature, RF and optically transparent AIMDs packaging technology.

MATERIALS AND METHODS: A package (Figure 1) was developed consisting of a bottom part ('substrate'), manufactured using a long-term biocompatible material (sapphire or alumina), with a cavity of size, big enough to integrate an electronic chip inside. The cavity is formed using micromachining techniques with sizes ranging from 500 µm to 4 mm square depending on the size of electronics to be integrated. Hermetically sealed feedthroughs are formed on the substrate, made using a biocompatible metal, which enables transfer of electrical signals in to and out of the package. The package includes a top part ('lid'), which is also manufactured using a long term biocompatible material (sapphire). The substrate and lid are manufactured in a batch (Figure 1) with hundreds of parts manufactured at the same time to enable low cost production.

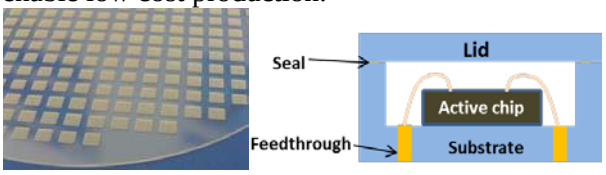

Figure 1: Micro-machined sapphire substrate with cavities (left); Package concept (right)

A post processing using lithography techniques and biocompatible thin film deposition are done on the substrate to enable hermetic sealing. The lid andsubstrate are sealed together after integration of the electronic chip in the cavity forming the package. The sealing is done using a proprietary laser based sealing method at a very low sealing temperature, so that the electronics components encapsulated endure no change in functionality.

Long term implantable pressure sensor[1] (MEMS based) and laser chip[2] (Vertical cavity surface emitting laser) were demonstrated using this packaging technique. The pressure sensor uses a membrane as thin as 25 µm as the lid and the laser package uses an optically transparent lid as shown in Figure 2. The substrate and the lid used are RF transparent enabling wireless communication.

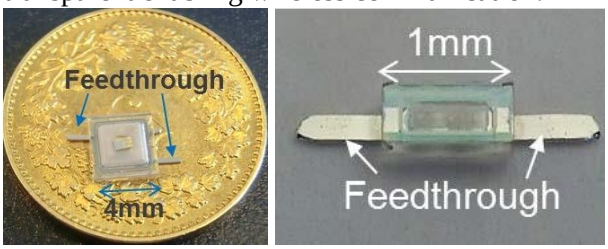

Figure 2: Implantable Pressure sensor (left); laser package (right) made of sapphire

RESULTS: The temperature inside the cavity, 200 µm away from the seal was measured to be less than 100 °C. The strength of the package was tested using shear testing method and an average shear strength of 110 MPa (3.5 Kg for the 500 µm square package) was measured. The hermeticity of the package was tested using helium spraying method and FTIR (Fourier transform Infrared spectroscopy, non-destructive). A hermeticity of less than 10^{-12} mbar*l/s (limit of the method) was measured with a yield of 90 %. Hot Saline immersion tests were done placing the samples in physiological saline at 95 °C, for 1100 hours. The hermeticity of samples were checked before and after the tests and there was no change measured.

DISCUSSION & CONCLUSIONS: Long-term implantable micro-packages was developed and tested for strength, hermeticity of the seal, temperature during sealing and hot saline immersion. The package was found to stable with a high yield. A non-destructive hermeticity testing based on FTIR was development for the package which enables testing of each and every package manufactured.

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BSP-coating of bone substitute materials and their influence on bone defect healing

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INTRODUCTION: Bone grafts are still the gold standard in the treatment of critical-size bone defects, but the availability of suitable and large size bone grafts is one major problem. Biomaterials, with osteoconductive and osteoinductive properties, are one approach solving this issue.[1] Nevertheless, the ideal biomaterial still has to be found. In this project we combine calcium phosphate cement (CPC) bone substitutes with Bone sialoprotein (BSP) and analyzed its osteoinductive properties. This protein, a major component of the extracellular bone matrix, is involved in the formation and remodeling of bone.[2] Therefore we hypothesize through BSP-coating a positive influence on bone healing.

METHODS: Pure CPC scaffolds were characterized through scanning electron microscopy (SEM). Bone substitutes were coated with BSP by physisorption. Subsequently, primary human osteoblasts (hOBs) were seeded on the scaffolds. Cell proliferation was analyzed by alamarBlue® assay and cell differentiation using real-time PCR. *In vivo* the effect on bone healing of BSP-coated bone substitutes was examined in a murine criticial-size calvarial bone defect model and evaluated employing µCt. Pure bone substitutes were used as controls both in the *in vitro* and in the *in vivo* experiments.

RESULTS: SEM images depict us the microstructure of the CPC scaffolds (Fig. 1). hOBs seeded on BSP-coated bone substitutes demonstrate an enhanced cell proliferation compared to the uncoated substitutes. Gene expression analyses show an increase of bone marker genes (Runx2, SP7) from hOBs seeded onto BSP-coated bone substitutes in reference to untreated samples after 14 days.

The measurement of bone thickness at the implant bone interface shows a greater thickness in the BSP group than in the control group (CPC scaffolds).

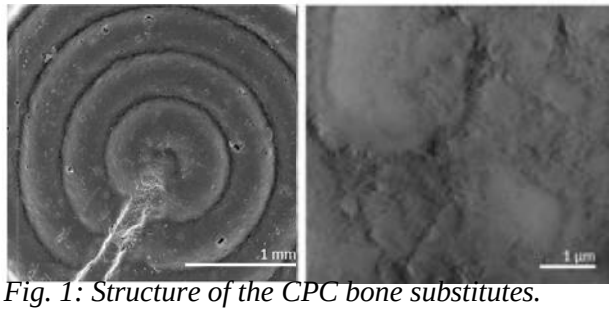

Fig. 1: Structure of the CPC bone substitutes.

DISCUSSION & CONCLUSIONS: Physisorption is an effective method for immobilizing BSP on bone substitutes. Particularly, the enhancement of gene expression of hOB cell differentiation markers (SP7, Runx2) through BSP-coating could influence bone healing in a positive way. However, the bone marker increases in gene expression, as well as the greater bone thickness in µCT scans, are not statistically significant. Therefore, further studies are needed to clearly demonstrate this positive impact on bone healing.

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Sulfated Glycosaminoglycan Derivatives Modulate the Biological Activity Profile of Angiogenic Growth Factors

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INTRODUCTION: Sulfated glycosaminoglycan (GAG) derivatives are promising candidates for functional biomaterials since their sulfate groups modulate the binding and thereby the biological activity profile of growth factors. The interaction of different GAGs with several growth factors has previously been reported. These studies demonstrated a sulfation- and carbohydrate back-bone-dependent binding of GAGs resulting in an influence on the interaction of mediators with receptors and therefore their bioactivity [1,2]. Angiogenic growth factors, like vascular endothelial (VEGF) and basic fibroblast growth factor (bFGF) play an important role during wound healing [3]. Heparin is known to distinctly regulate VEGF and bFGF signalling by altering their interaction with the respective receptors [4]. Hence we hypothesize that VEGF and bFGF interaction with different GAGs influences their bioactivity depending on their degree of sulfation (D.S.) and carbohydrate backbone. This might lead to important implications for the use of GAGs as part of biomaterials for controlling angiogenesis during wound healing in health compromised patients.

METHODS: The interaction of VEGF and bFGF with different sHA and CS derivatives was studied via surface plasmon resonance (SPR). VEGF or bFGF were immobilized onto a sensor chip surface and different concentrations of solute GAGs were injected to reveal their binding strength. Additionally, ELISA experiments with immobilized GAGs were performed to validate the SPR results. The influence of GAGs on the capability of growth factors to bind to their receptors was again investigated by SPR, while the consequences of the GAG/growth factor interaction on bioactivity were studied in cell culture experiments. Here pre-incubated GAG/growth factor complexes were added to HUVECs and growth factor-stimulated migration was determined.

RESULTS: In case of bFGF the binding strength of sHA and CS derivatives reveals a strong dependence on the D.S. but not on the carbohydrate backbone of the GAG.

For VEGF, however, sHA derivatives show a higher binding strength than CS derivatives with the same D.S. The binding of growth factors to their respective receptors was impaired in the presence of sulfated GAGs which implies an altered biological profile. This could be verified in cell culture experiments, since the presence of sulfated GAGs affected the growth factor-stimulated migration of HUVECs.

DISCUSSION & CONCLUSIONS: The results imply that the different molecular geometries in the carbohydrate backbone of the GAGs might render the respective sulfated groups to interact differently with the growth factors. This is further emphasized in investigations with TGF-β1 where naturally sulfated heparin demonstrated a comparable binding strength to high sulfated CS, even though it has a lower D.S. [1]. The specific interplay of GAGs with the angiogenic growth factors VEGF and bFGF modulates their receptor binding as previously reported for other mediators [2] and thus influences their biological activity profile.

The results imply that sulfated GAGs are promising candidates to be included in biomaterial coatings for controlling angiogenic and therefore wound healing processes, which would in particular be beneficial for health compromised patients.

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Magnetic blood purification: from research to clinic

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INTRODUCTION: A new approach for the specific removal of harmful substances from blood based on magnetic nanoparticles has been developed. These nanoparticles are chemically equipped with specific binding agents against harmful target substances and can be injected to an extracorporeal blood circuit. They will then selectively bind to target molecules (e.g. toxins) in the blood. Before it re-enters the patient's body again, the magnetic nanoparticles are separated from the blood via a magnetic filter unit thereby clearing the blood from the noxious target molecules (Fig. 1).

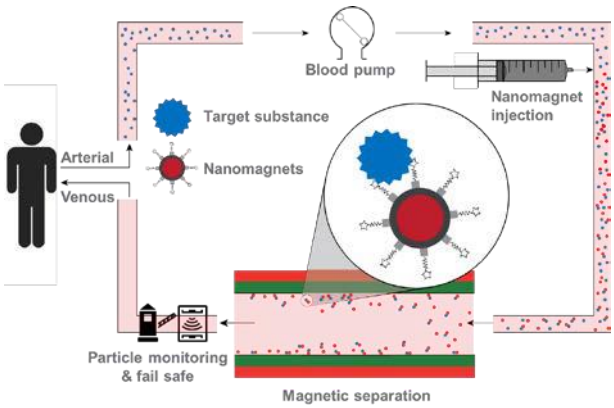

Fig. 1: Application of functionalized magnetic nanoparticles in an extracorporeal circuit for blood purification.

The magnetic nanoparticles have a diameter of around 30 nm and consist of an iron carbide core (Fe_3C) surrounded by a graphene-like carbon coating. The non-oxidic core provides high magnetization while the carbon coating not only ensures chemical and thermal stability, but can also be covalently functionalized. A very potent retrievable capturing vehicle is obtained by linking the magnetic core via a polyethylene glycol linker which improves biocompatibility to a highly specific binding agent (Fig. 2).

SCIENTIFIC BACKGROUND: The scientific background of the project has been published in numerous academic journals. A prototype was developed and tested *in vitro* and *in vivo*, whereby feasibility and efficacy were proven (removal of endotoxin, cytokines, drugs, heavy metals).[1,2] In addition, biocompatibility (e.g., blood compatibility, inflammatory response, cytotoxicity) and the long-term effects of injected nanoparticles over one year in mice were evaluated.[3,4] The preclinical tests indicated good biocompatibility and therapy tolerability.

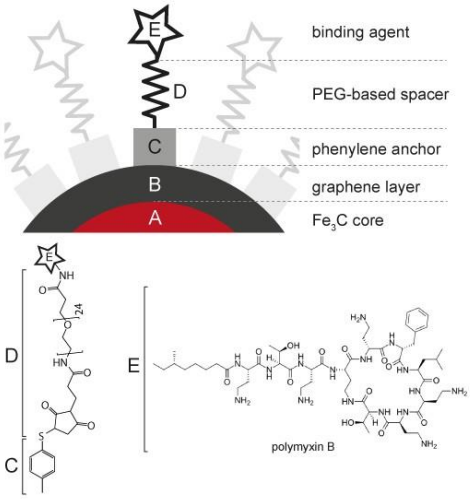

Fig. 2: Nanoparticle structure.

CLINICAL TRANSLATION: Circulating endotoxins are very common in sepsis, which is one of the most common causes of death in ICU. Polymyxin B, an approved antibiotic, is able to bind to endotoxins but its use is limited due to toxicity. However it is suitable for application in this blood purification as binding agent. In order to bring this technology to benefit patients, we want to design a medical device that can be connected to the existing medical equipment (e.g., dialysis machine) as an add-on. To gather clinical data in a first-in-man clinical trial with the aim to prove safety and efficacy of our therapy, we need to address the following points as next steps:

Particle manufacturing includes the establishment of a GMP conform production process of the particles, the corresponding functionalization, as well as packaging and shelf life evaluation.

Biocompatibility testing of particles implicates both *in vitro* and *in vivo* biocompatibility tests, compliant with GLP and ISO standards.

Optimization of device advances the prototype injector/separator towards human application and ensures safe and easy-to-use handling.

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Optimizing Attachment and Orientation of a Peptide Ligand to Plasma-Treated Polymers Using Molecular Dynamics

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INTRODUCTION: Biomedical devices often cause undesirable side-effects such as scarring or inflammation. Biomaterials offer the promise of integration by acting as a bridge between the non-organic implant and the organic tissue, thereby avoiding these responses. A technique developed at the University of Sydney based on plasma-immersion ion implantation uses dry plasma treatment to produce a carbonized, activated surface layer that is able to covalently bind to bioactive molecules such as proteins or peptides directly from buffer solution[1]. In this project, molecular dynamics computer simulations of variants of bioactive peptides derived from tropoelastin[2] are used to help explain both the cell activity of the variants and the conditions that lead to bioactivity on a surface.

METHODS: The molecular dynamics simulations were performed using the NAMD code and the CHARMM36 force field. Extended equilibrium simulations were performed for several peptide variants in solution, and these were analyzed using dimensionality reduction to determine the critical collective variables. Circular dichroism spectra were also produced to compare with experiment.
For the surface-bound simulations, an interfacial system consisting of water and a polymer surface was developed. The surface was parameterized using water contact angle simulations and surface energy values as training set data, since this is the main determinant of peptide adsorption. Steered molecular dynamics simulations were used to rank the binding strength of the different peptide variants, helping to explain their cell-activity behaviour in experiment.

RESULTS: Experiments have shown that some variants of exon 36 of tropoelastin are able to bind to cell-surface receptors and, when present on a surface in the correct orientation, can adhere cells to that surface. The equilibrium simulations of exon 36 show that the peptide is intrinsically disordered with low secondary structure and a broad conformational ensemble, in agreement with NMR measurements and circular dichroism spectra. Dimensionality reduction of the conformational data shows populations of unique conformations, corresponding to organisation of the likely binding region. These populations occur only in the bioactive variant.
The development of an interfacial system for simulating peptide-surface interactions showed that for additive force fields, some materials can be accurately represented. Using this interfacial system, SMD simulations ranked the order of adsorption strength for the peptide variants, which corresponded to their cell activity.

DISCUSSION & CONCLUSIONS: Control of the presentation and orientation of bioactive peptides on surfaces is vital to ensure their bioactivity. Conversely, without a detailed understanding of the molecular determinants of surface interactions, bioactivity can be lost because poor presentation of the bioactive region will block its interaction with cell surface receptors. Computer simulations can play an important role in forming a detailed, molecular understanding of surface interactions. The approach taken here is a novel method to parameterizing an interfacial system for simulations with additive force fields. The results show that such simulations, in conjunction with experiment, are useful to develop a more complete understanding of the performance of peptide-functionalised biomaterials.

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Alginate-sulfate Nanocellulose Bioinks for Cartilage Bioprinting Application

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INTRODUCTION: The lack of available materials for biopritnting applications is currently what is holding this promising technology back. A good bioink should not only be biologically active but also have the proper rheological properties. A combination of materials is often superior over single materials and necessary to fulfil both the biological and rheological requirements. Here we present a combination of the biologically active biopolymer alginate sulfate, which was shown to be chondrogenic and induce cell proliferation and spreading [1,2], and the viscosity modifying agent nanocellulose (NC).

METHODS: Rheological measurements were performed to assess the flow behaviour and gelation properties of alginate sulfate when mixed with NC. Bovine chondrocytes were encapsulated in alginate and alginate sulfate with or without NC and the viability and cell morphology was investigated at day 1, 14 and 28. Cells were also printed using the alginate sulfate-NC bioink with different needle diameters and shapes and cell viability and morphology were evaluated. COMSOL simulations were performed to see if differences between the printed and the encapsulated cells arise from differences in maximum and average shear stresses occurring in the different printing geometries.

RESULTS: Rheological measurements revealed that, despite the lower viscosity of alginate sulfate solutions compared to unmodified alginate, the flow properties of the final bioink with NC were only slightly influenced. Mechanical properties of alginate sulfate-NC were equal to alginate NC after increasing the alginate sulfate concentration in the bioink. Cells showed a round morphology at every time point in the alginate and alginate-NC gels, whereas in the alginate sulfate and alginate sulfate-NC samples the cells started spreading after 14 days in culture. Only when the printing geometry was optimized was the ability of cells in alginate sulphate to spread and proliferate maintained. Simulations revealed differences in the average and maximum shear stress between conical and straight needles of different diameters which might explain the differences in the cells ability to spread or not in the printed samples.

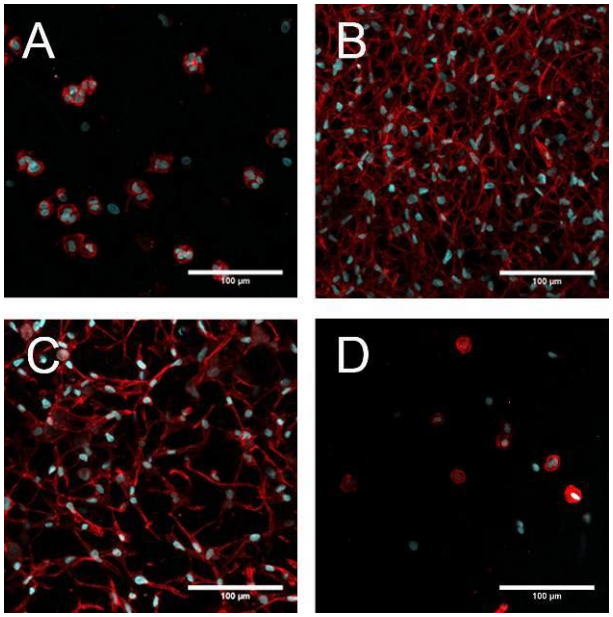

Fig. 1: Chondrocyte morphology at day 28 encapsulated in alginate-NC (A) or alginate sulfate-NC (B) or printed with alginate sulfate-NC with a straight 413 µm (C) or 159 µm (D) needle. Scale Bar is 100 µm.

DISCUSSION & CONCLUSIONS: Alginate sulfate maintained its mitogenic properties and effects on cell spreading in the presence of NC. The NC addition made alginate sulfate a printable material, but restrictions remain in regards to usable inner needle diameters and shapes for cells to survive and show the spreading behaviour.

ACKNOWLEDGEMENTS: This work was supported by ETH Research Grant ETH-23 14-1, FIFA/F-MARC and EU program Eureka and Vinnova.

Cell Response to Lateral Constraint

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INTRODUCTION: Cells at interfaces are not only exposed to biochemical stimuli, but also experience inhomogeneous mechanical environments. These may include spatial constraints for cells in multicellular arrangements or a reduced dimensionality on 2D cell culture substrates or even 1D confinement on single fibres in reconstituted *in vitro* fibrous scaffolds. Similar situations are also observed in various tissues *in vivo*. In previous work, we investigated human umbilical vein endothelial cells under varying lateral constraint and found a bimodal distribution of actin stress fibres, characterized by the uneven formation of exterior and interior stress fibres and their respective spacings [1].

METHODS: We use soft lithography to micro-pattern hard substrates and polyacrylamide hydrogels with fibronectin for subsequent culture of cells in defined geometry, i.e. on stripe-like patterns. Cells are either fixed and stained or monitored in a living state for several hours using SiR-Actin probes [2]. Actin cytoskeleton pattern and overall cell morphology are evaluated with and without biochemical inhibition to reveal cellular components and signalling pathways responsible for cell response to external geometry. In particular, myosin activity and overall cell contractility are targeted via blebbistatin and ROCK inhibitor Y-27632. Furthermore, cell traction force microscopy and cell stiffness measurements using atomic force microscopy are employed to investigate the contractile and mechanical state of cells under spatial constraint.

RESULTS: We observed that different actin stress fibre distributions, particularly on fibronectin stripes with widths below 40µm, persist against changes in biophysical and biochemical parameters. We found that inhibition of myosin activity or inhibition of ROCK pathway only lead to minor perturbations in the bimodal behaviour of actin stress fibre pattern.

Furthermore, cells in strong lateral confinement showed smaller projected areas, diminished traction forces and strain energies when compared to cells on less constraining substrates. However, no obvious correlation between cell contractility and the two distinct actin cytoskeleton patterns could be found.

Live cell imaging of actin stress fibre patterns revealed a fast switching between different stress fibre states on the timescale of minutes. Furthermore, a tendency of inward movement of stress fibres from the exterior was observed.

DISCUSSION & CONCLUSIONS: With our setup, we are able to measure the mechanical and morphological response of cells to geometric constraint, especially at the transition from 1D to 2D environments. We found that cells in quasi-1D confinement show a reduction of actin stress fibre occurrence, along with a decrease in cell size and contractility. It was found that contractility correlates with cell size, rather than with actin cytoskeleton pattern. Hence, we do not find traction force to be related to strong changes of actin stress fibre patterns in quasi-1D confinement and that such states are stable during perturbation of underlying signalling pathways. These observations support the idea that global biophysical parameters like surface tension, curvature and stiffness rather than intracellular molecular components are responsible for the bimodal stress fibre behaviour [1].

ACKNOWLEDGEMENTS: We gratefully acknowledge Prof Arndt form Jena University for donating SiR-Actin probe.

Influence of dissolved Zn ions on bio-degradation of Mg-based implants material.

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INTRODUCTION: Mg and its alloys are currently the most promising metallic candidates for biodegradable orthopaedic implants. Their excellent mechanical properties, very close to that of human bone, reduce stress shielding effects which otherwise could induce a considerable mismatch between implant and natural bone. They are degradable in the pH range of the body fluids and have inherent biocompatibility because Mg ions play an essential role in the cell/bone metabolism[1]. However, their low corrosion resistance has an influence on respective implant mechanical properties during the healing process, resulting in implant weakening before the regrown bone can fully take over load bearing functions. Recently, Zn has attracted attention as coalloying element to improve the mechanical properties and corrosion resistance Mg alloys[2].

METHODS: To study the influence of Zn in the corrosion behavior of Mg-Zn alloy, a model system targeting the interaction of ultrahigh pure Mg and a "simple" simulated body fluid (sSBF) containing (0, 1 and 5mM of Zn^{2+}) ions was investigated. Uhp Mg was produced via vacuum distillation at ETHZ[3]. In order to decrease the complexity of the sSBF system and to be able to separate the influence of Zn^{+2} ion on Mg corrosion from other components, 0.1M NaCl (non and Tris buffered) was initially used as sSBF system with an initial pH of 7.4. The corrosion process was monitored via H_2 evolution measurement, electrochemical/analytical methods (EIS, ICPMS), as well as X-ray photoelectron spectroscopy (XPS) and XRD.

RESULTS: Standardized 7 day immersion tests with H_2 evolution monitoring were performed for 6 different test solutions (Fig.1a) to evaluate the influence of Zn in the active corrosion pH domain (6-10.5) and during the transition to passivation (> 10.5). The pH-dependent speciation calculation was performed to estimate ionic composition and the thermodynamic state of the solutions which was then compared to the ICPMS elemental composition of the solutions after the immersion test. Fig.1b shows an overview of the samples after immersion test, whereas Fig 1c-e shows SEM micrographs (top and cross-section view).

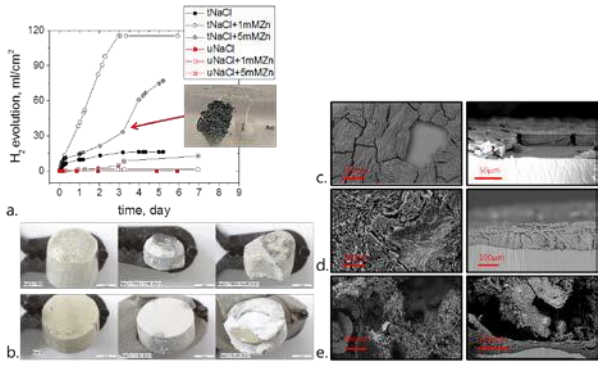

Fig. 1: Immersion test of uhp Mg: a. H_2 evolution vs. time for 6 different electrolytes containing: 0, 1 and 5mM Zn^{2+}, b. view of samples after immersion test: top row in buffered solutions and bottom row in non-buffered solutions, c-e. SEM images of corrosion product top and cross section view respectively in tNaCl, tNaCl+1mMZn^{2+} and tNaCl+5mMZn^{2+}.

DISCUSSION & CONCLUSIONS:

This study targeted the role of Zn^{+2} ions in the Mg degradation process. H_2 evolution (Fig1a) together with EIS measurements show a significant acceleration of the corrosion in presence of dissolved Zn^{+2} ions. For the highest Zn concentration (5mM), a short time of protection / passivation is observed before the corrosion rate increases. A reactivity increase upon Zn^{+2} additions to sSBF is even observed for a "passivated" sample. EDS mapping showed a uniform distribution of Zn across the corrosion product layer after immersion in 1mM Zn and a more local distribution after immersion in 5mM Zn^{+2} solution. Upon incorporation of Zn in the corrosion products, the layer thickness increases, exhibiting a complex porous structure (Fig.1d-e). The initial onset phase of Zn^{+2} / Mg interaction was studied by XPS after 90sec of immersion in different pH ranges (5-7.4). XRD analysis confirmed the presence of MgO, ZnO, $Mg(OH)_2$ and $Zn(OH)_2$ as well as metallic Zn, with the proportion of the different phases depending on the starting Zn^{+2} concentration in solution.

Poly(vinyl alcohol)/curdlan/silver nanoparticle nanofibers for wound healing

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INTRODUCTION: An ideal wound dressing material should be biocompatible, biodegradable, enhance the healing process and prevent the growth of exogenous7microorganism. Nanofibrous scaffolds produced via electrospinning are emerging as potential wound dressing materials, as they have high surface area to volume ratio, good porosity and also mimic the fibrillar nature of native extracellular matrix.

Curdlan is a linear glucan known to possess wound healing and immunomodulatory properties. In this study, a blend of PVA and curdlan has been electrospun and antimicrobial property has been imparted by the addition of silver nanoparticles.

METHODS: Curdlan was produced from Agrobacterium sp. ATCC 31750 as reported earlier[1]. It was dissolved in formic acid and blended with PVA dissolved in water at a weight ratio of 1:2 and was electrospun to obtain nanofibers. The electrospun mat was dried under vacuum at room temperature overnight. The mat was then made insoluble in water by glutaraldehyde vapour crosslinking for 24 hours.

Two different methods were used to introduce silver nanoparticles into and on the surface of the electrospun fibers. First, the prepared PVA/curdlan scaffold was soaked in 0.01M aqueous silver nitrate solution at 37 °C for 6h to enrich the scaffold surface with silver ions and the obtained scaffold (PVA/curdlan/AgNP) was dried [2]. Second, silver nitrate was added to the electrospinning solution, and treated with ultraviolet radiation for 8h post electrospinning to obtain reduced silver nanoparticles within the fibers of the scaffold (PVA/curdlan/$AgNO_3$) [3]. Physicochemical characterization, *in vitro* biocompatibility and antibacterial activity are investigated to evaluate PVA/curdlan/$AgNO_3$ and PVA/curdlan/AgNP nanofibers as ideal wound dressings.

RESULTS: The morphology of the electrospun PVA/curdlan/$AgNO_3$ and PVA/curdlan/AgNP fibers are shown in Fig. 1. The PVA/curdlan/$AgNO_3$ fibers had uniform fibers with mesh like appearance without any bead formation. PVA/curdlan/AgNP fibers had silver nanoparticles with an average diameter of ~140 nm over the fibers and on the pores. The presence of silver in the scaffolds was confirmed with Energy-dispersive X-ray spectroscopy and quantified using inductively coupled plasma optical emission spectrometry. Addition of silver nanoparticles to PVA/curdlan blend showed changes in FTIR spectra of PVA/curdlan blend in the 1500-1100 cm^{-1} region. The scaffolds exhibited significant log reductions in the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria after 24 hours treatment. They were also found to be nontoxic to mouse fibroblast cell line.

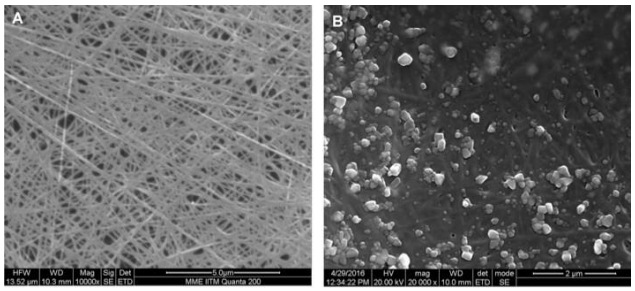

Fig. 1: SEM micrographs of electrospun (A) PVA/curdlan/$AgNO_3$ fibers and (B) PVA/curdlan fibers with AgNP coating.

DISCUSSION & CONCLUSIONS: The PVA/curdlan scaffolds with silver nanoparticles were fabricated in two different ways. SEM analysis revealed the nanotopographical features of the fibers and silver particles. The scaffolds were nontoxic and displayed excellent antibacterial activity against *S. aureus* and *E. coli*. Thus, they could favor wound healing due to the presence of curdlan and also minimize the risk of infection due to the presence of silver.

ACKNOWLEDGEMENTS: We thank SAIF, IIT Madras for SEM and ICP analysis. We thank Interdisciplinary program, Department of Biotechnology and Department of Metallurgical and Materials Engineering, IIT Madras for their support and DST-INSPIRE for fellowship.

Regenerative Potential of Copper Coated Implants

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INTRODUCTION: A current challenge in designing permanent implants for orthopedic applications is the modification of physical and chemical characteristics to stimulate the regenerative processes in the tissue. On the other hand, in about 3% of the patients with joint endoprosthesis a periprosthetic joint infection represents a devastating complication. To meet both challenges we tested both the anti-microbial activity and regenerative potential of copper coated titanium materials *in vitro* and *in vivo*. It is known that in dependence on its concentration copper plays a physiological role or can be cytotoxic.

METHODS: For the *in vitro* experiments copper ions were galvanically deposited to Ti6Al4V plates using a bath containing copper acetate. The generated surface contained a copper load of 1 µg/mm^2. *S. aureus* bacteria were used to test the anti-microbial activity and human bone marrow derived mesenchymal stem cells (MSC) were used to evaluate regenerative activity of the material surface. The same procedure was used to generate copper coated K-wires, which were applied to fix a tibia fracture in a rabbit model. A suspension of *S. aureus* bacteria were injected into the fracture site.

RESULTS: The *in vitro* experiments revealed that an increasing release of copper from the material surface with a maximum of 2.0 mM copper after 72 hours was able to kill planktonic bacteria. In addition, after 24 h a complete removal of adherent bacteria was observed. To test a regenerative potential of copper we found that at a concentration range of 0.1 to 0.3 mM, proliferation of MSC was observed. At a similar concentration copper was able to stimulate osteogenic differentiation, including mineralization of the cells. Mineralization of MSC was also induced when MSC were cultured on the copper coated material (Fig. 1). In the fracture model using rabbits we found that copper coated nails were completely cleaned from bacteria after 4 weeks at the end of the experiment. In contrast, copper uncoated nails contained attached bacteria films. This indicated an anti-microbial activity of copper coated nails *in vivo*. A regenerative potential of the copper coated nails was confirmed by an increased callus index during fracture healing, when copper coated nails were used.

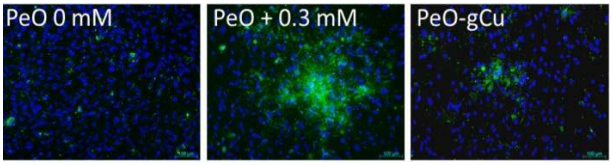

Fig. 1: Mineralization of MSC (green cells) at a copper concentration of 0.3 mM (middle) and on copper coated material (right)(reproduced from reference 1)

DISCUSSION & CONCLUSIONS: We demonstrated that copper ions released from the surface of a titanium implant are able to prevent the formation of a microbial biofilm. In addition, evidence was provided that copper induces regenerative processes, i. e. stimulation of MSC *in vitro* and an increased callus formation during fracture healing. Anti-microbial activity and stimulation of mesenchymal stem cells correlated with higher and lower copper concentrations, respectively. Therefore, we argue that using a copper coated implant, after implantation the material will first exert an anti-microbial activity at the vicinity of the implant surface, whereas later on the regenerative activity of the implant surface will predominate due to a lower copper concentration.

ACKNOWLEDGEMENTS: The study was financially supported by the Ministry of Economic Affairs Mecklenburg-Vorpommern and the European Fund EFRE (V 220-630-08-TFMV-S-016 and V220-639-08-TFMV-F-016 as well as V220-630-08-TFMV-1-001).

EFFECT OF CONCENTRATION OF CATIONS IN HYDROXYAPATITE FOR BIOMEDICAL APPLICATIONS

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INTRODUCTION: Hydroxyapatite (HAp) is an FDA approved bone substitute widely used for its osteoconductive and bioresorbable properties. These inherent properties can be further enhanced by addition of bioactive metals like strontium and zinc. Strontium is well known for preventing osteoporosis, stimulating bone formation and improving osteovascularization[1]. Zinc, acts as an antibacterial agent and also plays a vital role in osteogenesis[2].We hypothesize that by introducing these two ions in structure of hydroxyapatite, a tailored bioceramic can be obtained that serves a dual purpose of osteointegration and also inhibits microbial growth. By varying the concentration of ions used, it may be possible to alter the end application of the final product.

METHODS: The dual ion substituted hydroxyapatites (SrZnHAp) were synthesized by microwave accelerated wet chemical precipitation method. Strontium nitrate and zinc nitrate were used as cationic precursors, added to a solution of 1.67M of calcium nitrate. This solution was then reacted with 1M diammonium phosphate, at rate of 0.2ml/min and at an alkaline pH 10 – 11. Two different concentrations of cationic precursors were added to calcium nitrate solution thus varying the amount of ionic substituent in hydroxyapatite. The slurry obtained was subjected to microwave irradiation for 30 min to achieve homogenous nucleation. The precipitates were filtered, washed, oven dried and powdered to obtain nanoparticles. Physicochemical characterisation of these nanoparticles was carried out via XRD, FTIR, SEM/EDS, TEM and ICP–OES. In vitro antibacterial activity and biocompatibility are also assessed to investigate use of SrZnHAp as potential antimicrobial bone graft material.

RESULTS: The synthesized material was crystalline and monophasic. No carbonate impurities were present. SrZnHAp showed rod like morphology in TEM images with length of ~50nm and width of ~20nm. Morphology of SrZnHAp aggregates can be observed in Figure 1. EDS confirmed presence of ions in different concentration as shown in Table 1.

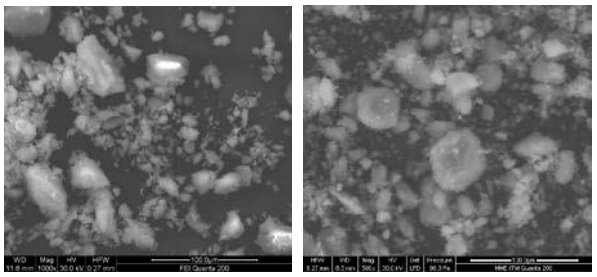

Fig. 1: SEM Micrographs of (1) SrZnHAp1 and (2) SrZnHAp2.

Table 1: EDS analysis showing % atomic substitution of ions in SrZnHAp

Elements	% atm	
	SrZnHAp1	SrZnHAp2
Ca	23.50	56.78
Sr	1.48	3.24
Zn	0.67	4.30
P	14.65	34.14
Ca/P	1.60	1.88

DISCUSSION & CONCLUSIONS: Microwave irradiation enabled effective synthesis of SrZnHAp nanoparticles in shorter span of time. Two different concentrations of ions incorporated in the structure could alter the properties of hydroxyapatite accordingly. This could have a significant effect on the biological properties of the bone substitute such as its inherent antimicrobial action, biocompatibility, cell proliferation and adhesion.

ACKNOWLEDGEMENTS: Rutvi Sanghavi acknowledges IITM and MHRD for fellowship.

Novel delivery system of bone morphogenetic protein 2 (BMP2) based on nanoporous silica nanoparticles and collagen matrices for bone and tooth regeneration

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INTRODUCTION: Bone morphogenetic protein 2 (BMP2) is a growth factor that has the potential to induce new bone formation by triggering of mesenchymal stem cell differentiation into osteoblasts.[1] A similar approach can be used for the regeneration of dental tissue such as enamel, dentin and periodontium. Due to the fact that BMP2 acts locally it should be delivered directly to the regeneration site via an appropriate carrier.[2] Hence, BMP2 is non-covalently immobilized on the surface of nanoporous silica nanoparticles (NPSNPs). With this simple and efficient approach it is possible to achieve a sustained release of BMP2.

METHODS: First, the nanoporous silica nanoparticles were loaded with 5 µg ml^{-1} BMP2 in PBS with 0.1% BSA by diffusional loading at 4 °C overnight. After the protein loading silica nanoparticles were subsequently combined with a collagen scaffold by immersing into the nanoparticle suspension for 24 hours. The *in vitro* release of BMP2 was carried out in PBS with 0.1% BSA in a convection oven at 37 °C. The release medium was refreshed every 24 hours. The total amount of immobilized BMP2 and the release profiles of BMP2 were determined by an enzyme linked immunosorbent assay (ELISA).

RESULTS: About 0.8 µg of BMP2 were loaded on the surface of both types of nanoporous silica nanoparticles. All samples show a sustained release of BMP2, where amino-modified NPSNPs demonstrate a faster BMP-2 release than non-modified silica nanoparticles.

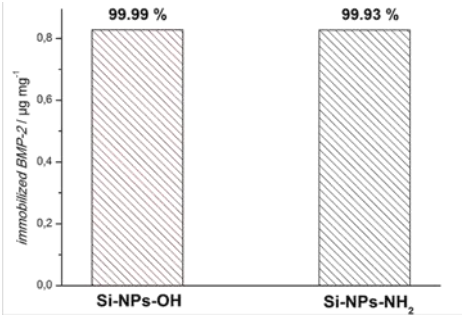

Fig. 1: BMP2 amounts that were loaded on the surface of 1 mg of unmodified and amino-modified nanoporous silica nanoparticles.

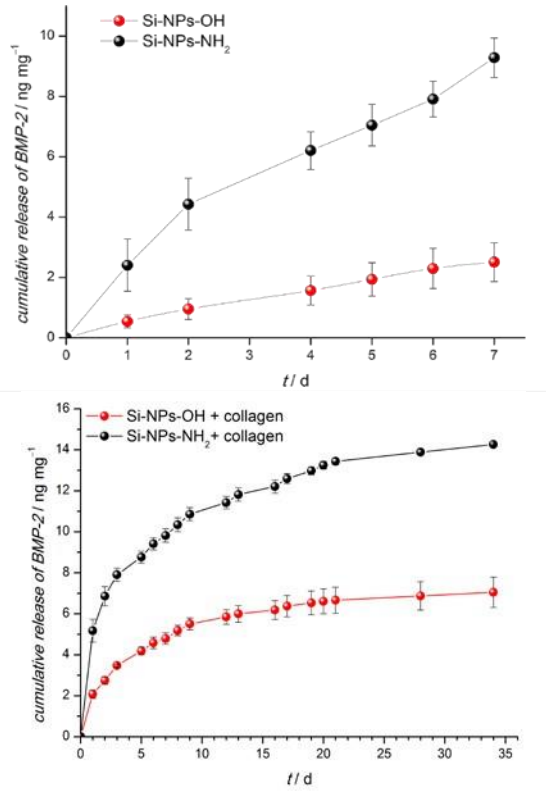

Fig. 2: Controlled BMP2 release from nanoporous silica nanoparticles and nanocomposites upon choosing an appropriate functional group.

DISCUSSION & CONCLUSIONS: Successful fabrication of nanocomposite structures between collagen and BMP2-loaded NPSNPs was achieved. Upon choosing an appropriate functional group on the surface of NPSNPs the release of BMP2 could be accelerated (amine groups) or slowed down (silanol groups). It could be shown that the release of BMP2 was sustained over 5 weeks.

ACKNOWLEDGEMENTS: This work is funded by NIFE (Niedersächsisches Zentrum für Biomedizintechnik, Implantatforschung und Entwicklung) within the interdisciplinary research program Biofabrication.

Foreign body giant cell response after implantation of the biodegradable polymer poly(L-lactide-co-D/L-lactide into rats depends on material structure

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INTRODUCTION: Implantation of biomaterials causes material-dependent inflammatory and wound healing responses and in the end-stage a foreign body reaction with occurrence of macrophages and foreign body giant cells (FBGC) originating from macrophage fusion. Furthermore, FBGC are also found in the implant vicinity if the implanted material is too large for phagocytosis[1].
The present study was aimed at examining occurrence and characteristics of FBGC after implantation of the biodegradable polymer poly(L-lactide-co-D/L-lactide as membranes and electrospun fibre meshes into rats.

METHODS: *In vivo study.* 24 rats received pieces (5×5 mm) of membranes or electrospun meshes from poly(L-lactide-co-D/L-lactide) (PLA) and PLA meshes coated with plasma-polymerized allylamine (PPAAm) by simultaneous i.m. implantation. After 7, 14 and 56 days, the peri-implant tissue was collected from 8 animals per experimental day. *Histological analysis.* Cryosections were either stained with Haematoxylin/ Eosin (HE) or with primary antibodies for $CD68^+$ macrophages/monocytes (ED1; M1-type), $CD163^+$ macrophages (ED2; M2-type) or the regeneration marker nestin. Bound primary antibodies were detected with the APAAP system. FBGC were counted in HE-stained slides and expressed as cells per mm^2. *Statistical analysis.* The non-parametric Mann-Whitney test and the Wilcoxon matched pairs test were used.

RESULTS: The number of FBGC did not differ between PLA meshes with or without PPAAm on any day, but was significantly higher for meshes compared to membranes (Fig. 1). Thus, material structure but not surface chemistry influenced the FBGC reaction. On day 56, the FBGC number was about 4-fold higher than on day 7 for both meshes, whereas no FBGC were found for membranes.
Furthermore, the FBGC response was negatively correlated with the predominantly M1-type pro-inflammatory $ED1^+$ monocytes/macrophages, but positively correlated with the M2-type anti-inflammatory $ED2^+$ macrophages (Fig. 2). Additionally, the $ED1^+$ monocytes/macrophages were negatively correlated with the regeneration marker nestin (r=-0.78; p=0.001). In contrast, $ED2^+$ macrophages showed a positive correlation trend with nestin expression (r=0.309; p=0.172).

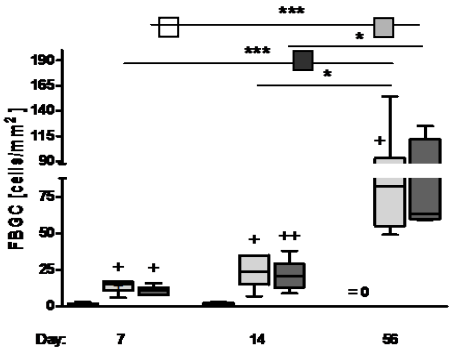

Fig. 1: *Number of FBGC after implantation of PLA membranes ( ), PLA meshes ( ) and PLA meshes coated with PPAAm ( ). * p<0.05; ** p<0.01, *** p<0.001 vs. PLA membranes*

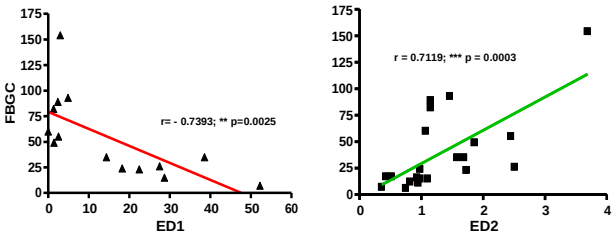

Fig. 2: *Negative correlation of $ED1^+$ monocytes/ macrophages but positive correlation of $ED2^+$ macrophages with FBGC response during chronic inflammation (days 14 and 56) within PLA meshes.*

DISCUSSION & CONCLUSIONS: The study demonstrates that the structure of PLA implants markedly influenced the FBGC response. The pronounced FBGC reaction for electrospun meshes was probably caused by the fibrous mesh character. Furthermore, clear correlations between the FBGC number and ED1, ED2 and nestin were observed indicating that "precursor cells" of FBGC are M2-type macrophages and express nestin as a sign of neoformation/reconstruction.

ACKNOWLEDGEMENTS: The study was supported by the Federal Ministry of Education & Research (project Campus PlasmaMed).

Growth of biocompatible structures on the surface of Ti-6Al-4V(α+β)

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INTRODUCTION: Ti-6Al-4V (α+β) has been used for decades in implants. This alloy has a good corrosion resistance, and its mechanical properties, such as Young's module and tensile strength, are more alike that of bones than other metallic biomaterials [1]. The metal itself does not bond directly to bone tissues. It is the oxide layer, native or anodized, which does the interface metal-bone. Essentially, the oxide layer has two functions. From the side of the metal, the layer works as a barrier preventing corrosion of the substrate and liberation of potentially harmful metal ions into body fluids. From the bone side, the oxide layer allows a spontaneous nucleation of apatite by consuming ions of calcium and phosphate present in the body fluid [2]. Moreover, micropatterning of the oxide layer with similar cellular length can improve regulation of the cell-cell contact and increase adhesion strength, [3]. Among several available methods for patterning the oxide surface, e.g. photolithography, adhesive tapes, one of the simplest approaches is to use the surface's own properties for extruding 3D microstructures. For instance, the phases α (hcp) and β (bcc) of Ti-6Al-4V (α+β) can have different oxidation rates [4]. Then, with heat treatment, it is possible to define the grain's geometry and, consequently, the 3D structures. This work presents the influence of phases α and β of Ti-6Al-4V (α+β) during the growth of the oxide layer using an anodization process. The oxide layer developed above the phases (α and β) may have an impact on the nucleation of apatite (Fig. 1).

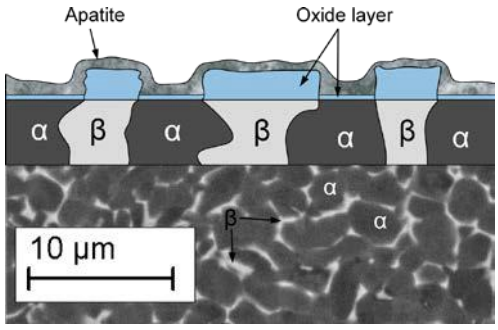

Fig. 1: Development of 3D-structures on the surface of Ti-6Al-4V (α+β) by anodic polarization using only the properties the substrate (phases α and β)

METHODS: Samples of Ti-6Al-4V (α+β) were anodized in 0,5M H_2SO_4 at different potentials up to 200V vs. Pt. The choice of the potentials was based on the results of a linear sweep voltammetry. After anodization, samples were characterized by the following techniques: XRD (Bruker D8 Advance), SEM (Zeiss EVO MA 15), EDX (Bruker Quantax 200) and atomic force microscopy - AFM (Park NX10).

RESULTS: The results showed that, for a limited range of potential (70 V to 103 V), atomic diffusion in β-phase was higher than in α-phase. Consequently, the growth of the oxide layer was not uniform. β-Phase generally grew 120nm more than α-phase (Fig. 2). An increase of voltage induced dielectric rupture, which produced uniform porous structures. This dielectric rupture also contributed to the oxide phase transformation (e.g. anatase to rutile).

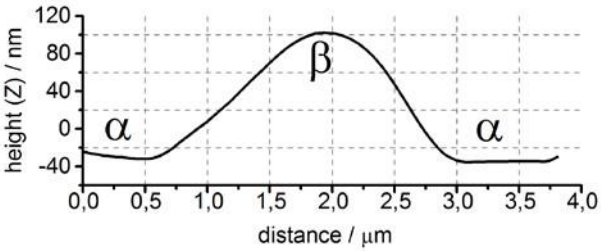

Fig. 2: AFM measurement of the anodized sample (cross-section)

DISCUSSION & CONCLUSIONS: The main outcome drawn from this work is that the crystal structure and the chemical composition of the alloy Ti–6Al–4V (α+β) may have an influence on the oxide formation. In addition, the formed oxide layer may affect the nucleation of apatite improving the biocompatibility of implants.

ACKNOWLEDGEMENTS: This project was financed as „GenMik" by the state of Ministerium für Wissenschaft, Forschung und Kunst B-W. And Hochschule Furtwangen (Campus Tuttlingen)

Hydrophilic, High Lubricious coatings for biomedical application

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INTRODUCTION: Medical devices, such as catheters, needles or syringes, typically require a lubricous coating that minimizes strain-induced discomfort when gliding within the blood vessel, penetrating the skin or enables smooth movement of the plunger, respectively.

In practical terms, when talking about potential technologies that are suitable to realize such low friction coatings on different medical devices, several technologies can be considered[1]. However, not all of those technologies can cope with the main requirements for an industrial process: advantageous functionality, high repeatability and low cost.

In the present contribution we will present an industrial, versatile approach for application of ultrathin lubricious thin films by means of jetting and spraying devices and compare this to other strategies commonly used in the MedTec field.

METHODS: Thin functional lubricous films are obtained by use of a three step process involving the deposition of a monomolecular adhesion promoting layer, the subsequent deposition of a functional polymer and the final curing with UV-light in order to ensure optimal bonding between substrate and coating.

In order to ensure high reproducibility of the coating process, an automated coating device based on a 4-axis robot system carrying dosing and jetting heads was used (Figure 1).

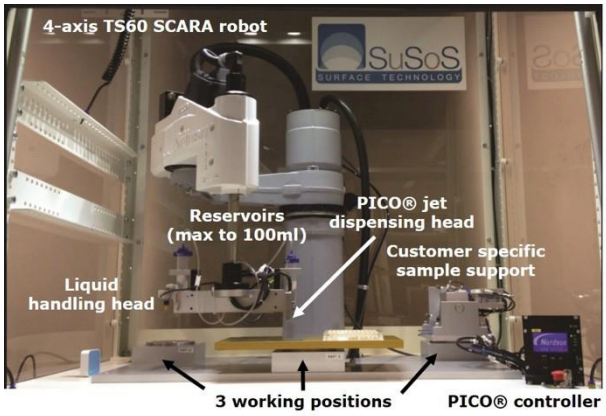

Fig. 1: Image showing the automated coating device used at SuSoS to apply thin film coating on various substrates. The device consists of a modified four axis pick and place SCARA Robot allowing fast and flexible handling with three different working platforms.

The coating thickness, composition and wettability has been determined by Ellipsometry, X-Ray Photoelectron Spectroscopy (XPS) and Contact Angle-measurements respectively.

Tribological tests of the different coatings have also been carried out under different conditions.

Coating wear resistance has been assessed by measuring number of particulate that is being transferred in the lubricant solution during long term wear testing, and the evolution of the friction properties.

RESULTS: Long term, high pressure (~100 MPa) wear investigation of different generations of coating (AziGRIP LUB) as well as a commercial coating was performed. First generation AziGRIP LUB and the commercial coating show increase in friction after different cycles, suggesting a wear-off of the coating. The highly crosslinked AziGRIP LUB 2, second generation, has an improved wear resistance (Figure 2).

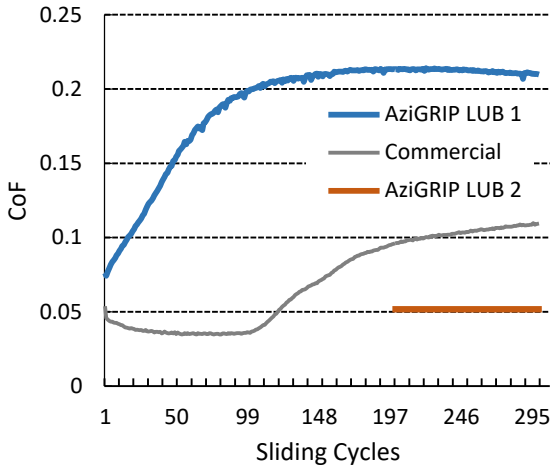

Fig. 2: Comparative wear testing (300 cylces,~100 MPa contact pressure) between AziGRIP 1 and 2 formulations and a commercial coating. The higher crosslinked AziGRIP LUB 2 formulation show improved wear resistance.

DISCUSSION: Highly Lubricious, wear resistant coatings can be generated by a simple industrial process and thus can compete with current technologies used for this type of applications.

Design of poly(N-isoproprylacrylamide)-silver nanocomposite for biomedical applications

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INTRODUCTION: In the last decades, polymeric materials have attracted a significant interest in the biomedical field especially in their use as implants. With the emergence of new multi-drug resistant bacteria and despite advances sterilization procedures, the contamination of implant surfaces by bacteria is a serious concern.[1] Thus, the design of new surfaces in order to prevent bacterial adhesion and biofilm formation is an important task. Silver has already been proven to be effective against bacteria even at low concentrations.[2]
In the framework of this project, we focus on the synthesis of a nanocomposite composed of silver nanoparticles (AgNPs) and poly(N-isopropyl-acrylamide) (PNiPAAm). This polymer is very suitable for biomedical applications owing to its good biocompability and thermosensitivity with a low critical solution temperature close to 32°C. [3]

METHODS: PNiPAAm was obtained by RAFT polymerization via a trithiocarbonate compound as chain transfer agent. Degrees of polymerization (DP) of 25, 50, 75, 100, 150, 200 were targeted.
AgNPs were synthesized by reducing Tollens reagent by glucose in a solution containing the PNiPAAm.[4] Several nanocomposites with different ratios of Ag/polymer (2/1; 1/1, 0.5/1) were prepared.

RESULTS:

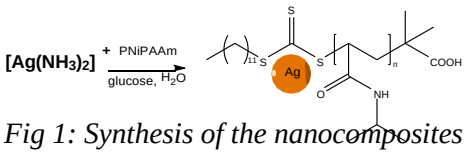

Fig 1: Synthesis of the nanocomposites

The polymers were obtained via a controlled polymerization as attested by a good correlation between the theoretical M_n and the experimental M_n as well as low polydispersity ($Đ < 1.3$).

Table 1: Number average molar mass (M_n) obtained for the different polymers.

DP targeted	M_n theo ($g.mol^{-1}$)	M_n exp ($g.mol^{-1}$)
25	2730	2790
50	5200	5570
70	7440	8340
100	10100	9990
150	14500	15700
200	19000	17300

AgNPS were synthesized in the presence of the polymer in order to prevent aggregation. The latter is believed to decrease the antimicrobial properties of the silver. As shown by the TEM pictures, better dispersion of AgNPs in the polymer was observed for longer chains compared to shorter ones.

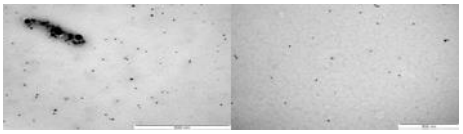

Fig. 2: TEM pictures of the nanocomposites with a ratio Ag/Polymer 1/1 (Mn 2790 $g.mol^{-1}$ left and 15700 $g.mol^{-1}$ right)

Ag content was determined by ICP-OES measurements showing a weight percentage less than 0.02 %. Thermogravimetric analyses showed a faster degradation of the polymer in the presence of silver.

DISCUSSION & CONCLUSIONS: Nanocomposites with different contents of Ag were successfully obtained. Ag^+ release kinetics are still under investigation in order to determine the influence of the temperature, the silver content as well as the length of the polymer. The antimicrobial properties are under investigation.

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Estimating Spring Constant Values Between the Adhesion of Bacteria and Substrates Using Three Different Spectroscopic Techniques

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INTRODUCTION: Bacteria are able to adhere to virtually any substrate giving rise to biofilms, which can lead to severe infections on biomedical implants. The initial adhesion of bacteria to a substrate is the first step in biofilm formation and this bond needs to be studied in detail in order to find ways for its prevention. Several methods thus far have been used in order to assess these viscoelastic bond properties; these include AFM, and optical tweezers. These techniques although informative are rather disruptive and unfortunately offer low throughput. In this study data will be presented where the spring constant (k) has been determined using three different spectroscopy methods: vibrational spectroscopy[1], quartz crystal microbalance with dissipation (QCM-D), and total internal reflection microscopy (TIRM). These methods offer fast, reliable and high throughput information about the viscoelastic bonds between bacteria and substrate. The data was obtained for two different strains of *S. salivarius*, HB7 containing 91 nm long fibrils whereas HBC12 only contains a layer of extracellular polymeric substances (EPS).

METHODS: The frequency and dissipation shifts at multiple QCM-D overtones facilitates determination of the viscoelastic bond properties while driving bacterial oscillations at MHz range. TIRM and phase-contrast microscopy was used to perform nano-scopic vibrational spectroscopy to determine the bond elasticity in 3 and 2 dimensions respectively. QCM-D was carried out using a window-equipped chamber. The window chamber containing the sensor was mounted underneath a microscope equipped with a CCD camera, enabling real time monitoring of the adhesion of bacteria. For 3D vibrational amplitude, a commercial TIRF microscope was modified to image scattering intensity. The scattered intensity was recorded on a camera, where 2000 frames were recorded at a rate of 33fps with an exposure time of 9 ms. This configuration resulted in a lateral resolution of 72nm/pixel. The vibrational spectroscopy in 2D was determined with a CCD mounted on a phase-contrast microscope. 2000 frames were recorded at a rate of 33fps. Experimental set up can be seen in *Fig. 1.*

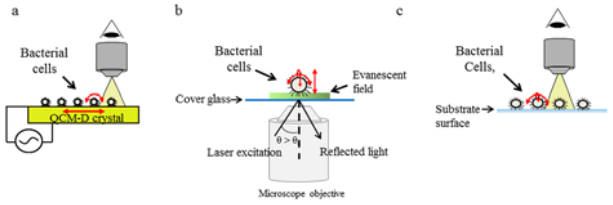

Fig. 1.Three different techniques used in this study to determine the spring constant (k), a) QCM-D, b) TIRM, and c) Vibrational spectroscopy.

RESULTS:

Table 1. Spring constant values for the adhesion of S. salivarius *onto hydrophilic glass at two different ionic strengths obtained by phase-contrast microscopy.*

Sample	**0.57 mM**	**57 mM**
	k (x 10^{-5} N m^{-1}	
HB7	**3.6 ± 0.04**	**2.1 ± 2.04**
HBC12	**0.3 ± 0.38**	**2.4 ± 0.84**

DISCUSSION & CONCLUSIONS: The network constituting the outermost bacterial cell layer is what determines the k of the bacteria. For HB7 k decreases with increasing fibril density on the bacterial cell surface. For HBC12, the outermost surface contains EPS, which when increases due to higher ionic strength, higher k is also observed. This shows the apparent differences in adhesion properties. This can thus aid in defining adhesion and detachment in the future.

Immobilization of rhBMP2 via Polydopamine Coating on Additively Manufactured Porous NiTi Alloy for Bone Tissue Engineering

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INTRODUCTION: The shape memory effect and superelasticity of NiTi alloys as well as their good biocompatibility makes them excellent candidates for biomedical implants. However, surface bio-functionalization is needed for better tissue integration. Thanks to recent progress in additive manufacturing technique that enable fabrication of highly porous NiTi alloys (Dadbakhsh *et al*, 2015), implants with precisely controlled micro-architectures and huge surface area can now be produced and bio-functionalized to enhance their bioactivity.

In this study, porous NiTi was made by selective laser melting (SLM) and post-coated by polydopamine crosslinked with rhBMP2 in order to promote osseointegration.

METHODS: A commercial MLab cusing Concept Laser SLM machine (100 W fibre laser, continuous wave, beam diameter ≈ 50 µm) was employed to manufacture 90% porous NiTi disk-shape specimens with a gyroid structure (AM sample). Manufacturing parameters and designed values are presented in Table 1.

Polydopamine was anchored to the surface of porous NiTi specimens via immersion in a 2 mg/ml solution of dopamine (10 mM Tris–HCl buffer, pH=8.5) and shaking in dark for 24h (DO sample) (Lee *et al*, 2016).

The coated scaffolds were dried at room temperature and subsequently immersed in 100 and 500 ng/mL concentrations of rhBMP2 solution (10 mM Tris-HCl buffer, pH = 8.5) for 24h, called as BL and BH respectively. To verify surface morphology and chemical structure of the coated samples, SEM and FTIR were used, respectively. *In vitro* studies were carried out by culturing hMSCs at a density of 10×10^4 cells/well. Cell viability was assessed by using a live/dead assay after 1 days of culturing. Alamar Blue assay was performed to evaluate cell proliferation.

RESULTS: Additively manufactured porous NiTi with the gyroid unit cell and the corresponding surface (coated with poly dopamine) are shown in Fig.1. FTIR results confirmed the presence of N-H bending on DO, amide N-H bending peak as well as amide C=O stretching peak on BL and BH samples. Based on the live/dead images and Alamar Blue assays (Fig. 2), there were no significant differences between various samples in terms of cell viability but slightly less proliferation in BH and BL after 5 days.

Table 1. Manufacturing parameters and designed values of AM samples

Laser power (W)	Scanning velocity (mm/s)	Hatch spacing (µm)	Layer thickness (µm)
30	200	77	30
Volume (mm^3)	Surface area (mm^2)	Strut size (µm)	Minimal pore size (µm))
18.7	393	110	1500

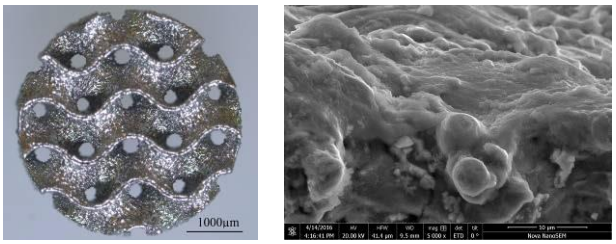

Fig. 1: Optical images of AM sample (left)and SEM (right) images of DO sample.

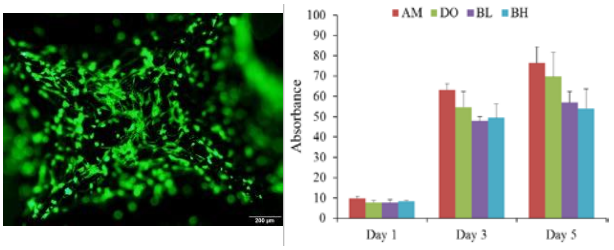

Fig. 2 a sample image of cell viability (left) and cell proliferation results (right)

DISCUSSION & CONCLUSIONS: For bio-functionalization of additively manufactured porous NiTi, it was shown that two different concentration of rhBMP2 can be immobilized by applying dopamine as a cross-linker on the surface of porous NiTi samples. Early time point *in vitro* results did not show a distinct difference between the groups. Further studies on the effect of rhBMP2 immobilizing on the ALP activity as well as rhBMP2 content of MSC cells seeded on the porous structures are in progress.

Interaction of RGD (Arg-Gly-Asp) polypeptide with titanium dioxide surfaces: A computational study

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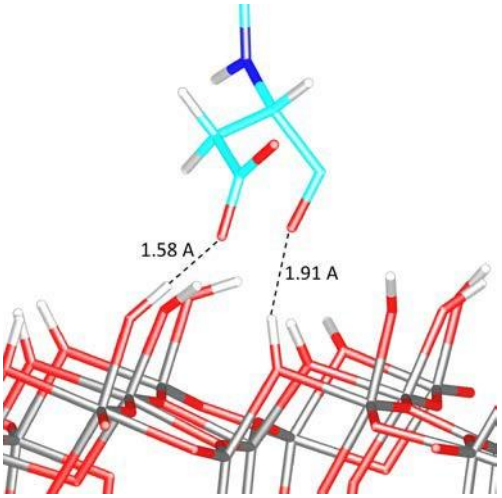
INTRODUCTION: The occasional difference between *in vivo* and *in vitro* test results calls for more in-depth studies. Two probable reasons for this discrepancy are: i) the solution; *in vivo* tests are carried out in blood while *in vitro* tests, an aqueous solution is used to mimic the human body fluid and ii) organic components which are present in the blood but not *in vitro* test solutions. In this project we have used computational methods to study the interaction between the biomaterial (titanium dioxide substrate) and a solvated organic component from an atomistic point of view. As the organic molecule, we have started with the RGD (Arg-Gly-Asp) polypeptide but we would like to study more complex units such as an albumin subdomain.

METHODS: Classical molecular dynamics methods were used to study the interaction of titanium dioxide surfaces with an organic component (RGD). For the inorganic slab, rutile (110) and anatase (101) surfaces were modelled using the force field developed by Preodta et al. [1]. The inorganic slab had a surface area of 30x30 $Å^2$. The SPC/E water model was used.

RESULTS: Even though at the initial orientation, RGD was put at a distance of 4Å from the anatase surface with both its amine and carboxylate groups close to it, the peptide underwent configurational changes. During the MD simulation, the carboxylate group interacts more dominantly with the anatase surface compared to the amine group. Hydrogen bonding (donor-acceptor distance < 3.7Å and donor-H…acceptor angle > 135°) was also observed several times between the oxygen atom of RGD carboxylate group and anatase surface hydroxyl groups (Fig. 1-a).

DISCUSSION & CONCLUSIONS: Comparison between the radial distribution functions and the spatial distribution of the amine and carboxylate group, in z-direction, reveals that RGD interacts differently with rutile and anatase. A noticeable peak is present in the RDF plot between the carboxylate group of RGD and the surface hydroxyl groups on anatase (Fig. 1-b). This was confirmed by the z-density graph for anatase in which the carboxylate group is closer to the surface compared to the amine group. These results confirm protein adsorption on titania surfaces which can influence apatite formation; this is currently being studied experimentally in our group.

a



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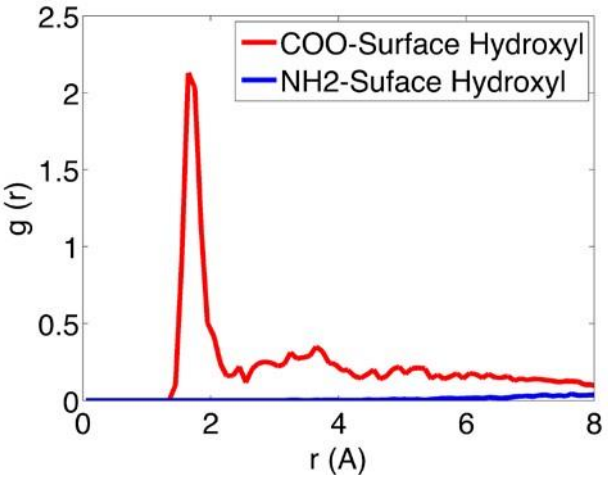

Fig. 1. a) The hydrogen bonding between the RGD carboxylate group and anatase surface hydroxyls (Colour code: C: cyan, N: blue, O: red, H: white, Ti: grey) and b) RDF plot for RGD functional groups and the anatase (101) surface hydroxyl groups.